FELIPE CAMURUGI ALMEIDA GUIMARÃES

BIOGEOGRAFIA DE ANUROS NEOTROPICAIS: FILOGEOGRAFIA, PADRÕES DE DISTRIBUIÇÃO E EVOLUÇÃO DE SINAIS ACÚSTICOS

João Pessoa
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Tese apresentada à Universidade Federal da Paraíba, como parte das exigências do Programa de Pós-Graduação em Ciências Biológicas (Zoologia), para obtenção do título de Doutor.

Orientador:
Dr. Adrian Antonio Garda

Co-orientador:
Dr. Marcelo Coelho Miguel Gehara

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Ata da 106ª Apresentação e Banca de Defesa de Doutorado de Felipe Camurugi Almeida Guimarães

Ao(s) vinte e dois dias do mês de fevereiro de dois mil e dezoito, às 14:00 horas, no(a) Sala 02 do DSE, da Universidade Federal da Paraíba, reuniram-se, em caráter de solenidade pública, membros da banca examinadora para avaliar a tese de doutorado de **Felipe Camurugi Almeida Guimarães**, candidato(a) ao grau de Doutor em Ciências Biológicas. A banca foi composta pelos seguintes professores/pesquisadores: **Dr. Adrian Antônio Garda (orientador), Dr. Sérgio Maia Queiroz Lima (titular), Dr. Fabrícius Maia C. B. Domingos (titular), Dr. Helder Farias Pereira de Araujo (titular) e Dr. Gustavo Henrique Calazans Vieira (titular).** Compareceram à solenidade, além do(a) candidato(a) e membros da banca examinadora, alunos e professores do PPGCB. Dando início à sessão, a coordenação fez a abertura dos trabalhos, apresentando o(a) discente e os membros da banca. Foi passada a palavra para o(a) orientador(a), para que assumisse a posição de presidente da sessão. A partir de então, o(a) presidente, após declarar o objeto da solenidade, concedeu a palavra a **Felipe Camurugi Almeida Guimarães**, para que dissertasse, oral e sucintamente, a respeito de seu trabalho intitulado **"Biogeografia de anuros neotropicais: Filogeografia, padrões de distribuição e evolução de sinais acústicos".** Passando então a discorrer sobre o aludido tema, dentro do prazo legal, o(a) candidato(a) foi a seguir arguido(a) pelos examinadores na forma regimental. Em seguida, passou a Comissão, em caráter secreto, a proceder à avaliação e julgamento do trabalho, concluindo por atribuir-lhe o conceito Aprovado__________. Perante a aprovação, declarou-se o(a) candidato(a) legalmente habilitado(a) a receber o grau de **Doutor em Ciências Biológicas**, área de concentração **Zoologia**. Nada mais havendo a tratar eu, **Dr. Adrian Antônio Garda**, como presidente, lavrei a presente ata que, lida e aprovada, assino juntamente com os demais membros da banca examinadora.

João Pessoa, 22/02/2018.

Dr. Adrian Antônio Garda (orientador)

Dr. Sérgio Maia Queiroz Lima (titular)

Dr. Fabrícius Maia C. B. Domingos (titular)

Helder F. P. de Araujo
Dr. Helder Farias Pereira de Araujo (titular)

Dr. Gustavo Henrique Calazans Vieira (titular)

Ciente do Resultado:
Felipe Camurugi A. Guimarães
Felipe Camurugi Almeida Guimarães

“O cabeção vai invadir o mundo
E tomar posse de todas as coisas surreais
Todos os mapas já estão sendo traçados na parte de
Cima da sua testa
Pois lá ele tem espaços para 37 mundos e uns
Poucos mais
Ninguém foge a capacidade de extensão que possui
Um cabeção
Já que ele está em todos os lugares óbvios e uns
Poucos mais
Se você acha que o computador é o futuro da
Humanidade
é porque você ainda não percebeu esta vasta
Cabecinha em expansão
E acredite
Mais cedo ou mais tarde não haverá mais
Pensamentos legais
Pois para cada cabeça existirá
Um piolho espião
Conectado diretamente com o cabeção
Que vai destruir todos os pensamentos
E unir
Todas as gerações
Numa lerda dimensão...
Lerda dimensão...
Talvez tenhamos no futuro
Um exército de 6 milhões de dólares
Que se acha porrada
Mas caminha no vácuo
De uma ilusão...
Uhuuu!”

Cabeção - Cidadão Instigado

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Resumo

GUIMARÃES, Felipe Camurugi Almeida, Universidade Federal da Paraíba, Fevereiro de 2018. **Biogeografia de anuros neotropicais: Filogeografia, padrões de distribuição e evolução de sinais acústicos.**
Orientador: Adrian Antonio Garda

A distribuição e diversificação das espécies podem ser influenciadas por uma gama de fatores históricos e ecológicos. Entender quais são esses mecanismos é peça chave para decifrarmos como a diversidade é distribuída em várias escalas, desde táxons, linhagens, fenótipos e genealogias. O objetivo principal da presente tese é verificar a importância de diferentes processos históricos e ecológicos na distribuição de anuros neotropicais, em diferentes escalas. No primeiro capítulo usei abordagens comparativas para investigar a influência da sobreposição de nicho climático na variabilidade dos sinais acústicos de um grupo de espécies proximamente relacionadas. Sendo assim, usei 15 espécies de anuros do grupo *Boana albopunctata* para testar as seguintes hipóteses: se espécies relacionadas são geograficamente sobrepostas, sinais acústicos importantes para reprodução e demarcação de territórios devem ser mais diferentes que o esperado por evolução neutra; alternativamente se os cantos possuem sinal filogenético elas devem divergir no nicho climático e distribuição geográfica evitando assim potencial hibridização ou competição interespecífica. Gerei uma árvore de espécie datada e construí modelos de nicho para boa parte das as espécies. Analisei 2088 cantos de anúncio de 212 indivíduos provenientes de 84 localidades. As espécies tenderam a apresentar baixa sobreposição de nicho, com divergência para a maioria dos pares de espécies. Os cantos de anúncio tiveram sinal filogenético. Os resultados sugerem que a maior parte da diversificação foi alopátrica com a conservação de sinais reprodutivos similares, suportando a segunda hipótese. No segundo capítulo, usei *Boana raniceps* para testar sob uma abordagem filogeográfica diferentes processos de diversificação na Diagonal de Formações Abertas da América do Sul (DFA). Utilizei múltiplos marcadores moleculares, métodos coalescentes, modelos de nicho ecológico, ferramentas de genética da paisagem e sinais acústicos para testar: 1) se existe apenas uma linhagem, amplamente distribuída e geneticamente coesa; 2) se o soerguimento do Planalto Central brasileiro ou características da paisagem da DFA tiveram influência na diversificação da espécie; 3) se mudanças climáticas no Quaternário influenciaram a história demográfica de *B. raniceps*; 4) se possíveis divergências genéticas foram seguidas por divergência em sinais acústicos. Identifiquei duas linhagens geograficamente estruturadas: uma do Chaco, sudoeste do Cerrado e savanas amazônicas; outra que compreende a Caatinga e a porção norte/leste do Cerrado. A divergência entre as populações data do Médio-Pleistoceno com alta migração entre as mesmas. Grandes áreas de potencial refúgio foram encontradas para ambas as linhagens. O nicho ambiental diferiu entre as linhagens, assim como os cantos de anúncio. *Boana raniceps* ocorre em áreas de baixa altitude e é possível que o soerguimento do Planalto Central, juntamente com um filtro climático, tenha funcionado historicamente como uma barreira semipermeável, reduzindo o fluxo gênico entre as duas populações. Esses resultados reforçam o complexo cenário de diversificação e de distribuição da biodiversidade na região mais biodiversa do mundo.

Palavras-chave: Anfíbios, *Boana*, Diversificação, Genética da paisagem, Interações bióticas, Isolamento por resistência ambiental, Modelos de distribuição de espécies.

Abstract

GUIMARÃES, Felipe Camurugi Almeida, Universidade Federal da Paraíba, Fevereiro de 2018. **Biogeografia de anuros neotropicais: Filogeografia, padrões de distribuição e evolução de sinais acústicos.**
Orientador: Adrian Antonio Garda

The distribution and diversification of species can be influenced by a variety of historical and ecological factors. Understanding these mechanisms is fundamental to deciphering how diversity is distributed at different scales, from taxa, lineages, phenotypes, and genealogies. The main goal of this thesis is to verify the importance of different historical and ecological processes in the distribution of neotropical anurans at different scales. In the first chapter, I used comparative approaches to investigate the influence of climatic niche overlap on the variability of the acoustic signals of a group of closely related species. Therefore, I used 15 anuran species from the *Boana albopunctata* group to test the following hypotheses: if related species are geographically overlapped, acoustic signals important for breeding and territoriality should be more different than expected by neutral evolution; alternatively, if calls present phylogenetic signal they must diverge in the climatic niche and geographic distribution, avoiding potential hybridization or interspecific competition. I generated a dated species tree and constructed niche models for most species. I analyzed 2088 calls of 212 individuals from 84 locations. Species tended to show low niche overlap, with divergence for most pairs of species. Advertisement calls had a strong phylogenetic signal. The results suggest that most of the diversification was allopatric with the maintenance of similar reproductive signals, supporting the second hypothesis. In the second chapter, I used *Boana raniceps* to test different diversification processes of the South American Diagonal of Open Formations (DOF) under a phylogeographic approach. I used multiple molecular markers, coalescent methods, ecological niche models, landscape genetics tools and acoustic signals to test: 1) whether there is only one lineage, widely distributed and genetically cohesive; 2) if the Central Brazilian Plateau uplift or landscape features in DOF influenced the species' diversification; 3) whether climatic changes in the Quaternary influenced the demographic history of *B. raniceps*; 4) if potential genetic divergences were followed by differences in acoustic signals. I identified two lineages geographically structured: one from the Chaco, southwest of the Cerrado, and Amazonian savannas; another comprising the Caatinga and the northern/eastern portion of the Cerrado. The divergence between populations dated to the Mid-Pleistocene with high migration between them. Large areas of potential refuge were found for both lineages. The environmental niche differentiated between lineages, as well as the advertisement calls. *Boana raniceps* occur in lowlands and it is possible that the uplift of the Central Plateau, along with a climatic filter, has historically acted as a semipermeable barrier, reducing the gene flow between both populations. These results reinforce the complex scenario of biodiversity diversification and distribution in the world's most biodiverse region.

Keywords: Amphibian, *Boana*, Diversification, Landscape genetics, Biotic interactions, Isolation by environmental resistance, Species distribution modeling.

INTRODUÇÃO GERAL

A distribuição geográfica e diversificação das espécies podem ser influenciadas por uma gama de fatores históricos e ecológicos. Aspectos como a capacidade de dispersão, restrições ao habitat, presença de competição ou predação, ou ainda características comportamentais, como a fidelidade a determinado sítio, são algumas das forças que moldam a distribuição dos organismos (Guisan & Thuiller, 2005; Peterson et al., 2011; Wisz et al., 2013). A maioria dos trabalhos recentes sugere uma interação sinergística entre as diversas hipóteses, que resultaram na alta diversidade encontrada nos Neotrópicos.

Além de úteis para a identificação de padrões biogeográficos, as ferramentas moleculares têm contribuído fortemente para o entendimento dos processos de diversificação dos organismos, desde escalas mais finas, através da genética de populações, até níveis supraespecíficos. A relação entre os requerimentos ambientais das espécies e aspectos comportamentais sob um contexto filogenético é pobremente compreendida. Além disso, relativamente poucos trabalhos foram dedicados a entender os processos de diversificação em biomas secos e abertos na América do Sul.

A presente tese apresenta o estudo biogeográfico de um grupo de espécies de anuros distribuído na região neotropical em diferentes escalas: desde uma escala filogenética, até a diversificação de linhagens/populações e fenótipos. No Capítulo 1 uso 15 espécies do grupo *Boana albopunctata* para verificar se a relação histórica entre as espécies influenciou na evolução dos nichos climáticos e sinais acústicos das mesmas. Para isso, construí um modelo teórico com três cenários alternativos e usei métodos filogenéticos comparativos para testar se a distribuição geográfica potencial de espécies filogeneticamente aparentadas de anuros possui efeito na divergência e evolução de características fenotípicas associadas a sinais reprodutivos. No Capítulo 2, usei *Boana raniceps*, um hilídeo amplamente distribuído na América do Sul, para testar diferentes hipóteses de diversificação na Diagonal de Formações Abertas. Para isso, usei uma abordagem filogeográfica e de genética da paisagem, com múltiplos locus, modelos de distribuição de espécies e sinais acústicos, contrastando diferentes cenários evolutivos levando em conta o tempo e as características da paisagem da DFA.

Distribuição das espécies: aspectos históricos, ambientais e comportamentais

Alfred R. Wallace (1852, 1855) já havia notado que espécies intimamente aparentadas tendiam a ocorrer próximas, mas não sobrepostas. Atualmente, com hipóteses filogenéticas associadas a métodos comparativos recentes, é possível inferir se espécies relacionadas possuem características ecológicas conservadas ou não (Blomberg & Garland, 2002; Wiens, 2004; Pyron et al., 2014). O sinal filogenético, por exemplo, é baseado na premissa que o grau de parentesco entre espécies influencia na semelhança de características ecológicas (Blomberg & Garland, 2002). O conservantismo de nicho, portanto, seria a tendência de espécies aparentadas apresentarem nichos mais parecidos que o esperado (Wiens, 2004; Pyron et al., 2014).

Modelos de nicho ecológico (MNE), que detectam características ambientais adequadas e projetam no espaço áreas potenciais para a ocorrência de determinada espécie, têm sido recentemente empregados em estudos que investigam os padrões e processos de distribuição e diversificação dos organismos (Alvarado-Serrano & Knowles, 2014). Essa ferramenta tem sido usada em pesquisas de biologia evolutiva (Graham et al., 2004; Pyron et al., 2014; Vodă et al., 2015), biogeografia (Carnaval & Moritz, 2008; Werneck, 2011), ecologia (Leibold & McPeek, 2006) e conservação (Rice et al., 2003; Werneck et al., 2012b). Os MNE são geralmente baseados no nicho Grinelliano. Nessa abordagem, a distribuição geográfica das espécies é dada principalmente por variáveis ambientais (e.g. temperatura, umidade, precipitação e etc.), e são usados em padrões macro da distribuição (Soberón, 2007; Anderson, 2013). Contudo, além da adequabilidade ambiental a ocorrência de uma espécie é também limitada por interações bióticas locais (nicho Eltoniano), e pela sua capacidade de dispersão e colonização (Peterson, 2011).

Vários modelos de especiação têm sido propostos a partir do nicho ecológico das espécies (Wiens, 2007; Pyron et al., 2014). Nesse sentido, padrões de diversificação de nicho podem fornecer ideias sobre alguns dos fatores importantes na distribuição geográfica das espécies (Knouft et al., 2006). Nos MNE é assumido que as espécies mantêm seus nichos (conservantismo de nicho), com poucas modificações no decorrer do tempo (Wiens et al., 2010). Sendo assim, esse modelo seria aplicado em casos de especiação alopátrica, uma vez que se espécies aparentadas com forte sinal filogenético no nicho potencialmente co-ocorrem, elas poderiam competir por recursos similares, ou mesmo hibridizar.

Embora efeitos vicariantes, através de barreiras físicas ou climáticas, sejam provavelmente a forma mais comum de especiação, as interações bióticas podem também atuar como barreiras diminuindo ou quebrando o fluxo gênico entre populações (Brown & Lomolino, 1998; Gutíerrez et al., 2014). Nesses casos, as interações podem promover a distribuição parapátrica dessas espécies e influenciar tanto na composição das comunidades, quanto no fenótipo (Moen & Wiens, 2009; Wisz et al., 2013; Gutiérrez et al., 2014). Alguns trabalhos indicaram que espécies irmãs podem ter preferências climáticas distintas, ocorrendo em ambientes distintos (Rissler & Apodaca, 2007; Florio et al., 2012). Entre espécies irmãs de borboletas e aves, a divergência na distribuição geográfica tem sido explicada tanto por preferências climáticas quanto por interações bióticas (Pigot & Tobias, 2013; Vodă et al., 2015).

Interações bióticas além de regularem a distribuição geográfica de espécies podem desencadear diversificação nos fenótipos (Moen & Wiens, 2009; Wisz et al., 2013; Gutíerrez et al., 2014; Vodă et al., 2015). Por exemplo, em populações simpátricas de espécies aparentadas de salamandras, interações agressivas aparentemente promoveram modificações na forma do crânio, que são mais robustos em populações sobrepostas geograficamente (Adams et al., 2007). Anfíbios anuros são particularmente interessantes para testar como a relação filogenética entre as espécies pode influenciar a diversificação do nicho climático (e a potencial distribuição geográfica), e de traços fenotípicos. Além de prontamente responderem a mudanças no ambiente, dado que são ectotérmicos e dependem da umidade para reproduzirem, a maioria das espécies se comunica por sinais acústicos (Duellman & Trueb, 1986; Gómez-Rodríguez et al., 2015).

Evolução de atributos fenotípicos em anuros

Dentre os diversos tipos de sinais acústicos emitidos pelos anuros, o canto de anúncio se destaca pela sua dupla função. Ele serve primariamente para atração de parceiras para reprodução, e secundariamente para mediar o espaçamento entre machos da mesma espécie e de espécies diferentes (Duellman & Trueb, 1986). Esse sinal, em geral, assegura a reprodução de coespecíficos geneticamente compatíveis, evitando heteroespecíficos e o custo envolvido na formação de híbridos (Wilczynski & Ryan, 1999; Köhler et al., 2017). Esta possibilidade de barreira reprodutiva pré-zigótica pode ser fundamental para o processo de especiação e manutenção do isolamento de espécies (Duellman & Pyles, 1983).

O canto de anúncio apresenta um grande potencial para seleção sexual, visto que fêmeas claramente apresentam preferências por diferentes cantos, tendo sido demonstradas pressões direcionais e estabilizadoras sobre diferentes parâmetros acústicos desta vocalização (Gerhardt & Brooks, 2009). Além disso, há fortes evidências que essa seleção sexual pode levar à especiação em anuros (Boul et al., 2007). Ao mesmo tempo, diversas pressões seletivas ambientais já foram propostas e/ou demonstradas como atuantes na evolução do canto de anúncio dos anuros, podendo ser consideradas como principais: eficiência de propagação em diferentes hábitats (Kuczynski et al., 2010); presença de predadores (Page & Ryan, 2005) e parasitas (Bernal et al., 2006); possibilidade de hibridização com espécies simpátricas de vocalização com sobreposição acústica (Lemmon, 2009); barulho ambiente biótico (Love & Bee, 2010) e abiótico (Röhr et al., 2016). Este cenário abre portas para estudos de variação intra e interespecíficas que auxiliam na compreensão dos fatores envolvidos na evolução de sistemas de comunicação (Panhuis et al., 2001).

Comparações entre variação genética e diversidade fenotípica podem trazer informações relevantes sobre a influência de processos adaptativos e neutros na evolução de algum comportamento, como o canto de anúncio. No entanto, por si só estas comparações não são suficientes para avaliar quais pressões seletivas podem estar modulando estes sinais acústicos (Ohmer et al., 2009). Uma das maneiras para detectar quais são estas pressões é testar quais fatores ambientais e/ou ecológicos podem estar relacionados com a possível discordância entre a variação genética e fenotípica (Richards & Knowles, 2007). Alguns trabalhos demonstraram que há correlação entre variabilidade genética e a diversidade fenotípica e comportamental (como sinais acústicos) em anuros de diferentes populações de uma mesma espécie (Pröhl et al., 2006), ou entre diferentes espécies dentro de um mesmo clado (Erdtmann & Amézquita, 2009).

A correlação entre as variações genética e acústica sugere uma grande importância de processos estocásticos, enquanto discordâncias podem ser atribuídas a processos adaptativos como seleção natural e sexual (Erdtmann & Amézquita, 2009). Estas inferências são feitas com base na proposta de que sinais acústicos podem apresentar taxas evolutivas mais rápidas, uma vez que parâmetros comportamentais tendem a apresentar um menor sinal filogenético do que outras características como morfologia, história natural e fisiologia (Blomberg et al., 2003). Enquanto alguns autores não encontraram covariação entre a variação genética e estrutura do canto de anúncio (Cannatella et al., 1998; Castellano et al., 2002; Pröhl et al., 2006; Ohmer et al., 2009), outros detectaram sinal filogenético neste sinal acústico (Ryan et al., 1996; Wollenberg et al., 2007; Erdtmann & Amézquita, 2009; Goicoechea et al., 2010). Além disso, intraespecificamente a divergência acústica pode também corresponder à estrutura filogeográfica em que parte da variação dos cantos é correlacionada com a divergência genética (Boul et al., 2007; Guerra & Ron, 2008; Funk et al., 2009).

Testando hipóteses biogeográficas com estudos filogeográficos

A filogeografia permite o estudo independente de cada espécie e de espécies co-distribuídas, na qual diversas hipóteses biogeográficas podem ser testadas. Assim, estudos filogeográficos têm contribuído para a compreensão dos processos históricos da distribuição de genealogias, cruzando aspectos micro e macroevolutivos e integrando sistemática filogenética, genética de populações, demografia, geologia, modelos paleogeográficos e paleoclimáticos e história natural das espécies (Avise, 2000). Paradoxalmente, a região mais biodiversas do mundo é uma das menos estudadas (Beheregaray, 2008; Turchetto-Zolet et al., 2013). Mesmo com um aumento expressivo na quantidade de trabalhos sobre os processos que moldaram a história evolutiva da biota Sul-Americana, esses ainda são insuficientes (Turchetto-Zolet et al., 2013).

Na América do Sul os padrões de diversificação observados foram decorrentes de processos geológicos e climáticos antigos (Terciário) e recentes (Quaternário) (Rull, 2008). Alguns dos principais eventos são o soerguimento dos Andes e incursões marinhas no Mioceno, fechamento do Istmo do Panamá durante o Plioceno e ciclos glaciais do Pleistoceno (Rull, 2008; Santos et al., 2009). A Teoria dos Refúgios Pleistocênicos (TRP) baseia-se nessa última (Haffer, 1969). Na TRP, durante os períodos secos e frios do Pleistoceno, áreas de floresta úmida teriam retraído e ficado restritas a refúgios, enquanto os ambientes de formação aberta teriam expandido. Esse processo pode ter causado vicariância de populações de espécies associadas a ambientes de floresta úmida. Espera-se então, que exista uma alta diversidade genética dentro dos refúgios e uma alta estruturação filogeográfica quando diferentes refúgios são comparados. A confirmação desses dois padrões seria um indício de estabilidade em áreas de refúgio e de isolamento entre essas áreas (Carnaval et al., 2009). Diversos estudos têm suportado a TRP (Carnaval et al., 2009; Fitzpatrick et al., 2009; Barlow et al., 2013). No entanto, esses estudos são concentrados principalmente nos biomas de floresta úmida (Carnaval et al., 2009; Fitzpatrick et al., 2009; Thomé et al., 2010), enquanto os biomas abertos e/ou secos têm sido negligenciados (Werneck, 2011), mesmo sendo peça importante no estudo de diversificação de espécies Neotropicais, já que apresentam uma história própria e complexa de diversificação. Essa negligência ocorre, em parte, devido à dificuldade de estabelecer possíveis refúgios nessas regiões (identificados como áreas de maior pluviosidade nas florestas, por exemplo; Haffer, 1969).

A Diagonal de Formações Abertas da América do Sul (DFA) é composta por três tipos de vegetação principais, que são sazonalmente estressados pela seca: as Florestas tropicais sazonalmente secas (e.g. Caatinga), as savanas (e.g. Cerrado) e o Chaco (Pennington et al., 2006). Várias espécies ocorrem amplamente e são endêmicas da DFA (Werneck, 2011). No entanto, estudos recentes têm revelado que há uma alta diversificação e especiação críptica, mesmo em organismos que ocorrem em apenas um desses biomas (Domingos et al., 2014; Oliveira et al., 2015; Werneck et al., 2015). A complexidade do cenário de diversificação das espécies que ocorrem nesses biomas pode ser observada nos resultados opostos de vários estudos. Diferentes eventos históricos e biogeográficos, inclusive conflitantes, têm sido evocados para explicar a diversificação nessas regiões. Para a herpetofauna da caatinga, por exemplo, a hipótese do Rio São Francisco como barreira, com efeitos na estruturação das populações, tem sido suportada por alguns trabalhos (Nascimento et al., 2013; Werneck et al., 2015) e refutada em outros (Recoder et al., 2014; Oliveira et al., 2015). No Cerrado, um dos eventos mais marcantes foi o soerguimento do Planalto Central (7-5 Ma), que teria promovido a diversificação de espécies adaptadas aos platôs e às depressões (Colli, 2005). Para algumas espécies de anfíbios, tanto de baixadas quanto de áreas mais elevadas, períodos de diversificação correspondem com o final do soerguimento do Planalto Central (Maciel et al., 2010; Prado et al., 2012; São-Pedro, 2014). Durante o mesmo período, incursões marinhas nas áreas de depressão da bacia Chaco-Paraná, que alagaram grandes áreas do Chaco, devem ter promovido alopatria em algumas populações, e extinguido outras nas áreas de baixada (Garda & Cannatella, 2007; Maciel et al., 2010; Werneck, 2011; Werneck et al., 2012a). Além disso, ciclos glaciais do Pleistoceno devem ter afetado a biota nos três biomas, promovendo expansões e constrições populacionais. Os ciclos climáticos devem também ter conectado e desconectado as áreas de savanas amazônicas com os biomas da DFA (Cardoso Da Silva & Bates, 2002; Werneck, 2011). Estudos filogeográficos com espécies amplamente distribuídas e endêmicas da DFA são escassos, mas necessários para entender se todos esses eventos são importantes ou se as idiossincrasias dos organismos podem revelar diferentes padrões e processos de diversificação.

Alguns estudos filogeográficos prévios sobre a herpetofauna endêmica da diagonal de formações abertas detectaram uma forte associação entre agrupamentos genéticos e os três principais biomas (Caatinga, Cerrado e Chaco) (Werneck et al., 2012a; Recoder et al., 2014), que poderia corresponder a um potencial padrão biogeográfico. No entanto, trabalhos subsequentes demonstraram que os processos de diversificação ao longo da diagonal aberta são complexos e podem depender tanto dos eventos históricos como da história natural das espécies. Para lagartos do complexo de espécies *Phyllopezus pollicaris* e *Vanzosaura*, a forte associação ao habitat também pode ter sido importante no agrupamento genético por bioma (Werneck et al., 2012a; Recoder et al., 2014). A serpente *Phyllodryas nattereri*, por outro lado, não apresentou estruturação geográfica, o que seria esperado considerando sua história de vida, já que é uma espécie altamente dispersiva (Silveira-Filho, 2017). Já o sapo fossorial *Dermatonotus muelleri* teve sua história de diversificação relacionada principalmente a uma forte barreira vicariante, segregando duas linhagens que compreendem Chaco e sul do Cerrado, e norte do Cerrado e Caatinga (Oliveira et al., 2018a).

Pelo seu comportamento filopátrico e baixa capacidade de dispersão, espera-se que os anfíbios não tenham uma ampla distribuição geográfica (Reading et al., 1991; Beebee, 2005). São assim, excelentes modelos para testar hipóteses biogeográficas e modelos de especiação, pois se espera que elementos da paisagem exerçam influência significativa na estruturação genética das espécies do grupo. Não é incomum que espécies com ampla distribuição, teoricamente mais tolerantes à degradação de hábitat e, portanto, com status de conservação como menos preocupante, sejam na verdade complexos de espécies crípticas com múltiplas linhagens evolutivas (Rissler & Apodaca, 2007; Fouquet et al., 2013; Gehara et al., 2014). Algumas dessas espécies, ou mesmo linhagens, podem estar inclusive altamente ameaçadas (Gehara et al., 2013). Estudos como esses podem alterar nossa percepção sobre a riqueza de espécies potencial na região tropical e ter impacto nas ações conservacionistas locais. Após revisões taxonômicas de complexos de espécies crípticas, algumas podem ter sua distribuição geográfica reduzida a menos de 1% do que se achava previamente (Caminer & Ron, 2014). Os impactos dessa diversidade críptica podem afetar o que conhecemos sobre a riqueza de espécies local, biogeografia, evolução, conservação e taxas de extinção (Angulo & Icochea, 2010).

A paisagem da diagonal de formações abertas e a diferenciação genética

Além dos processos históricos, as características da paisagem também podem afetar a estrutura espacial e genética das populações, diminuindo o fluxo gênico, um processo conhecido como isolamento pela resistência ao ambiente (IBE; Cooke et al., 2012; Wang &

Bradburd, 2014). Consequentemente, a interação entre processos históricos e da paisagem, levando em conta a heterogeneidade ambiental (ex. mudanças climáticas no Pleistoceno, fragmentação de habitat, presença de rios e topografia) pode conduzir à diferenciação genética e, inclusive, levar à especiação (Carnaval et al., 2009; Cooke et al., 2014; McCartney-Melstad & Shaffer, 2015). Se uma determinada característica da paisagem atua como barreira ou facilitadora do fluxo gênico dependerá da biologia do organismo. Diferentes habilidades dispersivas, modo reprodutivo, tamanho corporal, fisiologia e habitat de ocorrência também podem influenciar o grau de estrutura genética das linhagens (Fouquet et al., 2012; Orsini et al., 2013; Papadopoulou & Knowles, 2016; Sandberger-Loua et al., 2018; Zamudio et al., 2016). As espécies generalistas e altamente dispersivas de ambientes abertos podem usar sítios reprodutivos imprevisíveis, gerando divergência genética intraespecífica rasa (Rodríguez et al., 2015). Por outro lado, em paisagens topograficamente complexas, há uma tendência para estrutura filogeográfica profunda devido à maior resistência ambiental derivada da heterogeneidade de habitats (Giordano et al., 2007; Guarnizo & Cannatella, 2013; Jablonski et al., 2016; Rodríguez et al. 2015). Consequentemente, espécies de áreas abertas distribuídas em regiões topográficas complexas podem mostrar cenários contrastantes de diversificação. Na DFA, o soerguimento do Planalto Central, com a formação de platôs e vales, adicionou uma maior complexidade na paisagem do Cerrado, por exemplo (Ab'Sáber, 1998; Colli, 2005).

Oscilações climáticas durante o Quaternário também são evocadas para explicar parte da diferenciação genética intraespecífica. As mudanças paleoclimáticas do Pleistoceno impactaram a biota da DFA em ao menos duas maneiras: alteraram a demografia de populações em diferentes períodos e/ou estruturaram populações devido à fragmentação do habitat (e formação de áreas de refúgio). Esses efeitos podem ser observados em vários organismos, como plantas (Ramos et al., 2007; Bonatelli et al., 2014; Correa Ribeiro et al., 2016), insetos (Franco & Manfrin 2013), aracnídeos (Bartoleti et al., 2017), e a herpetofauna (Santos et al., 2014;

Gehara et al., 2017). De fato, os modelos de distribuição de biomas previstos para a América do Sul sugerem uma contração e fragmentação de savanas e florestas sazonalmente secas durante o Último Máximo Glacial, há aproximadamente 20 mil anos (Costa et al., 2018). Nesse período, observou-se expansão demográfica de populações de diferentes espécies na DFA, ou mesmo em escala de comunidade na Caatinga (Gehara et al., 2017). Áreas de instabilidade climática na DFA passaram por constantes mudanças climáticas e poderiam levar a um padrão de isolamento no qual o fluxo gênico entre essas áreas é reduzido. No entanto, outras características da paisagem, como rios, também podem estruturar a população na DFA.

Grandes rios da Amazônia têm sido utilizados como modelos para testar a hipótese dos rios como barreiras. Essa hipótese pressupõe que para organismos terrestres rios amplos e profundos poderiam estruturar populações (Wallace, 1852; Ayres & Clutton-Brock, 1992; Gascon et al., 2000; Moraes et al., 2016). Para a fauna da diagonal da fauna de formações abertas, barreiras formadas por rios também têm desempenhado um papel importante na diferenciação genética. Na Caatinga, o rio São Francisco (um dos maiores da América do Sul) foi responsável pela estrutura genética de alguns lagartos e mamíferos (Nascimento et al., 2011, 2013; Werneck et al., 2015; Oliveira et al., 2018b). No entanto, um determinado rio pode constituir uma barreira para uma espécie terrestre, mas conectar populações de espécies ribeirinhas. Para os anfíbios, a estrutura genética pode ser associada à hidrologia considerando os seguintes fatores: o histórico da drenagem (Kuchta et al., 2016); as bacias hidrográficas e rios que podem funcionar como corredores de dispersão (Lind et al., 2011; Lawson, 2013); os rios atuam como modelo clássico de vicariância, com populações estruturadas em margens opostas dos rios (Fouquet et al., 2012; Ortiz et al., 2018); e dada a natureza do rio (por exemplo, profundidade, largura, velocidade do fluxo e sazonalidade) ser barreira ou corredor (Bittencourt-Silva et al., 2017).

Grupo de estudo

O grupo *Boana albopunctata* apresenta sinapomorfias baseadas apenas em dados moleculares (Faivovich et al., 2005). Até o momento 16 espécies são incluídas no grupo: *B. albopuncatata* (Spix 1824), *B. caiapo* Pinheiro et al., 2018, *B. calcarata* (Troschel 1848), *B. dentei* (Bokermann 1967), *B. fasciata* (Günther 1858), *B. heilprini* (Noble 1923), *B. lanciformis* (Cope 1871), *B. leucocheila* (Caramaschi & Niemeyer, 2003), *B. multifasciatus* (Günther 1859), *B. paranaiba* (Carvalho, Giaretta & Facure, 2010), *B. steinbachi* (Boulenger 1905), *B. alfaroi*, *B. almendarizae*, *B. maculateralis*, *B. tetete* (Caminer & Ron 2014). Com exceção de *B. heiplrini* que ocorre em Hispaniola, na América Central, todas as outras espécies ocorrem na América do Sul, com espécies ocupando uma variedade de ambientes com regimes de temperatura, precipitação, cobertura vegetal e altitudes distintas.

Aparentemente há alta diversidade críptica em alguns complexos de espécies do grupo (Funk et al., 2012). Após uma revisão taxonômica do que se achava ser um complexo de espécies amazônicas reunidas em *B. calcarata/B. fasciata*, baseada em dados genéticos, morfológicos e acústicos, foram identificadas onze espécies candidatas, seis delas espécies confirmadas (Caminer & Ron, 2014). Em estudo filogeográfico com *B. albopunctata*, espécie característica de áreas abertas, foram identificadas três linhagens e uma alta diversificação genética, sendo possível que existam ao menos duas espécies (Prado et al., 2012).

Das espécies do grupo, *Boana raniceps* é a que possui maior distribuição geográfica. Ela é amplamente distribuída na América do Sul e a utilizamos para testar diferentes hipóteses de diversificação sob uma perspectiva filogeográfica. É uma espécie grande, típica de regiões de baixa altitude, ocorrendo principalmente na DFA, mas que pode ser encontrada também nas áreas de savanas amazônicas. Sua população é considerada estável e pode ser encontrada também em habitats degradados e áreas urbanas (La Marca et al., 2004). É principalmente associada a rios (áreas alagadas) e grandes pântanos (Fouquet et al., 2007). O objetivo geral da presente tese é verificar a importância de diferentes processos históricos e ecológicos na distribuição de anuros neotropicais, em diferentes escalas. No primeiro capítulo investigo, através de métodos filogenéticos comparativos, como a diversificação do canto de anúncio e distribuição de espécies aparentadas é associada à relação histórica das espécies. No segundo capítulo, estudo quais são os principais processos históricos relacionados a diversidade e diferenciação genética de um anfíbio amplamente distribuído na América do Sul.

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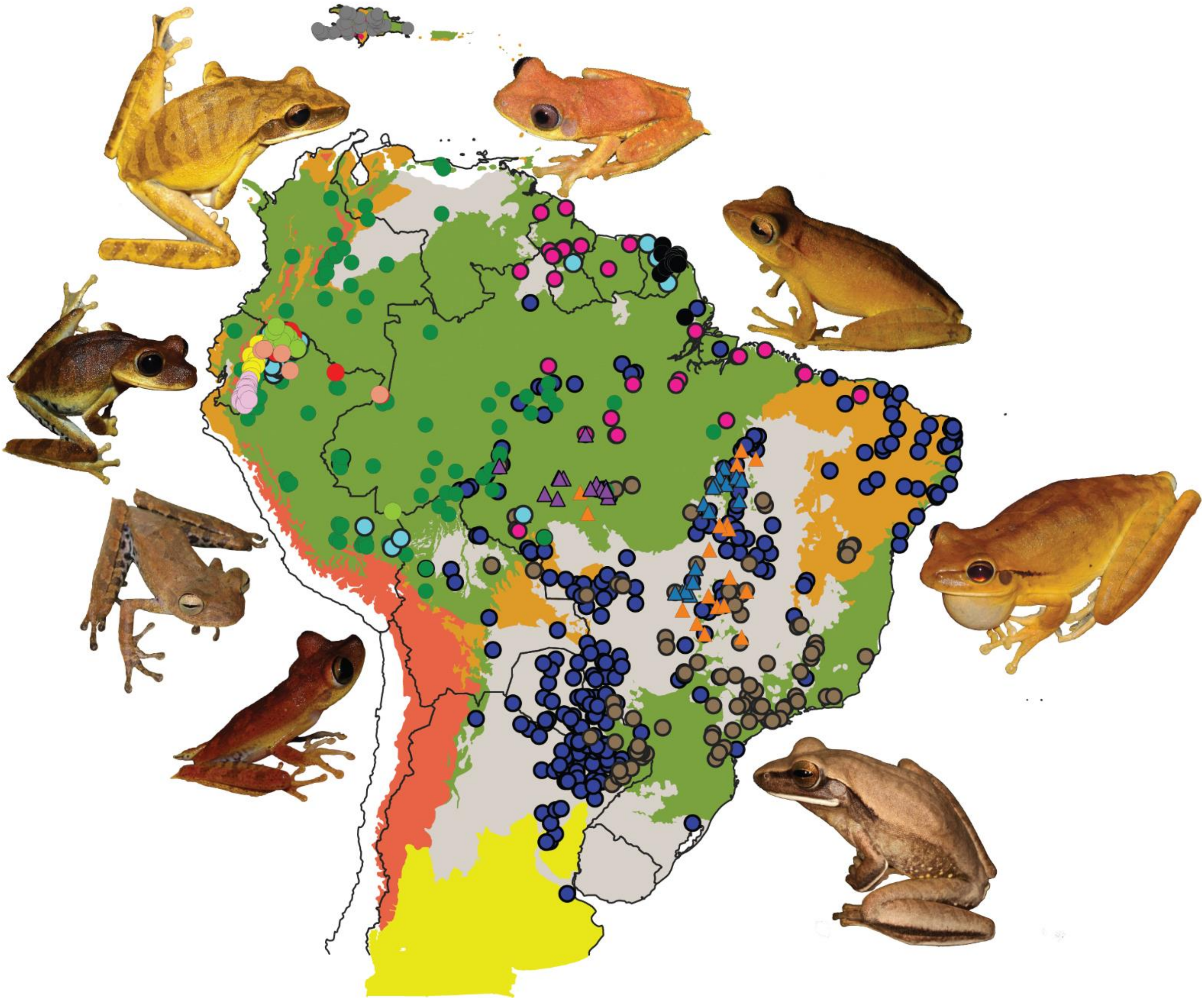
EVOLUTION OF ADVERTISEMENT CALLS AND ENVIRONMENTAL NICHE DIVERGENCE IN NEOTROPICAL TREE FROG SPECIES



Evolution of advertisement calls and environmental niche divergence in a Neotropical tree frog clade

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Abstract

Environmental changes and biotic interactions can drive phenotypic evolution. Although closely related species tend to share similar ecological attributes (e.g. environmental niche), reproductive traits used in species recognition and mate attraction (e.g. anurans' advertisement calls) must differ enough to hinder hybridization. Environmental niche features (and potential geographic overlap) could drive the diversification of advertisement calls among closely related species. We test whether the advertisement calls of closely related species are related to species distributions and evolutionary histories. We constructed a dated species tree for the long-legged tree frogs of the *Boana albopunctata* group. Next, we built species distribution models for each species and compared them using multidimensional analyses to assess whether species environmental niches are similar. Finally, we tested the phylogenetic signal of environmental niches, advertisement calls and their covariation with environmental variables. Pairwise comparisons in the multivariate climatic space supported niche divergence among species. Advertisement calls showed a strong phylogenetic signal, with calls evolving near to the expected by neutral evolution. We found a pattern of niche divergence, with closely related species occurring in parapatry or allopatry. Our results are consistent with a primarily allopatric model of evolution and suggest that the evolution of calls and environmental niches are decoupled.

Keywords: bioacoustics – biotic interactions – *Boana* – diversification – Hylidae – species distribution modeling.

1. Introduction

The degree to which species phylogenetic relationships influence ecological and phenotypic diversification has been a matter of debate for over a century. Wallace (1855) and Darwin (1859) suggested that sister species tend to have similar geographical distributions but usually with non-overlapping ranges, and that phenotypic divergence would be favored by reducing costs associated with competition, aggression, and/or hybridization. The coexistence of congeners can impact diversification of phenotypic traits, generally considered to be driven by competition (Schluter, 2000). Conversely, closely related species tend to have similar ecological attributes, behaviors, morphology, and many other inheritable traits (Blomberg and Garland, 2002).

According to the classic view, competition drives speciation to be allopatric or parapatric, and this is one of the reasons why closely related species rarely co-occur. (Schluter, 2000). Co-occurrence is possible at finer geographic scales, as long as phenotypes have diverged, usually due to ecological character displacement (ECD) (Pfennig and Pfennig, 2009; Schluter, 2000;

Wisz et al., 2013). Moreover, traits related to reproduction and/or strong selection may rapidly evolve in response to biotic interactions and changes in the environment (Pfennig and Pfennig, 2009; Zamudio et al., 2016). Thus, conservatism or divergence in phenotypic traits can covary with phylogenetic relatedness and degree of geographic overlap among species.

The species' range is in a broad sense the combination of intrinsic factor (e.g. dispersion capability and environmental tolerances) and interspecific interactions (e.g. competition and predation) (Holt and Keitt, 2005). Both environmental and biotic factors can, by different processes, ultimately promote speciation. For example, speciation can occur with climatic niche conservatism when sister lineages are primarily allopatric due to physical or climatic barriers, with daughter lineages sharing similar niches over time (Kozak and Wiens, 2006; Wiens and Graham, 2005). Alternatively, allopatric or parapatric lineages may show niche divergence when speciation occur with adaptation to distinct environments (Hua and Wiens, 2010; Schluter, 2009), generally as a result of ecological speciation or species interactions (Gutiérrez et al., 2014; Twomey et al., 2008; Vodă et al., 2015). The presence of a stronger competitor in sympatric species can promote spatial or temporal segregation and ultimately the exclusion of less dominant species from a given assemblage (Borzée et al., 2016).

Both environmental and biotic factors can, through different processes, ultimately influence speciation. For example, speciation can occur with climatic niche conservatism when sister lineages are primarily allopatric due to physical or climatic barriers, with daughter lineages sharing similar niches over time (Kozak and Wiens, 2006; Wiens and Graham, 2005). Alternatively, allopatric or parapatric lineages may show niche divergence when speciation occurs with adaptation to distinct environments (Hua and Wiens, 2010; Schluter, 2009), generally as a result of ecological speciation or species interactions (Gutiérrez et al., 2014; Twomey et al., 2008; Vodă et al., 2015).

Biotic interactions can generate parapatric or allopatric species distributions and influence population density, community composition and phenotype diversification (Moen and Wiens, 2009; Vodă et al., 2015; Wisz et al., 2013). Traits can be conserved through time as a result of stabilizing selection, high rates of gene flow, physiological constraints or biotic interactions (Blomberg and Garland, 2002; Kamilar and Cooper, 2013; Losos, 2008). Trait divergence, conversely, can be driven by genetic drift, pleiotropy, natural selection, and geographic isolation (Blomberg et al., 2003; Wilkins et al., 2013). Characteristics capable of acting as pre-zygotic reproductive barriers can be critical to the process of speciation and maintenance of species isolation (Wilkins et al., 2013). In phenotypes associated with reproductive behavior, such as anuran's advertisement calls, divergence due to character displacement decreases the chances of hybridization among closely-related species and should be subject to diversifying selection under sympatric speciation (Gerhardt and Huber, 2002; Lemmon, 2009; Pfennig and Pfennig, 2009).

For some species, advertisement calls function as important pre-zygotic barriers and are sexually selected traits, being able therefore to evolve rapidly in geographic isolation (Gerhardt and Huber, 2002; Yasumiba et al., 2016). They serve primarily to attract mates and secondarily to delimit male territory (Duellman and Trueb, 1994). Thus, efficient communication is important to ensure reproduction with conspecifics, avoiding costs associated with the formation of hybrids (Oliveira et al., 1991). Indeed, acoustic signals play an important role in the diversification of many taxa, such as primates, birds, and amphibians (Wilkins et al., 2013).

Using anurans to test the associations among ecological, phenotypic and genetic variation is particularly interesting because they have low dispersal capacity, high fidelity to reproductive sites, and relatively high potential for inter and intra- population differentiation (Beebee, 2005; Gerhardt, 1994). Such characteristics suggest that behavioral traits can shape geographic distribution or vice-versa. For closely-related sympatric species, it is expected that calls will be more dissimilar (Köhler et al., 2017). Thus, phylogenetic relationships, geographic distributions, and phenotypic features of species should synergistically interact in the evolution of these organisms.

Here, we used phylogenetic comparative methods along with species distribution modeling and bioacoustics to investigate diversification processes in Neotropical anurans of the *Boana albopunctata* group (Faivovich et al., 2005). This is a widely distributed clade that harbors 16 species with narrow to broad geographic ranges across different environmental conditions. We reconstructed phylogenetic relationships in this group under the multi-species coalescent model and used the resulting phylogeny to investigate acoustic and environmental niche evolution. Different diversification mechanisms may lead to alternative patterns of trait evolution (Fig. 1A; Table 1). Thus, in the present paper we tested three non-mutually exclusive hypotheses: (i) under a system of predominantly allopatric speciation without climatic adaptation we expect the phylogeny to explain advertisement calls and niche divergence; (ii) if speciation is predominantly ecological with adaptation to new environments, we expect highly divergent niches that evolved faster than expected by the phylogeny. In this case, advertisement calls should evolve under neutrality because the predicted small geographic overlap obviates forces that would otherwise promote character displacement; (iii) if speciation is predominantly sympatric, we expect high divergences in advertisement calls in species pairs where environmental niches overlap significantly. Given these hypotheses, we expect that calls of closely-related sympatric species will diverge more than expected by neutrality for frogs in the *B. albopunctata* group (Fig. 1B). Conversely, calls of parapatric and/or allopatric species may be conserved, divergent or evolve according to a Brownian motion model as a result of different processes related to trait evolution (Fig. 1C-D, Table 1).

2. Material and methods

2.1 Study organisms

To test our hypotheses, we used a monophyletic clade of hylid species from South and Central America (Hispaniola), the *Boana albopunctata* group. This clade harbors 16 species with narrow to broad geographic ranges (Caminer and Ron, 2014; Frost, 2018): *B. albopunctata* (Spix, 1824), *B. alfaroi* (Caminer & Ron, 2014), *B. almendarizae* (Caminer & Ron, 2014), *B. caiapo* Pinheiro *et al*., 2018, *B. calcarata* (Troschel, 1848), *B. dentei* (Bokermann, 1967), *B. fasciata* (Günther, 1858), *B. heilprini* (Noble, 1923), *B. lanciformis* (Cope, 1871), *B. leucocheila* (Caramaschi & Niemeyer, 2003), *B. maculateralis* (Caminer & Ron, 2014), *B. multifasciata* (Günther, 1858), *B. paranaiba* (Carvalho & Giaretta, 2010), *B. raniceps* (Cope, 1862), *B. steinbachi* (Boulenger, 1905) and *B. tetete* (Caminer & Ron, 2014). With the exception of *B. heilprini* (restricted to Hispaniola), all other species occur in several South American Biomes (AmphibiaWeb, 2018). The species occupy a wide range of environmental conditions and different biomes, such as moist, dry and coniferous forest, grasslands, savannas and shrublands. All species are distributed, at least partially, in tropical and subtropical moist broadleaf forests and only *B. heilprini* occurs in coniferous forests. We did not use *B. steinbach* in the analyses because there is no acoustic data available for this species. In addition, this species is only known from its type locality (Caminer and Ron, 2014), therefore it would not influence our hypothesis testing framework.

2.2 Divergence time estimation

To test our hypothesis of conservatism or divergence linked to environmental niches and advertisement calls under a comparative framework, and to estimate species divergence times, we built a dated tree for the *Boana albopunctata* group. We used three mitochondrial (mtDNA) and two nuclear (nuDNA) genes deposited in GeneBank (Supporting Information

Table S1) from twelve of the 16 species. For mitochondrial fragments we used the protein coding cytochrome oxidase subunit I (COI) and ribosomal genes 12S and 16S; and the protein-coding nuclear fragments proopiomelanocortin (POMC) and the recombination activating protein 1 (RAG1). There is no molecular data available for *B. caiapo*, *B. leucocheila* and *B. paranaiba*, but according to morphological and acoustic descriptions the last two species are more related to *B. lanciformis* and *B. multifasciata*, respectively (Caramaschi and Niemeyer, 2003; Carvalho et al., 2010).

We aligned sequences with Muscle algorithm (Edgar, 2004) in Geneious v8.1, excluded gaps with GBLOCKs0.91b (Castresana, 2000) and estimated the best substitution model with jModelTest v2.1.7 (Darriba et al., 2012). We phased nuDNA with Phase algorithm (Stephens et al., 2001) using the default configuration as implemented in DnaSP 5.10 (Librado and Rozas, 2009). We estimated a species tree with StarBEAST2 (Ogilvie et al., 2017) using linked trees with the ribosomal genes 12S and 16S and cytochrome oxidase I (COI). We used the uncorrelated lognormal relaxed clock with a Yule speciation-process prior for the mtDNA, and a strict clock for the nuDNA. We calibrated the species tree using the following substitution rates from the literature: 7.35×10^{-3} mutation/site/million years for Hylidae 16S fragment (Gehara et al., 2014) and 0.0147 (two times faster) for COI (Vences et al., 2005). We combined three independent MCMC runs with 2×10^{8} generations each, sampling every 2×10^{4} generation with a 10% burn-in, and checked chain mixing (ESS values $\geq$200) in TRACER (Rambaut et al., 2014). We used TreeAnnotator to generate a maximum clade credibility tree (Fig. 2).

2.3 Advertisement Calls

We assessed 289 recordings obtained from public sound collections and recorded by us (N=40). Due to poor quality and/or misidentification we excluded 70 of these recordings. We analyzed 2,218 calls from 219 individuals recorded in 86 different localities (Supporting

Information Table S2). We measured seven acoustic parameters: call duration (CD; s), note duration (ND; s), number of notes (NN), number of pulses (NP), note rate (NR = NN/ CD; notes/s), pulse rate (PR = NP/ND or CD; pulses/s) and dominant frequency (DF; kHz) in RAVEN Pro 1.5 (Bioacoustics Research Program, 2014). Spectrograms were produced with 512 FFT resolution points and overlap of 50%. All calls were normalized at 0 dB. For statistical analyses we used the average value of each parameter per individual. Advertisement calls of all species present a pulsatile structure, except for *B. tetete*, which exhibits a tonal structure and for this reason was excluded from the analyses because of comparability issues.

To test if advertisement calls are different among species we performed a non-parametric ANOVA (Collyer et al., 2014) in 'geomorph' v.3.1.2 package (Adams & Otárola-Castillo, 2013). A *post hoc* test for pairwise comparison was performed to test which species had different calls, using 10,000 permutations in 'geomorph'.

2.4 Species distribution modeling

We constructed species distribution models (SDMs) using occurrence data available in digital databases (SpeciesLink, http://splink.cria.org.br/; Global Biodiversity Information Facility, http://www.gbif.org/; July 2016), bibliographic references, and scientific collections (Table 2). We applied a spatial filtering on occurrence points at a spatial distance of 5 km with 'spThin' package (Aiello-Lammens et al., 2015). We used 19 bioclimatic variables from Worldclim (http://www.wordclim.org) and forest canopy height (Simard et al., 2011) at a spatial resolution of 2.5 arc-min. To generate SDMs, we used the maximum entropy algorithm MaxEnt (Phillips et al., 2006). We measured the correlation between variables, keeping biologically relevant variables with Pearson correlation coefficients < 0.8. These variables included: forest canopy, mean diurnal range temperature (BIO2), max. temp. of warmest month (BIO5), min. temp. of coldest month (BIO6), precipitation seasonality (BIO15), precipitation of wettest (BIO16), driest (BIO17), warmest (BIO18), and coldest quarter (BIO19). For MaxEnt model tuning and evaluations we used 'ENMeval' package (Muscarella et al., 2014). Models were built considering different transformations (i.e. linear – L, quadratic – Q, hinge – H, product – P, and threshold – T), with six feature classes (FC) combinations: L, H, LQ, LQH, LQHP and LQHPT, with 10,000 background points. We set the range of regularization multiplier (RM) from 0.5 to 4 at increments of 0.5, resulting in 48 combinations considering the six FCs, for each species. We used the Akaike's information criterion corrected for small samples sizes (AICc) to rank models among all candidate models. To partition occurrence and background data we used the "checkboard1" method (see Fig. 1 Muscarella *et al.*, 2014). We also used the area under the curve (AUC) to assess model performance, with average AUC values ranging from 0.82 to 0.99, presenting good to excellent predictive power (Table 2; Fig. 3). Because *Boana tetete* had few occurrence records (N = 5) we did not build SDM for this species.

2.5 Conservatism and divergence analyses

2.5.1 Environmental overlap

We made pairwise comparisons with an ordination approach PCA-env (Broennimann et al., 2012) to evaluate whether species environmental niches are more or less similar than expected by chance, taking into account their environmental background. This method summarizes the multivariate environmental space into two-dimensional principal components (PCA). In PCA-env the density of occurrences is compared with the bi-dimensional environmental space available (100 x 100 cells). We standardized the density of species occurrences employing a kernel smoother in the environmental space (background area). Pairwise niche overlap was estimated by Schoener's *D* similarity index with 'ecospat' R package (Di Cola et al., 2017): *D* metric varies from 0, indicating no overlap, to 1, complete overlap, with the following classes: 0–0.2 = no or very limited overlap, 0.2–0.4 = low, 0.4–0.6 = moderate, 0.6–0.8 = high, 0.8–1.0 = very high overlap (Rödder and Engler, 2011). To test whether niches are more similar or dissimilar than expected by chance ($P \leq 0.05$), the observed *D*-value of pairwise combination was compared to a null distribution (1,000 pseudoreplicates). We used geographical minimum convex polygon (MCP) around occurrence points as the background for each species. Next, we evaluated the relationship between niche overlaps (*D* index) and species divergence times using age-range correlation (ARC) (Fitzpatrick and Turelli, 2006) in 'phyloclim' package (Heibl and Calenge, 2013), with 10,000 random permutations.

2.5.2 Phylogenetic signal in advertisement calls

We calculated the phylogenetic signal of calls (mean per species) using K_{mult} (Adams, 2014) in 'geomporph' package. K_{mult} is an adaptation of Blomberg's K (Blomberg et al., 2003) for multivariate data and considers the evolution of traits under a Brownian motion model (BM). K_{mult} varies continuously from 0 to ∞, with K_{mult} = zero indicating absence of phylogenetic signal, $K_{mult} = 1$ indicating that the traits are under BM, and $K_{mult} > 1$ the traits of related species are more similar than expected by BM. We standardized the data and used 10,000 phylogenetic permutations to access K_{mult} value. To represent the patterns of call evolution in the acoustic space, we used 'phylomorphospace', in which the first two principal components are projected with the phylogeny (Sidlauskas, 2008).

2.6 Covariation between acoustic signals and environment

To test whether calls covary with environmental features we performed two-block partial least squares analyses corrected for phylogenetic relationships (pPLS) (Adams & Felice, 2014). For this, bioclimatic data were extracted for each advertisement call recording point and used mean values for each species. The significance was assessed using 10,000 permutations in 'geomorph'. All analyses were performed in R 3.6 (R Core Team, 2018). Because we did not have access to environmental temperature during calling behavior for most of the recordings (55%) we did not incorporate this measure in our analysis. Because temperature affects call parameters, such as call rate, pulse rate and call duration (Köhler et al., 2017), we removed these variables from the pPLS analysis.

3. Results

3.1 Divergence time estimation

We found a pattern of diversification that started during the Miocene with the split of the Hispaniolan green treefrog *Boana heilprini* from South American species approximately 12 Mya (95% HPD; 6.62–17.44: Fig. 2). The South American clade (SA) diversified during the late Miocene and Pleistocene with its first branching about seven Mya (95% HPD; 4.66–9.72 Mya), originating two clades: clade I comprising *B. alfaroi*, *B. almenderizae*, *B. calcarata*, *B. dentei*, *B. fasciata*, *B. maculateralis* and *B. tetete*; clade II *B. albopunctata, B. lanciformis, B. multifasciata* and *B. raniceps* (Fig. 2). Most SA species appeared during the Pleistocene, from 2.21 to 0.73 Mya.

3.2 Advertisement calls

Advertisement calls differ significantly among species ($F_{13, 208}$= 34.09; *P* < 0.001; Table 3). Species calls are different in 71% of the 91 pairwise combinations (Table 4). Some comparisons in which non-significant differences are observed (*P* > 0.05) may be explained by the small number of samples (i.e. *B. leucocheila* and *B. maculateralis*; see also z-values smaller than or close to 1). Species belonging to clade I have higher note rates, while clade II have higher calls and notes durations (Table 3). Calls of *B. heilprini* are characterized by having the highest number of notes. Calls of *B. tetete* are similar to clade I, but differ from all species analyzed by its tonal structure, while *B. caiapo* is similar to species from clade II (except from *B. raniceps*) but differs from all species by having ascending amplitude modulation and maximum intensity at the final pulses.

3.3 Species distribution modeling

MAXENT models performed well with AUC values equal or greater than 0.86 (Table 2). Clade I is entirely composed of species that occur in the Amazon (Fig. 2A), while species from Clade II are widely distributed along open and/or forested biomes (Fig. 2B). Within clades, species show patterns of parapatric distributions. Furthermore, Clade I has species with more restricted distributions, some of them potentially occurring in sympatry (Fig. 2). The potential for species co-occurrences according to SDMs was identified in clade I, where *Boana calcarata* may be found with all species from its clade (except *B. fasciata*), *B. tetete* with *B. alfaroi* and *B. maculateralis,* and *B. fasciata* with *B. almendarizae*. Likewise, clade II species from open environments (*B. albopunctata*, *B. paranaiba*, and *B. raniceps*) potentially occur in parapatry, whereas those from the Amazon (*B. lanciformis* and *B. multifasciata*) occur in allopatry. *Boana leucocheila* is distributed at the ecotone area between the open Cerrado biome and the Amazon and shares a small contact zone with *B. caiapo*. *Boana caiapo* potentially overlaps with *B. paranaiba* and *B. raniceps.*

3.4 Conservatism and divergence analyses

The ‘PCA-env’ analyses support no or low overlap among all pairwise comparisons: Schoener’s $D \leq 0.1$ is supported for 88% (N = 80/91); 9% with $0.1 < D \leq 0.2$; and 3% with $0.21 < D \leq 0.23$ (Table 5; Fig. 4). Significant niche similarity ($P < 0.05$) with $D > 0.10$ is observed in five cases: *B. alfaroi/B. calcarata*, *B. almendarizae/B. calcarata*, *B. almendarizae/B. fasciata*, *B. caiapo/B. paranaiba* and *B. heilprini*/*B. raniceps*. This latter is composed of allopatric species. In seven cases significant *P*-values with low overlaps are found ($D < 0.1$; see Table 5). All other observed niche overlap values fall within the confidence limits of the null distribution (Fig. 4). ARC analysis detected no phylogenetic signal for overlap ($P > 0.05$), with low intercept and negative but non-significant slope (Fig. 5).

Calls have high phylogenetic signal and evolved near to expected by Brownian motion model ($K_{mult} = 1.09$; $P_{rand} < 0.001$). Phylomorphospace analysis shows the species at the root (*B. heilprini*) occupying a different range in the acoustic space, followed by the split of major species groups along PC1 (Fig. 6), but with the sister species *B. almendarizae* and *B. fasciata* nearly overlapping. The calls of the three species without phylogenetic information, *B. caiapo*, *B. leucocheila* and *B. paranaiba*, are grouped in the acoustic space with those from clade II (Fig. 6). Phylogenetic signal tests for each acoustic parameter separately is presented in Fig. 7.

In species pairs that diverged recently, two patterns considering the potential distribution and call evolution are observed: allopatry and similar calls (*Boana albopunctata* and *B. multifasciata*) and parapatry and similar calls (*B. fasciata* and *B. almendarizae*). Three species from clade II (*B. albopunctata*, *B. paranaiba* and *B. raniceps*) can co-occur, but calls differ among species. *Boana leucocheila*, on the other hand, does not differ in acoustic signals but occurs in allopatry with all other species. While *B. caiapo* and *B. tetete* can potentially co-occur with other species (see above), but their advertisement calls differ.

3.5 Covariation between acoustic signals and environment

The pPLS analysis did not detect a relationship between call variation and environmental conditions (r-PPLS = 0.81, $P_{rand} = 0.07$; Fig. 8). The first vector of calls is composed of positive scores mainly due to note duration, while note rate and dominant frequency have negative values. For the environmental vector, negative values are related mainly to precipitation of the coldest and warmer quarter and minimum temperature of coldest month, while positive scores were mostly due to mean diurnal range and temperature seasonality.

4. Discussion

We found that in the *B. albopunctata* group, species mostly diverged in respect to their environmental niches while acoustic signals evolved near neutrality. This suggest a predominantly allopatric speciation mechanism with adaptation to new environmental conditions, potentially with secondary contact in some cases. Different mechanisms of evolution of bioacoustics characters appear to be ruling the diversification of advertisement calls, considering each clade separately. In Clade I, for example, the sister species *B. almendarizae*/*B. fasciata* have similar calls, which suggests a slower divergence rate than *B. alfaroi*/*B. tetete*, that diverged more recently but with calls structurally different. For these species the possibility of geographic overlap can be related to the observed differences. Most of the Clade II species are widely distributed in different environments, with sister species mainly allopatric. For them, both history and different environmental conditions can impact the call diversification.

Mixed signals of divergence and conservatism are expected in clades with taxa of varying ages (McCormack et al., 2010; Pyron and Burbrink, 2009). Patterns of ecological niche divergence in South and Central American faunas were previously reported for closely related species that also diverged during Pleistocene (Posso-Terranova and Andrés, 2016; Zurano et al., 2017). Unlike clade I (excepts *B. calcarata*/ *B. fasciata*), species that diverged recently during Pleistocene in clade II (*B. albopunctata* and *B. multifasciata*) showed divergences with very limited niche overlap. Although ARC analysis showed inconclusive results on the speciation mode, given the general tendency of low environmental overlap among species pairs, allopatric speciation with secondary range overlap can be associated to diversification process of the group. With exception of *B. dentei*, older lineages within subclades are in general more widely distributed. Because older clades have more time, secondary range overlap in species that diversified allopatrically or parapatrically can be expected in such cases (Warren et al., 2014). On the other hand, for the sister species *B. almendarizae*/*B. fasciata*, environmental niches are more similar than expected by the available background. In this case is hard to distinguish whether their current distribution resulted from similar climatic tolerances due to phylogenetic relatedness and/or biotic interactions. The absence of phylogenetic signal and inconclusive ARC can be expected when there are signals of sympatric and nonsympatric speciation in a given group (Fitzpatrick and Turelli, 2006).

4.1 Advertisement call evolution

Anuran advertisement calls have been suggested to present weak or no phylogenetic signal because of sexual selection (Cannatella et al., 1998). Accordingly, many studies have reported no correlation between genetic variation and advertisement call structure (Cannatella et al., 1998; Castellano et al., 2002). Others, in contrast, detected significant phylogenetic signals in anuran calls (Gingras et al., 2013). In species of the *Boana albopunctata* group, advertisement calls evolving near neutrality can be due to different rates of trait evolution among and within clades. While calls of species from clade II were more dispersed in the acoustic space, calls of species from clade I were more limited, occupying a narrower range in acoustic space. However, values of phylogenetic signal near to Brownian Motion Model (BMM) of evolution could be the resulted of different evolutionary processes or different rates of trait evolution (Revell et al., 2008). It is important to consider and analyze calls as multidimensionally complex traits. For multidimensional phenotypes, where traits are highly correlated, natural selection is expected to act on them as a whole (Collyer et al., 2014). In general, studies have measured the phylogenetic signal in advertisement calls for each parameter separately. Different acoustic parameters in a given call can show distinct patterns of evolution, such as absence or strong phylogenetic signal (Gingras *et al.*, 2013; Fig. 7). For *B. albopunctata* group, traits showed different patterns of evolution. While strong and significant phylogenetic signal was found for temporal traits such as CD, ND, NN and NP, no phylogenetic signal was shown for DF, NR and PR.

Advertisement calls have been used by herpetologists as an important taxonomic tool for describing species (Köhler et al., 2017). In many cases, morphologically cryptic species show distinguishable calls (Funk et al., 2008; Magalhães et al., 2014). However, call similarity alone is not sufficient to assign species as conspecifics (Köhler et al., 2017; Santana et al., 2016). We found no significant differences between the advertisement calls of some closely related species. For example, *B. fasciata*, *B. almendarizae* and *B. maculateralis* (Fig. 6) showed surprisingly similar calls, suggesting a pattern of conservatism. This result also contrasts with the multiple secondary contact zones among these species, which suggest a typical scenario where character displacement should have taken place (Köhler et al., 2017). Conversely, advertisement calls can be constrained by a set of conditions, including intrinsic factors such as morphology and physiology along with sexual selection and environmental requirements. For example, bigger males have calls with lower dominant frequencies, while temperature can affect muscular contraction and hence pulse rate (McLister, 2001; Ryan, 1985). However, several selective pressures can act on call diversification. Locally, sexual selection, signal transmission efficiency, or acoustic competition may be determinant in the more rapid evolution of sexual signals (Pröhl et al., 2007; Wollerman and Wiley, 2002).

4.2 The effects of geography and behavior on phenotype diversification

Hybridization between closely related frog species has been widely reported (Lemmon and Lemmon, 2010; Oliveira et al., 1991). Some authors suggest that, in contact zones, reproductive character displacement may decrease the chances of hybridization or signal interference between neighbors, promoting diversification (Blair, 1964; Lemmon, 2009). For the species pairs *B. albopunctata*/*B. multifasciata* and *B. alfaroi*/*B. tetete*, hybridization can be avoided by different processes: in the first pair, these species with similar calls do not co-occur; in the latter, species can co-occur, but calls are very distinct and structurally different. When there is parapatric distribution and similar calls, such as *B. fasciata* and *B. almendarizae*, hybridization is possible along contact zones because calls are not different enough to segregate them. *Boana maculateralis* had calls similar to calls of these species, but did not overlap geographically. Conversely, *B. caiapo* and *B. raniceps* can occur in syntopy (Pinheiro et al., 2018), but have distinct calls, decreasing the possibility of hybridization between them. For both clades the number of species that can potentially co-occur varies from two to five. This raises questions on how related species behave at the assembly level, diversifying their signals for mate interaction and agonistically with hetero- and conspecific males. At a metacommunity scale, for example, some sister-species pairs can diverge in spectral parameters that are expected to be phylogenetic and morphologically constrained (Garey et al., 2018). Locally, however, species can partition niches temporally, breeding at specific times throughout the year or day, or spatially by occupying different microhabitats (Wells, 2007). When there is no none of these partitions, species can engage in aggressive interactions.

Aggressive interactions mediated by acoustic signals between different species can generate changes in the vocal behavior of one or both species (Gerhardt & Huber, 2002). Studies have shown that *B. albopunctata* males can change acoustic parameters of the advertisement call in syntopy with *B. paranaiba,* apparently in an aggressive context, and fighting against *B. raniceps* in territorial disputes (Guerra et al., 2018; Vieira et al., 2016). Interspecific competition may promote biotic exclusion and/or divergence in evolutionary unstable traits, such as behavioral features (Pfennig and Pfennig, 2009). Agonistic behaviors followed by aggression may have shaped various phenotypic traits, even those hypothetically more conserved such as external morphology. In salamanders, aggressive behavior among related species was been shown to exert a strong selective pressure on cranial shape for populations in sympatry (Adams et al., 2007).

4.3 Calls and environment

In addition to social interactions, previous studies have reported the influence of environmental factors such as temperature on vocal behavior in anurans at a local scale (Blair, 1958). However, as in the present study, others did not find evidences for call variation associated to broad scale environmental variables (Forti et al., 2017). It is expected by the Acoustic Adaptation Hypothesis (AAH) that species have their calls adapted to propagate better in the habitat (e.g. open or closed habitats) in which they evolved (Ey and Fischer, 2009). Therefore, if habitat features exert some constrain over acoustic communication, decreasing the acoustic space available, the diversification of this trait can be limited (Wilkins et al., 2013). Moreover, frogs can handle acoustic environment constraints by adjusting some acoustic properties to have a better sound propagation (Lardner and bin Lakim, 2002). Tests comparing calls of species widely distributed in different biomes should help explain the importance of environment micro and macroecological features on call variation. We found no significant covariation between macroenvironmental characteristics and call. This could be due to the fact that closely related species, with similar calls, generally tend to diverge in their climatic niche.

4.4 Perspectives

Many questions regarding the actual species distribution remain unresolved. For example, how does speciation occur along climatic gradients without strong vicariant barriers? How can phenotypic traits evolve in contact zones of closely related species? How did demographic history affect the current pattern of species distributions? Studies on comparative phylogeography, focusing on closely related species that occur in parapatry, can test historical demographic patterns of population contraction/expansion, including if there is gene flow among those populations. Future studies should also focus on the fine aspects of phenotypic variation, considering closely related species in parapatry. We found evidences for divergence in environmental niches and advertisement calls evolving near to the expected by neutral evolution in Neotropical tree frogs. These results help understand part of the complex processes involved in the evolution of phenotypic traits that are likely under strong natural and sexual selection and how they can be used to test hypotheses considering the species distributions.

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Table 1. Scenarios of advertisement call evolution based on geographic distributions of closely related species and possible associated mechanisms.

Species distribution	**Mechanism**	**Scenario of advertisement call evolution**
Sympatry	Character displacement due to disruptive selection favoring mechanisms of pre-zygotic isolation	Divergent calls
Parapatry	There are no selective pressures driving call evolution	Brownian motion model
	Different subclades are under different selective forces (e.g. selection towards divergence and/or conservatism)	
	Selection against hybrids is stronger than stabilizing selection	Divergent calls
	Environmental conditions affect call variation	
	Male morphology constrains call variation	Conserved calls
Allopatry	Species splitted by vicariance and no selective pressures driving call evolution (similar to parapatric model)	Brownian motion model
	Species are adapted to distinct environments that drive call variation	Divergent calls

Table 2. Number of occurrence points per species and average values of test and training AUC for the species distribution models of the long-legged tree frogs *Boana albopunctata* group.

Species	N points	Test AUC	Training AUC
B. albopunctata	98	0.88	0.92
B. alfaroi	18	0.95	0.95
B. almendarizae	22	0.99	0.99
B. caiapo	17	0.97	0.97
B. calcarata	18	0.94	0.96
B. dentei	13	0.99	0.99
B. fasciata	23	0.99	0.99
B. heilprini	46	0.99	0.99
B. lanciformis	168	0.91	0.94
B. leucocheila	18	0.98	0.99
B. maculateralis	22	0.98	0.99
B. multifasciata	25	0.86	0.90
B. paranaiba	23	0.91	0.96
B. raniceps	303	0.87	0.87

Table 3. Acoustic parameters (mean ± SD) and number of individuals analyzed (in parenthesis) of the long-legged tree frogs *Boana albopunctata* group. Cdur – call duration, Dfreq – dominant frequency, NN – number of notes, Ndur – note duration, NP – number of pulses, Nrate – note rate, Prate – pulse rate. Species are abbreviated: alb – *B. albopunctata, alf - B. alfaroi, alm - B. almenderizae, cal - B. calcarata, den - B. dentei, fas - B. fasciata, hei - B. heilprini, lan – B. lanciformis, leu - B. leucocheila, mac - B. maculateralis, mul - B. multifasciata, par - B. paranaiba, ran - B. raniceps* and *tet - B. tetete.*

	Cdur (s)	Dfreq (kHz)	NN	Ndur (s)	NP	Nrate (NN/s)	Prate (NP/s)
alb (65)	0.90± 0.50	2.03± 0.45	1.44± 0.52	0.53± 0.11	74.67± 18.75	1.77± 0.51	146.39± 53.70
alf (5)	0.15± 0.02	2.10± 0.07	1.00± 0.00	0.02± 0.01	3.98± 0.46	6.71± 0.89	159.88± 24.84
alm (5)	0.43± 0.05	2.03± 0.17	3.38± 0.29	0.07± 0.00	14.40± 3.20	8.00± 0.41	209.49± 37.73
cai (7)	1.14± 0.80	2.18± 0.08	1.60± 0.74	0.48± 0.10	105.99± 15.12	1.90± 15.12	228.53± 26.12
cal (10)	0.07± 0.03	1.81± 0.09	1.07± 0.14	0.06± 0.01	15.62± 1.71	16.12± 3.33	268.19± 18.27
den (4)	0.07± 0.00	1.86± 0.06	1.00± 0.00	0.07± 0.00	28.21± 0.77	14.10± 0.17	397.67± 14.51
fas (5)	0.36± 0.07	1.88± 0.17	3.15± 0.72	0.06± 0.01	11.96± 1.41	8.73± 0.41	192.50± 15.28
hei (4)	2.22± 0.29	1.74± 0.15	16.70± 1.67	0.08± 0.01	14.59± 6.55	7.53± 0.28	194.27± 9.59
lan (18)	0.77± 0.48	1.74± 0.40	2.15± 0.88	0.31± 0.12	70.96± 22.64	3.22± 1.22	243.45± 37.17
leu (2)	1.03± 0.23	1.60± 0.15	4.15± 0.21	0.17± 0.03	30.10± 0.99	4.14± 0.74	186.03± 16.48
mac (2)	0.36± 0.06	2.14± 0.02	3.34± 0.47	0.09± 0.01	24.62± 0.07	9.35± 0.33	299.99± 25.22
mul (5)	0.79± 0.30	2.41± 0.15	1.80± 0.52	0.39± 0.12	72.13± 12.73	2.45± 0.80	211.87± 51.08
*par (*21)	2.07± 0.82	2.35± 0.26	2.56± 0.82	0.63± 0.09	132.24± 23.55	1.29± 0.23	209.91± 33.28
ran (57)	0.89± 0.83	1.77± 0.55	2.90± 2.25	0.17± 0.04	6.96± 1.77	3.89± 1.45	40.43± 4.25
tet (9)	0.08± 0.01	1.86± 0.06	1.00± 0.00	0.08± 0.01	-	12.08± 0.72	-

Table 4. Matrix showing pairwise tests for advertisement calls of species of the long-legged tree frogs *Boana albopunctata* group. Values in lower diagonal represent the *P*-value for each comparison. In bold *P*-values > 0.05. Effect size estimate is shown in upper diagonal with z-scores. Possible due to the small sample size (N=2 for each) *leu* and *mac* calls did not differ in most comparisons. *alb – Boana albopunctata, alf* - *B. alfaroi, alm* - *B. almenderizae, cal* - *B. calcarata, den* - *B. dentei, fas* - *B. fasciata, hei* - *B. heilprini, lan – B. lanciformis, leu* - *B. leucocheila, mac* - *B. maculateralis, mul* - *B. multifasciata, par* - *B. paranaiba* and *ran* - *B. raniceps.* * indicates pairs of sister species.

	alb	alf	alm	cai	cal	den	fas	hei	lan	leu	mac	mul	par	ran
alb		4.81	4.83	0.71	12.16	7.70	4.99	11.26	4.28	1.07	2.60	0.38	7.04	12.47
alf	0.00		-0.54	4.16	2.73	2.64	-0.55	7.97	2.98	0.32	-0.05	2.07	8.00	2.34
alm	0.00	**0.67**		3.62	2.18	1.91	-1.87	6.54	2.26	-0.40	-1.14	1.92	7.32	2.88
cai	**0.21**	0.00	0.00		7.92	5.44	4.16	9.26	1.27	1.19	2.00	-0.60	1.48	6.95
cal	0.00	0.01	0.03	0.00		0.11	1.84	10.04	7.87	2.40	0.37	5.82	13.39	10.03
den	0.00	0.02	0.04	0.00	**0.41**		1.94	8.41	4.72	2.21	-0.11	4.30	8.72	7.71
fas	0.00	**0.68**	**0.99***	0.00	0.05	0.04		6.93	2.58	-0.29	-0.85	2.35	7.90	2.53
hei	0.00	0.00	0.00	0.00	0.00	0.00	0.00		9.33	3.75	4.68	8.22	10.45	9.49
lan	0.00	0.01	0.03	**0.11**	0.00	0.00	0.02	0.00		-0.34	0.95	0.70	7.55	8.19
leu	**0.13**	**0.34**	**0.61**	**0.12**	0.02	0.03	**0.56**	0.00	**0.57**		-0.04	0.56	2.84	0.06
mac	0.02	**0.48**	**0.90**	0.05	**0.32**	**0.49**	**0.80**	0.00	**0.17**	**0.49**		1.03	3.98	2.31
mul	**0.32***	0.03	0.04	**0.69**	0.00	0.00	0.02	0.00	**0.22**	**0.28**	**0.14**		2.47	4.06
par	0.00	0.00	0.00	**0.08**	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02		14.79
ran	0.00	0.03	0.01	0.00	0.00	0.00	0.02	0.00	0.00	**0.42**	0.03	0.00	0.00	

Table 5. Results of niche similarity test of the species of the *Boana albopunctata* group. Schoener's *D* values of niche overlap with significant *P*-values indicated by * ($P < 0.05$) and ** ($P < 0.01$). Species are abbreviated as: *alb – B. albopunctata, alf - B. alfaroi, alm - B. almenderizae, cai – B. caiapo, cal - B. calcarata, den - B. dentei, fas - B. fasciata, hei - B. heilprini, lan – B. lanciformis, leu - B. leucocheila, mac - B. maculateralis, mul - B. multifasciata, par - B. paranaiba* and *ran - B. raniceps.*

	alb	alf	alm	cai	cal	den	fas	hei	lan	leu	mac	mul	par	ran
alb														
alf	0.00													
alm	0.00	0.18												
cai	0.01	0.00	0.00											
cal	0.00	0.19**	0.21*	0.00										
den	0.00	0.00	0.00	0.00	0.00									
fas	0.00	0.02	0.23*	0.00	0.02	0.00								
hei	0.02	0.00	0.00	0.00	0.00	0.00	0.00							
lan	0.01	0.02*	0.11*	0.00	0.14	0.02**	0.02*	0.01						
leu	0.01	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00					
mac	0.00	0.03	0.03	0.00	0.01	0.00	0.10	0.00	0.00	0.00				
mul	0.00	0.08*	0.00	0.00	0.01	0.01	0.00	0.01	0.04	0.00	0.01			
par	0.03	0.00	0.00	0.21*	0.00	0.00	0.00	0.08	0.01	0.17	0.00	0.01		
ran	0.18	0.00	0.00	0.01**	0.00	0.00	0.00	0.16**	0.02	0.01**	0.00	0.05*	0.03	

FIGURES

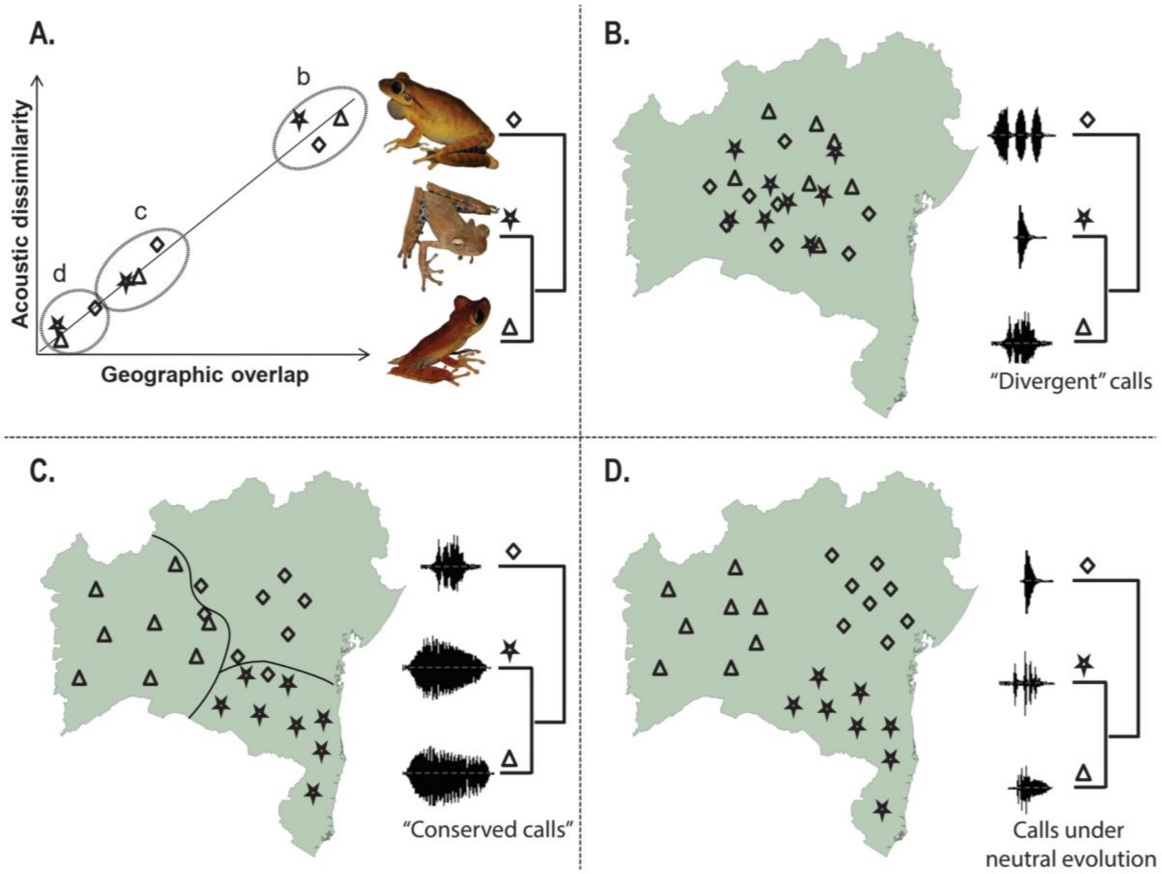


Figure 1. Hypotheses for call evolution in frogs based in our expectations of how species tend to diverge or conserve phenotypic traits considering species distributions and phylogenetic relationship (panel A). Given a phylogeny, we expect six alternative scenarios: If closely related species have similar environmental niche and are sympatric their advertisement call should be more dissimilar – diversifying selection on calls to avoid hybridization among closely related species (panel B); calls of parapatric species can be conserved, evolve neutrally or be more divergent than expected by neutral evolution (panel C); calls of allopatric species could differ proportionally to the divergence time of species, or be more divergent if adapted to different environments (panel D). Each symbol represents the mean of calls per individual per species per site.

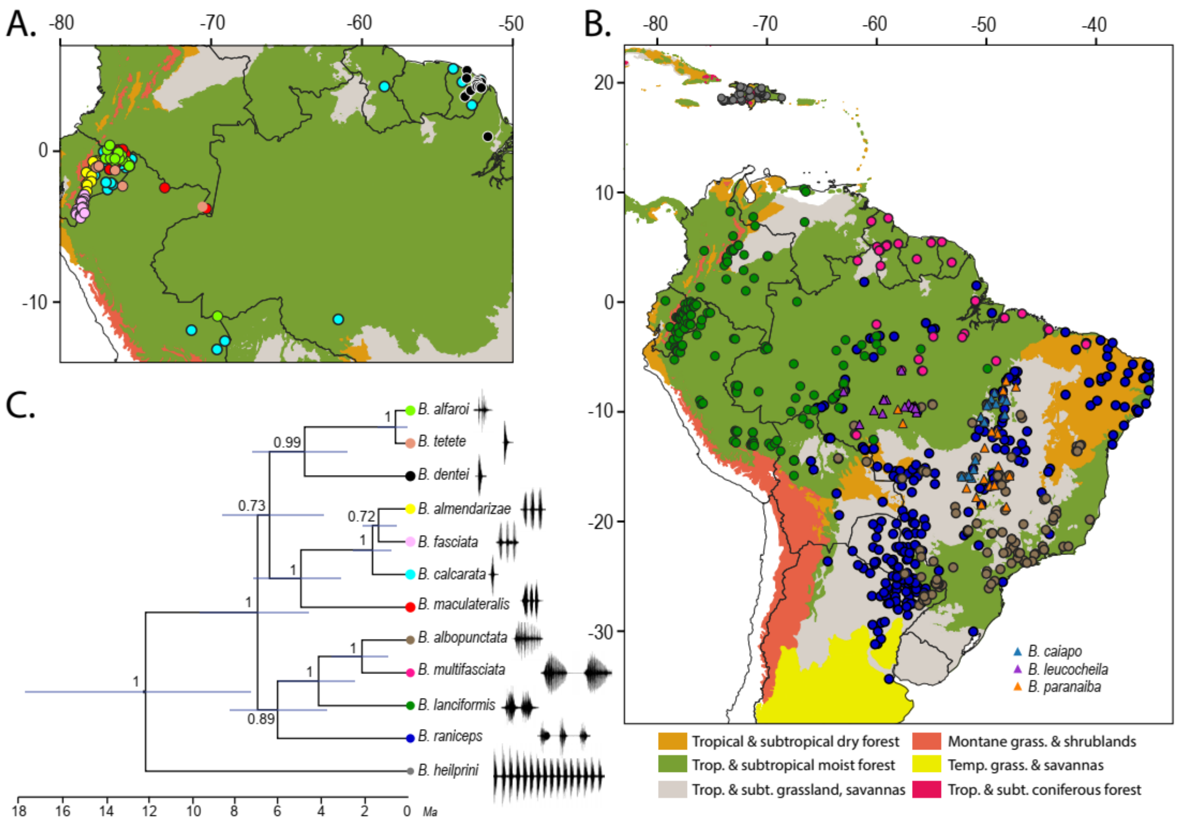


Figure 2. Distribution and phylogenetic relationships among species of the long-legged tree frogs of the *Boana albopunctata* group. Panels A and B show geographical sample points and ecoregions of occurrence for each species. A) Distribution of the species from the western/northern Amazon (clade I). B) Distribution of the most external species *Boana heilprini* and species of broader distribution from forest and open/dry biomes (II). Triangles represents species not presented in the phylogeny (see Methods). C) Species tree, under a coalescent model implemented in *BEAST, and advertisement call per species. Bars at nodes represent means and 95% confidence intervals for node ages (in Mya). Posterior probabilities are shown on each node.

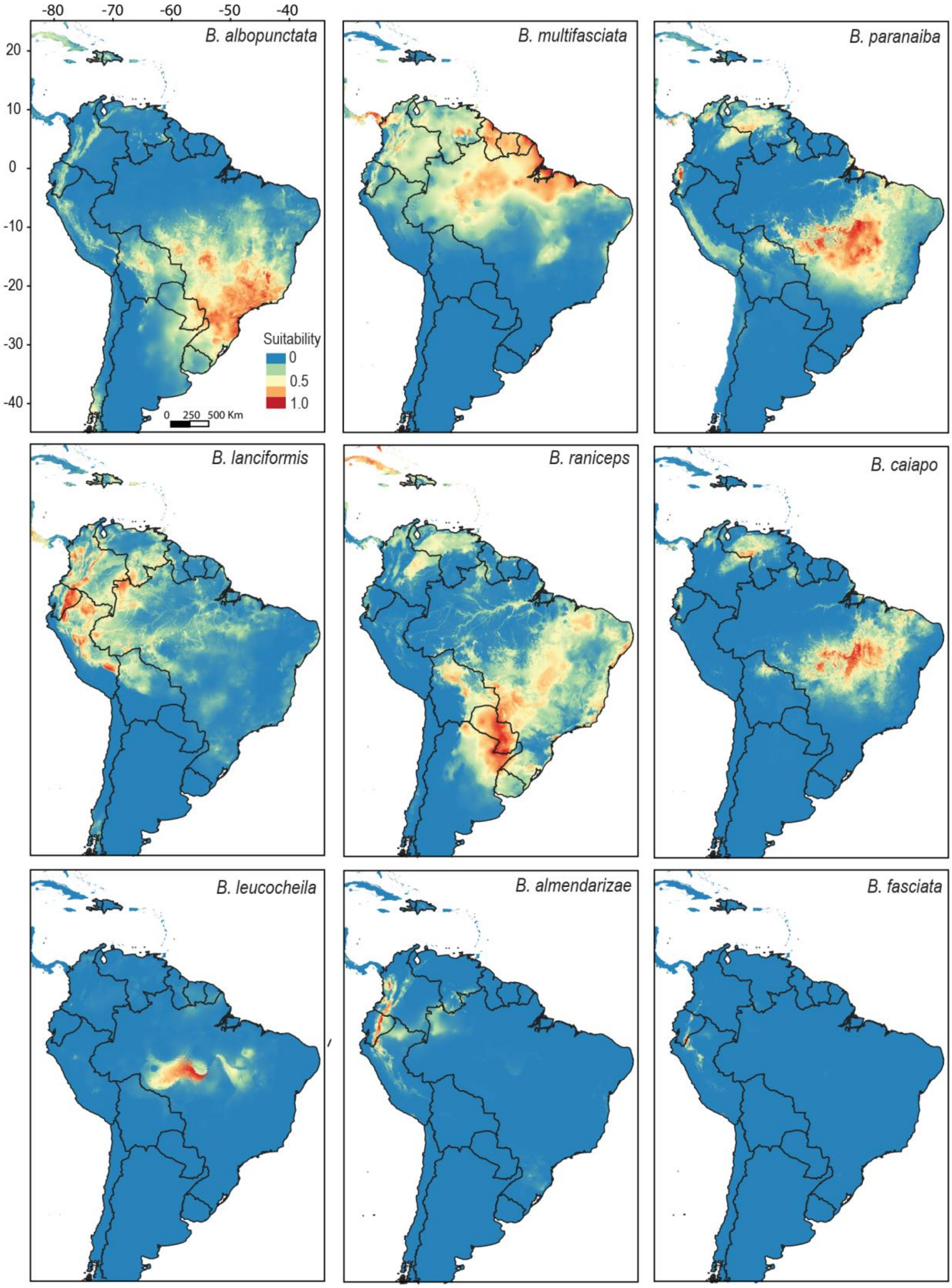
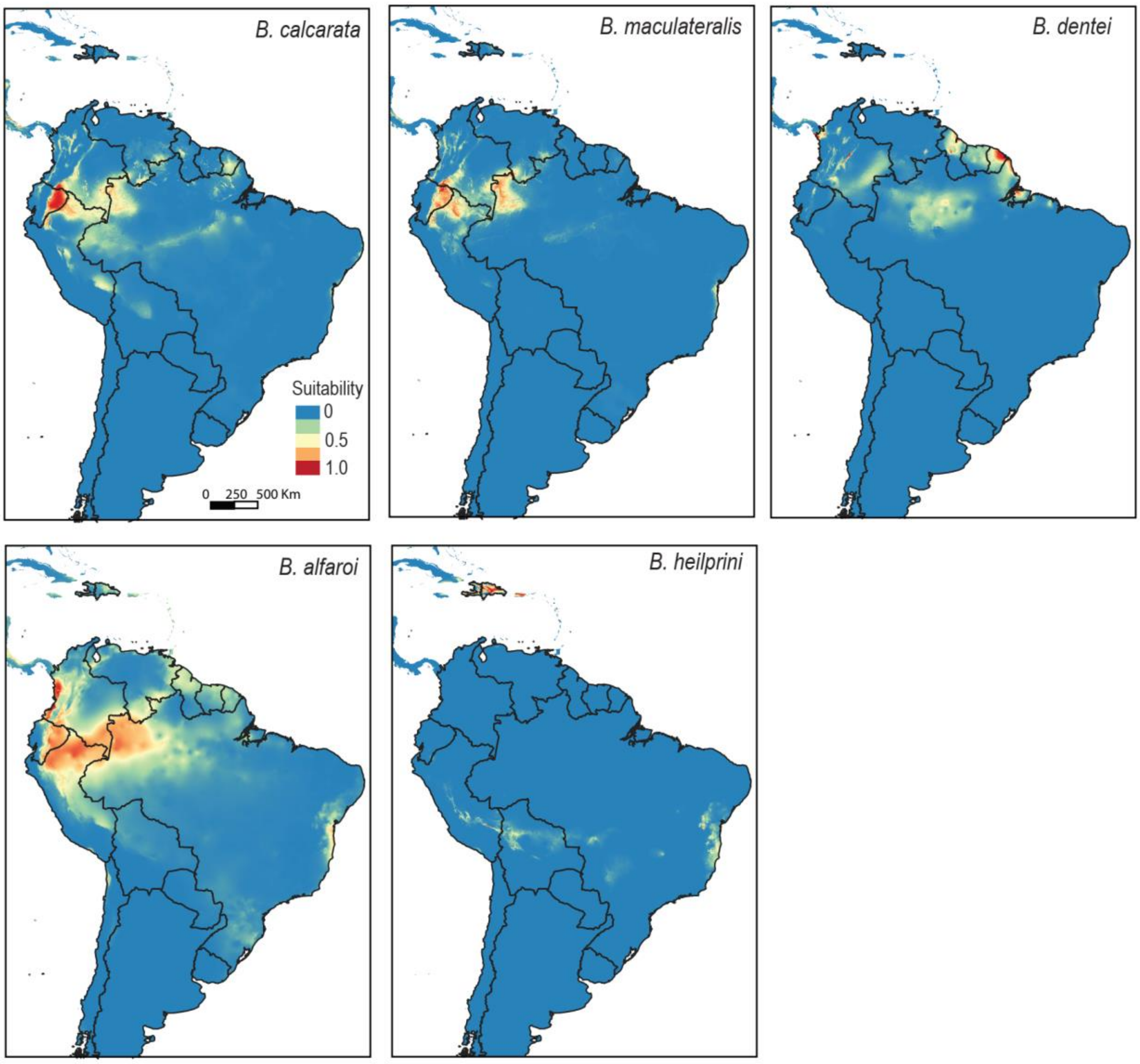


Figure 3. Environmental suitability for species of the long-legged tree frogs *Boana albopunctata* group. Values of suitability varies from 0 to 1.

Figure 3. *Cont.*



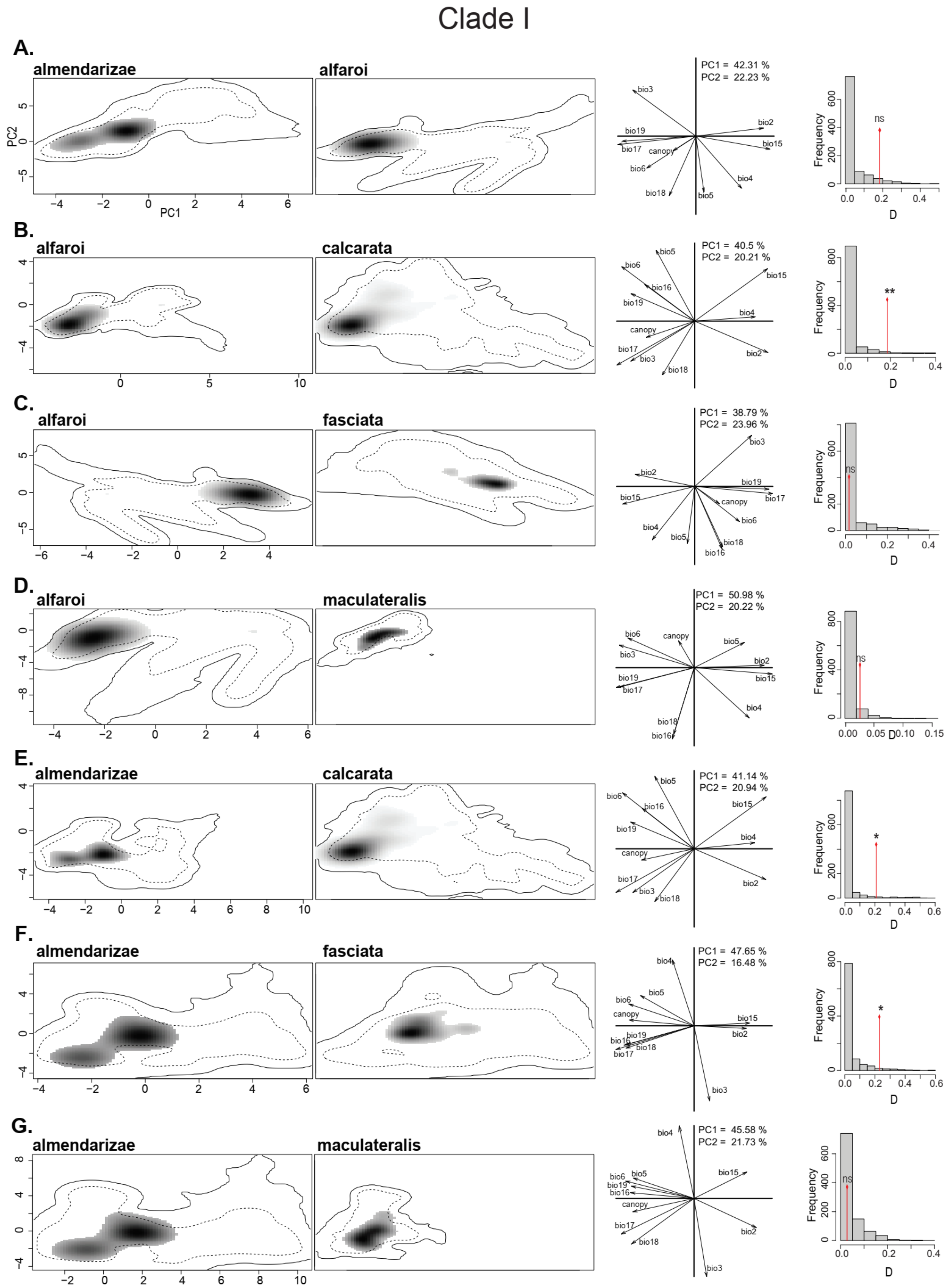


Figure 4. Niche of tree frog species of the *Boana albopunctata* group from Clade I. Panels A-K presents the environmental space occupied by pairs of species that can potentially overlap in the geographic space with a PCA-env approach. Two first plots show the environmental space

occupied by each species in the pairwise comparison, with the highest density of the occurrences in darker cells. Solid and dashed lines indicate the available background. The contribution of each variable (see Methods) is presented in the PC plots. Histograms show the observed niche overlap (in red) for each pairwise comparison. ns – non-significant divergence or similarity (*P* > 0.05); * *P* < 0.05; ** *P* < 0.01.

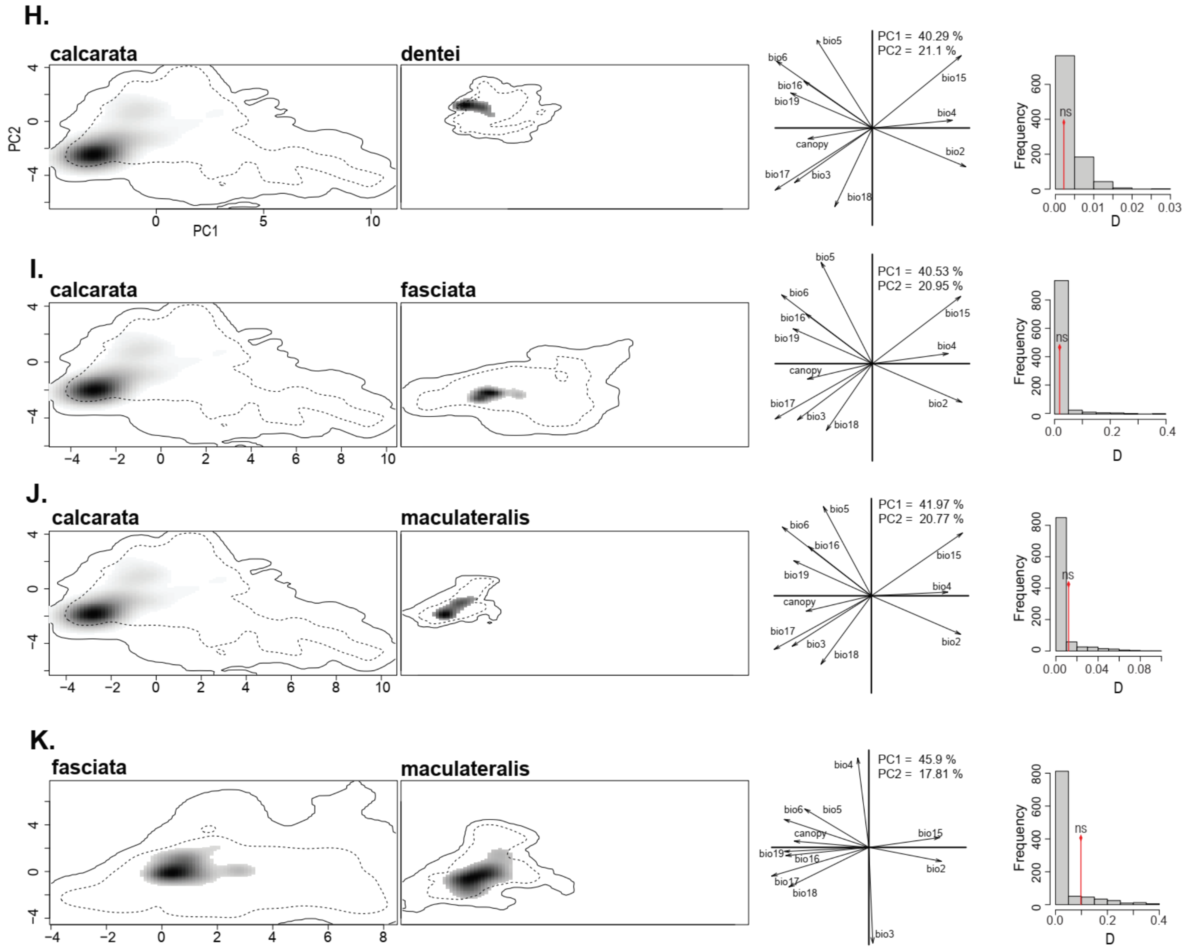


Figure 4. *Cont.*

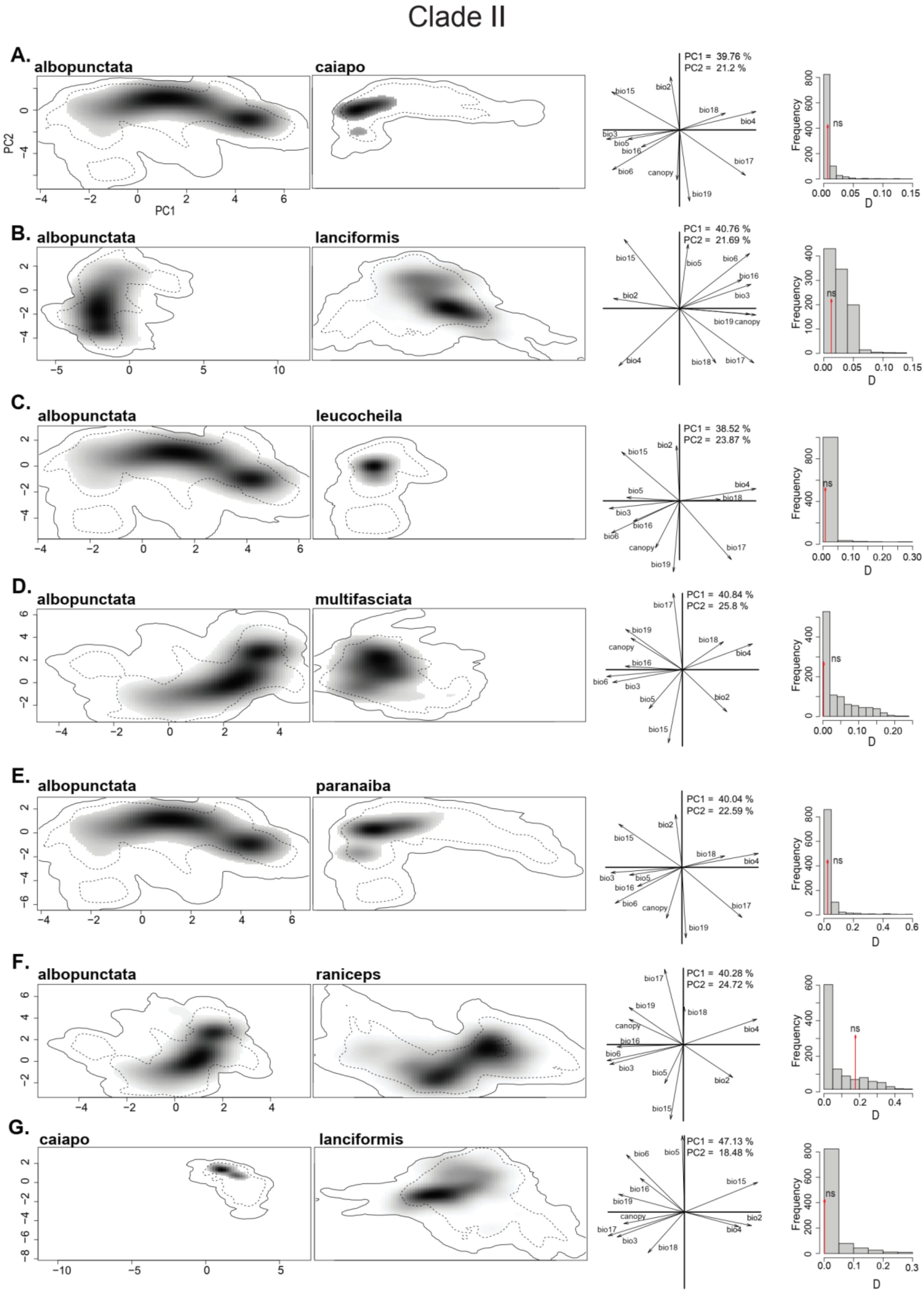


Figure 4. Niche of tree frog species of the *Boana albopunctata* group from Clade II.

Figure 1.4. Clade II. *Cont.*

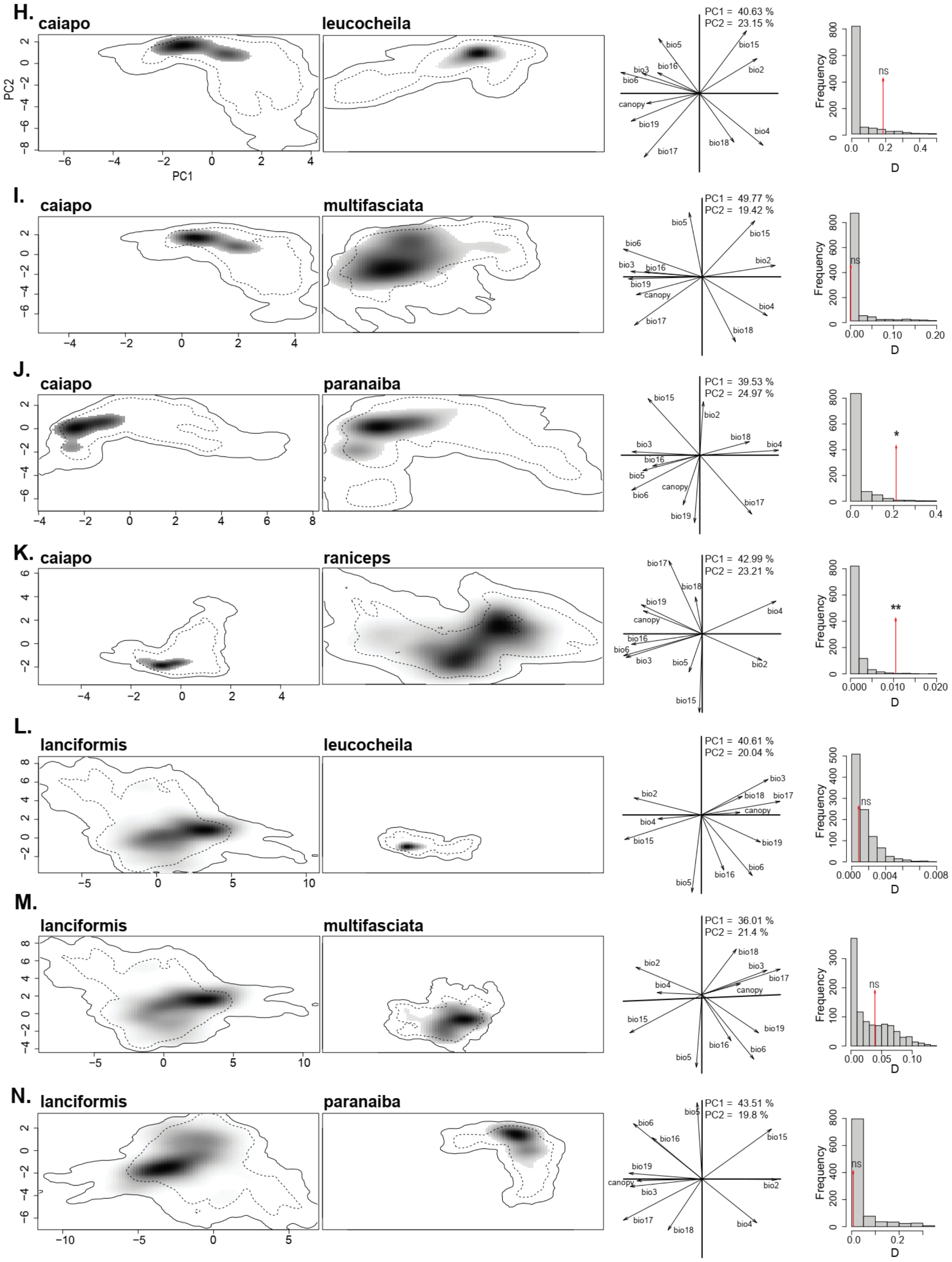
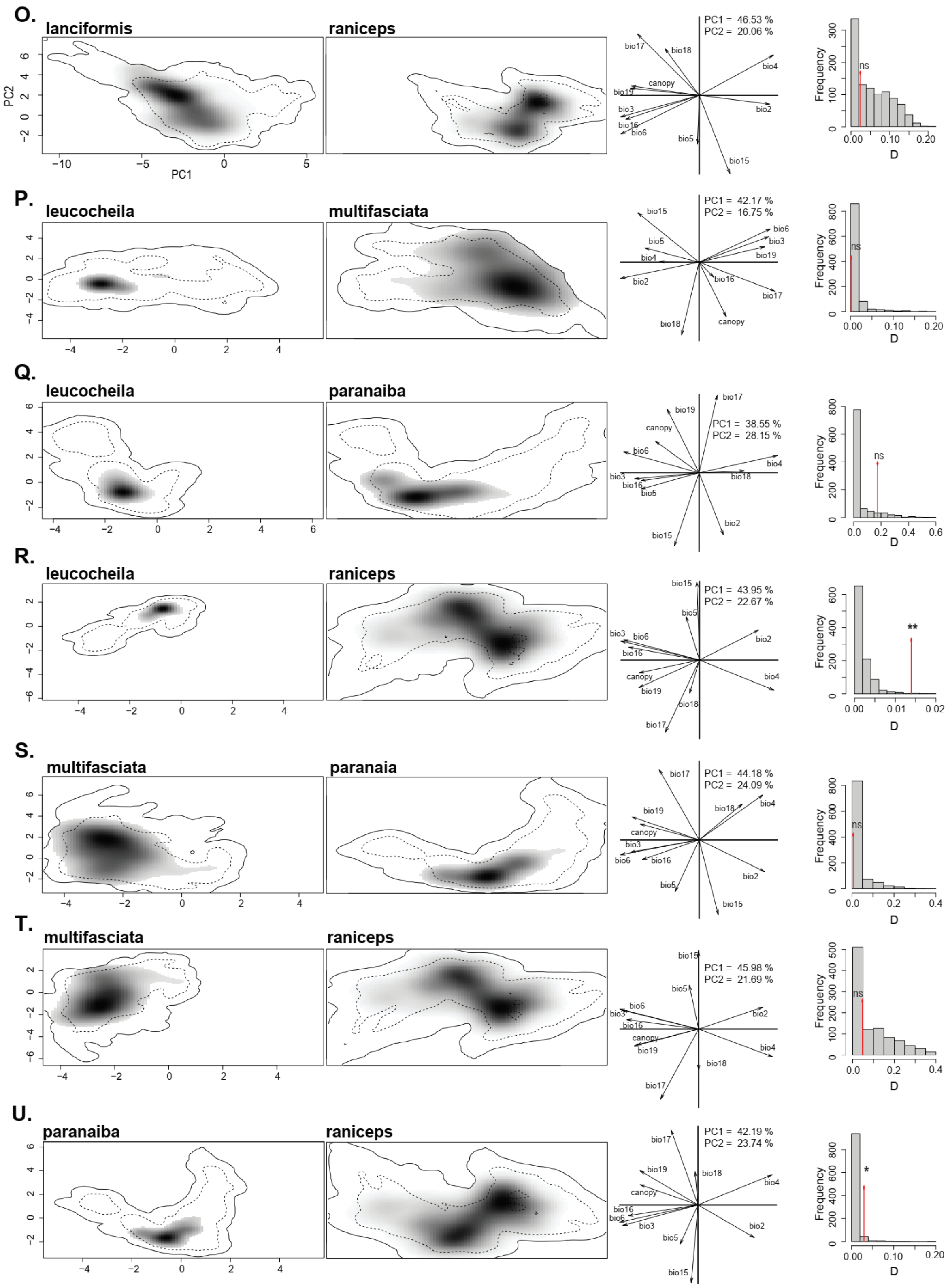


Figure 1.4. Clade II. *Cont.*



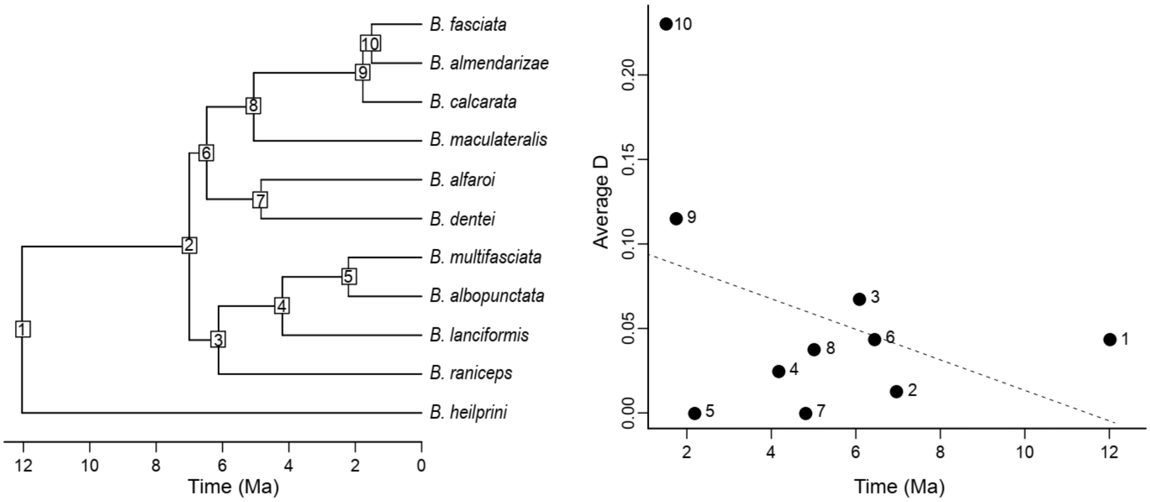


Figure 5. Age-range correlation analysis considering divergence time (in millions of years - Ma) and mean of niche overlap (*D* index) among species of the long-legged tree frogs *Boana albopunctata* clade. Numbers in the scatter plot represent the nodes pinpointed on the phylogenetic tree. *P*-value = 0.07, Intercept = 0.10, slope = -0.01.

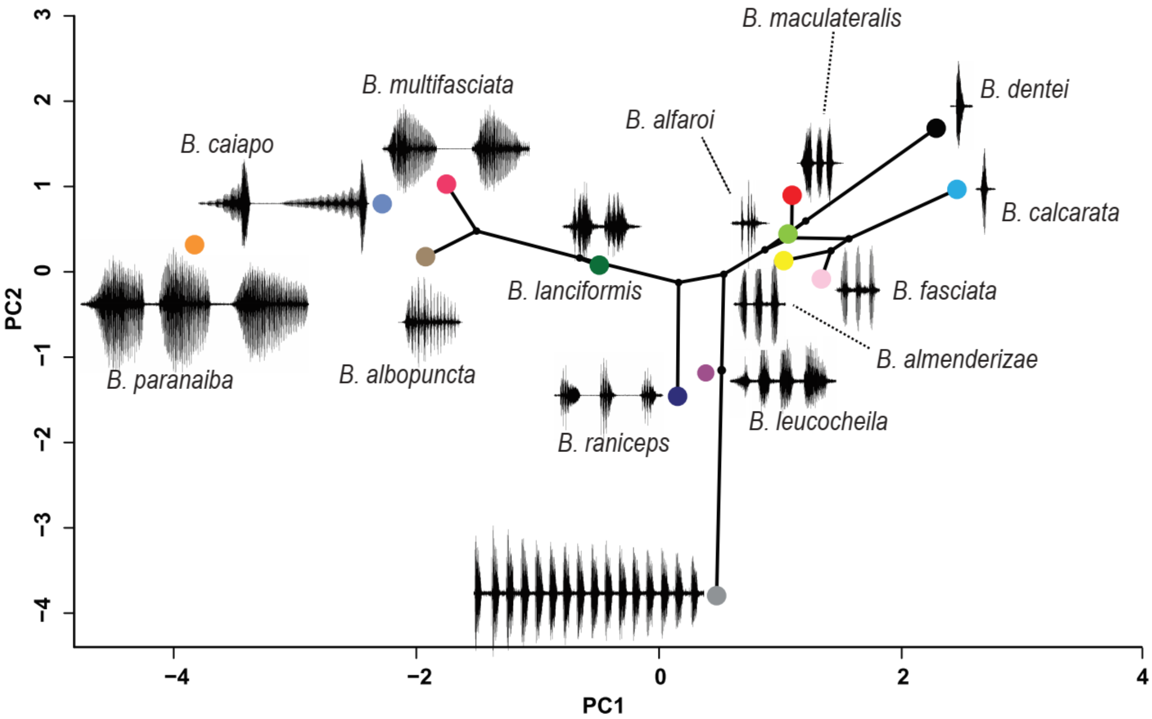


Figure 6. Call diversification of species of the long-legged tree frogs of the *Boana albopunctata* group. Projection of the phylogenetic tree into the acoustic space (considering the two first principal components) with Phylomorphospace. PC1 represents a gradient from call and note durations in the negative values, note and pulse rates in positive values; PC2 represents a gradient from number of notes and pulses. Figures of the calls (oscilograms) from each species and are at the same temporal scale. *Boana caiapo*, *B. leucocheila* and *B. paranaiba* are not included in the phylogeny (see Methods), however, their calls are projected in the acoustic space. Acoustic data were centered and standardized.

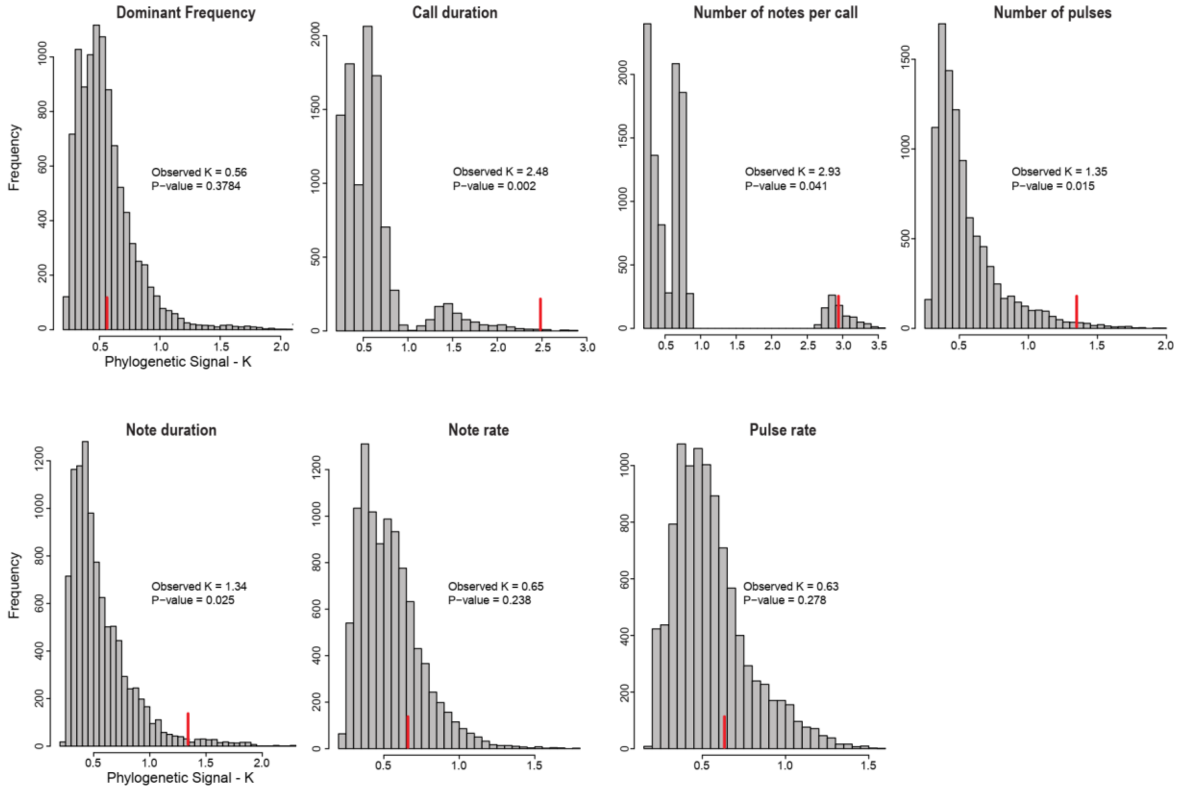


Figure 7. Phylogenetic signal (*K*) per acoustic parameter of the advertisement call of the species the long-legged tree frogs *Boana albopunctata* group. Values of *K* higher than 1 suggest traits phylogenetically conserved. In red is the observed *K* value.

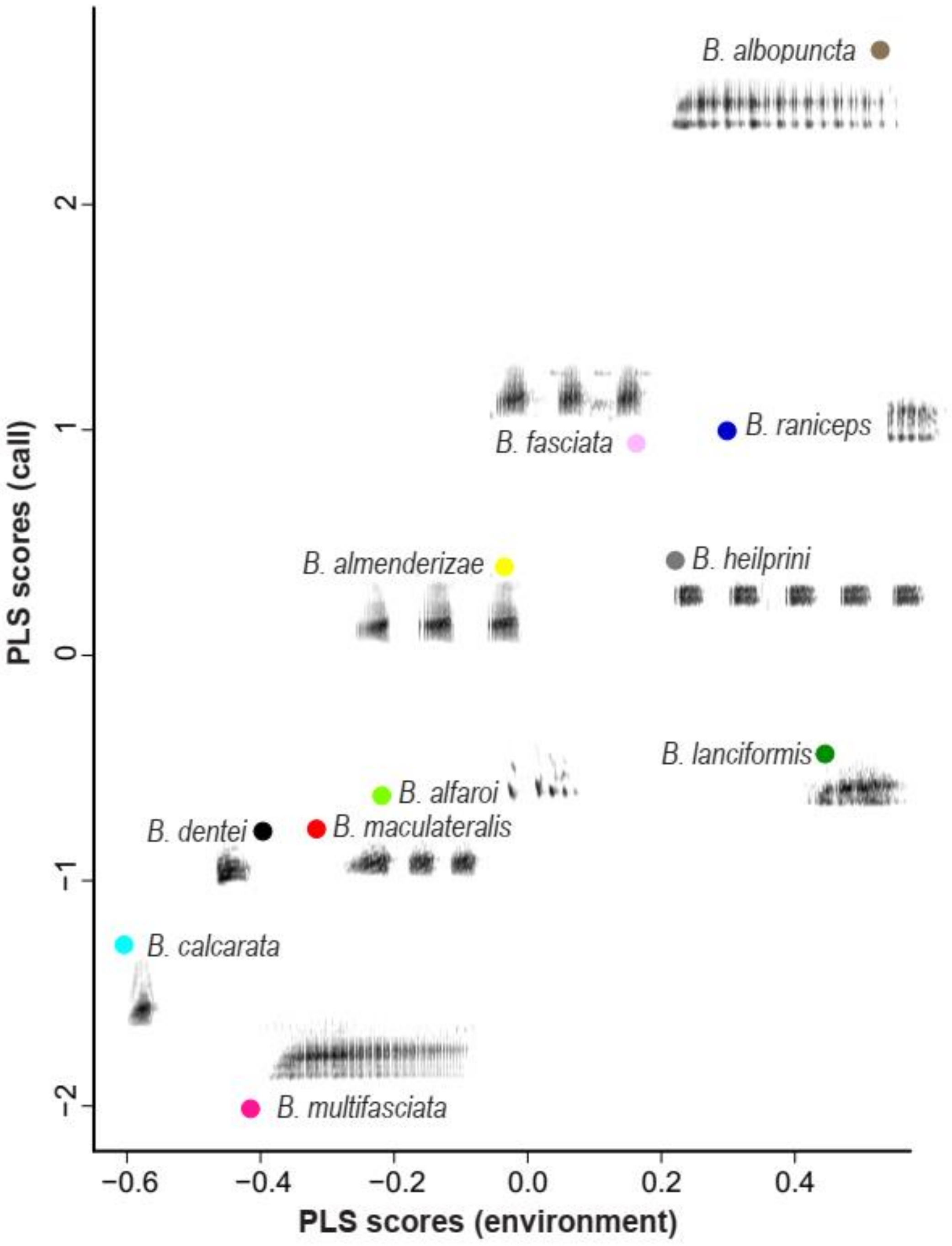


Figure 8. Phylogeny-adjusted partial least square (pPLS) analysis showing absence of covariation among calls and environmental variables of species of the long-legged tree frogs *Boana albopunctata* group.

Table S1. GeneBank accession numbers of species of *Boana albopunctata* group.

	12S	16S	CO1	POMC	RAG1
B. albopunctata	AY549317	AY549317	JQ627301	AAGARDA765 (This work)	--
B. alfaroi	JN970413	JN970549	JN970682	JN970804	KF955320
B. alfaroi	JN970415	JN970551	JN970684	JN970806	KF955321
B. alfaroi	--	JN970605	JN970737	JN970860	KF955322
B. alfaroi	KF955303	KF955305	KF955306	KF955307	--
B. almendarizae	JN970482	JN970618	--	JN970873	KF955311
B. almendarizae	JN970386	JN970522	JN970658	JN970777	KF955312
B. almendarizae	JN970394	JN970530	JN970665	JN970785	KF955313
B. calcarata	--	JN970580	JN970713	JN970835	KF955314
B. calcarata	JN970412	JN970548	JN970681	JN970803	KF955315
B. calcarata	--	JN970553	JN970686	JN970808	KF955316
B. dentei	EF376018	AF467270	--	--	--
B. fasciata	--	JN970535	JN970669	JN970790	KF955310
B. fasciata	JN970490	JN970626	--	JN970881	KF955308
B. fasciata	JN970388	JN970524	--	JN970779	KF955309
B. heilprini	AY843632	AY843632	--	EU034114	--
B. lanciformis	--	JN970648	JN970767	JN970898	KF955325
B. lanciformis	JN970510	JN970646	JN970765	JN970896	KF955326
B. maculateralis	JN970405	--	JN970675	JN970796	KF955317
B. maculateralis	--	JN970559	JN970692	JN970814	KF955318
B. maculateralis	JN970416	JN970552	JN970685	JN970807	KF955319
B. multifasciata	AY843648	--	JN970643	GQ366036	--
B. raniceps	--	KU495281	KU494488	CFBH11516 (This work)	--
B. tetete	JN970403	JN970539	JN970673	JN970794	KF955323
B. tetete	JN970404	JN970540	--	JN970795	KF955324

Table S2: Source and recording site of the advertisement call of *Boana albopunctata* species group.

Species	Longitude	Latitude	Recording Number	Source
B. albopunctata	-52.2619	-15.8916	ASUFRN046	Arquivos Sonoros da Universidade Federal do Rio Grande do Norte
B. albopunctata	-41.7675	-13.1522	ASUFRN542	Arquivos Sonoros da Universidade Federal do Rio Grande do Norte
B. albopunctata	-47.1572	-23.3155	FNJV0007691	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-43.9178	-19.6479	FNJV0012744	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-43.9178	-19.6479	FNJV0012745	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-46.6261	-20.256	FNJV0012747	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-47.1163	-22.8504	FNJV0012748	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-47.1163	-22.8504	FNJV0012749	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-47.1331	-23.3075	FNJV0012750	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-47.1331	-23.3075	FNJV0012751	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-46.9422	-22.8848	FNJV0012752	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-55.4635	-15.4849	FNJV0012753	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-44.5827	-20.7696	FNJV0012754	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-46.3013	-23.777	FNJV0012755	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-53.028	-24.185	FNJV0012756	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-47.7232	-23.8079	FNJV0013100	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-48.4442	-22.8841	FNJV0031824	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-43.6194	-19.3492	FNJV0032054	Fonoteca Neotropical Jacques Vielliard
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo1	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo11	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo3	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo4	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo5	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo6	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo7	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo8	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-49.0394	-15.0966	FONO-ZUFG1Halbo9	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND1	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND2	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND3	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND4	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND5	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND6	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND7	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND8	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.6133	-16.6681	FONO-ZUFGRPB21IND9	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás

B. albopunctata	-48.965	-15.8457	FONO-ZUFGRPB54Ind1	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.965	-15.8457	FONO-ZUFGRPB54Ind2	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.965	-15.8457	FONO-ZUFGRPB54Ind3	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.965	-15.8457	FONO-ZUFGRPB54Ind4	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.965	-15.8457	FONO-ZUFGRPB54Ind5	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-48.965	-15.8457	FONO-ZUFGRPB54Ind6	FONOTECA da Coleção Zoológica da Universidade Federal de Goiás
B. albopunctata	-43.8983	-19.6303	ML 22501	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-43.8983	-19.6303	ML 22511	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-43.8983	-19.6303	ML 22517	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-43.6198	-19.35	ML 22518	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-43.6198	-19.35	ML 22520	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-43.6198	-19.35	ML 22524	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-43.6198	-19.35	ML 22526	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-43.6198	-19.35	ML 22527	The Macaulay Library at the Cornell Lab of Ornithology
B. albopunctata	-41.7678	-13.1558	SUEFS100_403	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.7678	-13.1558	SUEFS100_405	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.7678	-13.1558	SUEFS100_408	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.3729	-13.0182	SUEFS22_04	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.485	-13.0978	SUEFS4_06	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.8136	-13.5858	SUEFS4_09	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.8136	-13.5858	SUEFS5_16	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.7032	-13.6007	SUEFS8_02	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-41.7032	-13.6007	SUEFS8_03	Sonoteca da Universidade Estadual de Feira de Santana
B. albopunctata	-55.7525	-15.4583	UFMT_17A-02	Banco de Registros Bioacústicos - Laboratório de Herpetologia UFMT
B. albopunctata	-56.0872	-9.86715	UFMT_33A-07	Banco de Registros Bioacústicos - Laboratório de Herpetologia UFMT
B. albopunctata	-53.2612	-17.8601	UFMT_41A-01	Banco de Registros Bioacústicos - Laboratório de Herpetologia UFMT
B. albopunctata	-57.2367	-15.5287	UFMT_LH 039	Banco de Registros Bioacústicos - Laboratório de Herpetologia UFMT
B. albopunctata	-56.9799	-14.3752	UFMT_LH 225	Banco de Registros Bioacústicos - Laboratório de Herpetologia UFMT
B. albopunctata	-59.9477	-15.0055	UFMT_LH 330	Banco de Registros Bioacústicos - Laboratório de Herpetologia UFMT
B. alfaroi	-76.429	-0.6893	MR-FWA3	Museo de Zoología QCAZ
B. alfaroi	-76.429	-0.6893	QCAZ43260	Museo de Zoología QCAZ
B. alfaroi	-76.429	-0.6893	QCAZ43261	Museo de Zoología QCAZ
B. alfaroi	-76.429	-0.6893	QCAZ43262	Museo de Zoología QCAZ
B. alfaroi	-76.429	-0.6893	QCAZ43263	Museo de Zoología QCAZ
B. almendarizae	-78.4415	-2.9796	QCAZ39645	Museo de Zoología QCAZ
B. almendarizae	-78.4415	-2.9796	QCAZ39647	Museo de Zoología QCAZ
B. almendarizae	-78.4415	-2.9796	QCAZ39648	Museo de Zoología QCAZ
B. almendarizae	-78.4415	-2.9796	QCAZ39649	Museo de Zoología QCAZ
B. almendarizae	-78.4415	-2.9796	QCAZ39650	Museo de Zoología QCAZ

B. caiapo	-49.9476	-9.35056	MAP2981	Mapinguari - UFMS
B. caiapo	-49.9476	-9.35056	MAP2982	Mapinguari - UFMS
B. caiapo	-49.9476	-9.35056	MAP2983	Mapinguari - UFMS
B. caiapo	-49.9412	-9.43059	MAP3365	Mapinguari - UFMS
B. caiapo	-49.9412	-9.43059	MAP3506	Mapinguari - UFMS
B. caiapo	-49.9412	-9.43059	MAP3507	Mapinguari - UFMS
B. caiapo	-49.9412	-9.43059	MAP3508	Mapinguari - UFMS
B. calcarata	-76.4535	-0.6535	MR-FWA3	Museo de Zoología QCAZ
B. calcarata	-77.4412	-0.9895	QCAZ40055	Museo de Zoología QCAZ
B. calcarata	-77.4412	-0.9895	QCAZ40084	Museo de Zoología QCAZ
B. calcarata	-77.4412	-0.9895	QCAZ40085	Museo de Zoología QCAZ
B. calcarata	-76.6194	-0.4062	QCAZ43131	Museo de Zoología QCAZ
B. calcarata	-76.4005	-0.6713	QCAZ43247	Museo de Zoología QCAZ
B. calcarata	-76.4005	-0.6713	QCAZ43256	Museo de Zoología QCAZ
B. calcarata	-76.4005	-0.6713	QCAZ43257	Museo de Zoología QCAZ
B. calcarata	-76.4005	-0.6713	QCAZ43258	Museo de Zoología QCAZ
B. calcarata	-76.4005	-0.6713	QCAZ43259	Museo de Zoología QCAZ
B. dentei	-51.6517	0.966389	ASUFRN197	Arquivos Sonoros da Universidade Federal do Rio Grande do Norte
B. dentei	-51.6517	0.966389	ASUFRN198	Arquivos Sonoros da Universidade Federal do Rio Grande do Norte
B. dentei	-51.6517	0.966389	ASUFRN471	Arquivos Sonoros da Universidade Federal do Rio Grande do Norte
B. dentei	-51.6517	0.966389	ASUFRN472	Arquivos Sonoros da Universidade Federal do Rio Grande do Norte
B. fasciata	-78.9508	-4.0669	QCAZ48583	Museo de Zoología QCAZ
B. fasciata	-78.6504	-3.3365	QCAZ48584	Museo de Zoología QCAZ
B. fasciata	-78.6504	-3.3365	QCAZ48585	Museo de Zoología QCAZ
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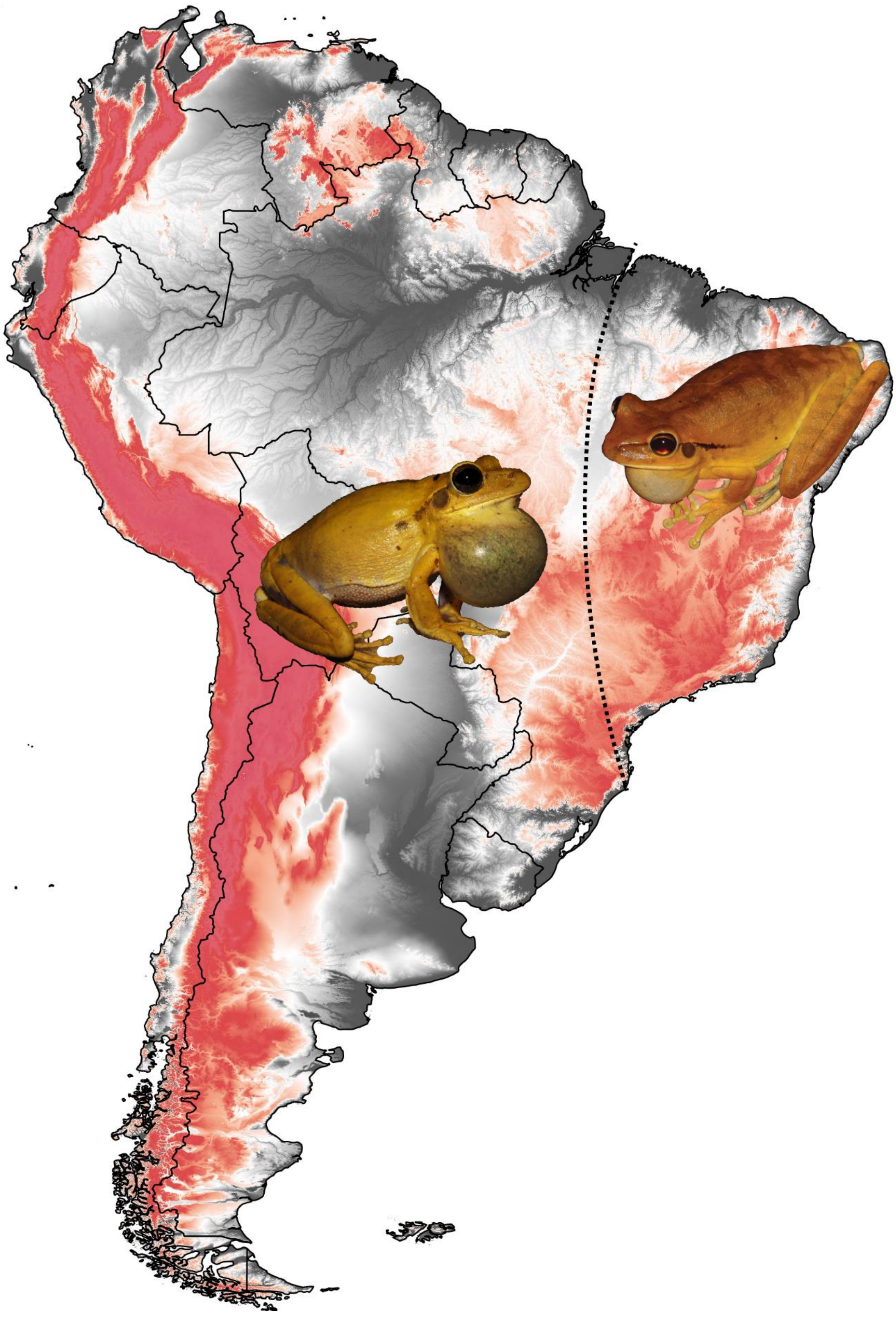
ISOLATION BY ENVIRONMENT AND RECURRENT GENE FLOW SHAPED THE EVOLUTIONARY HISTORY OF A CONTINENTALLY DISTRIBUTED NEOTROPICAL TREEFROG



Isolation by environment and recurrent gene flow shaped the evolutionary history of a continentally distributed Neotropical treefrog

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Abstract

Phylogeographic studies have shown how landscape evolution shaped the distribution of genetic diversity in many species of animals and plants. In particular, for the Diagonal of Open Formations (DOF), the compartmentalization of the Central Brazilian Plateau (CBP) during the Tertiary and climatic oscillations during the Quaternary have often been invoked to explain the origin and current patterns of biodiversity. We investigate how landscape evolution shaped the distribution of a widespread South American treefrog. We used a multilocus dataset from 115 localities along most of the species distribution. We used population assignment analysis, species distribution models, demographic models, approximate Bayesian computation, and landscape genetics analyses to test alternative hypotheses of diversification. We found two genetically structured lineages that diverged during middle Pleistocene with gene flow. Approximate Bayesian computation supported a scenario of isolation with migration and gene flow until the Last Glacial Maximum and recent population expansion in northeastern Brazil. Additionally, isolation by environment was the best predictor of genetic distance between populations. As *B. raniceps* is a lowland species, steep slopes in the Central Brazilian Plateau likely had a major impact on gene flow. We found evidence for ample climatically suitable areas in the Late Pleistocene, with a major shrinkage during the Last Glacial Maximum. Our findings highlighted how landscape evolution can shape the diversification of DOF biota. Our results show how past climatic changes and environmental resistance due to topography acted in concert, forming a semipermeable barrier to gene flow and shaped current patterns of genetic diversity and differentiation in a continentally distributed species.

Keywords: Anura, approximate Bayesian computation, *Boana raniceps*, isolation by environment, landscape genetics, lowland species, Quaternary climatic fluctuation, riverine effects, South America, topography

1. Introduction

The origin of the high Neotropical biodiversity has been related to a complex history of geological events and climatic dynamics (Rull, 2008). In particular, the Andes uplift, marine incursions and the completion of the Panamanian Isthmus during the Tertiary, followed by climatic oscillations during the Quaternary, appear to have shaped current patterns of biodiversity in the Neotropical region (Hoorn et al., 2010; Montes et al., 2015; Rull, 2011; Santos et al., 2009; Teixeira et al., 2016; Zamudio and Greene, 1997). Landscape evolution affects the spatial and genetic structure of populations by decreasing or facilitating population connectivity and gene flow (Cooke et al., 2012; Wang and Bradburd, 2014).

The South American Diagonal of Open Formations (DOF) is a large and well-connected belt of seasonally dry biomes between Amazonia and the Atlantic Forest (Werneck et al., 2011). The DOF is composed of three biomes, seasonally stressed by drought, and stretching from northeastern to southwestern South America: the Caatinga (seasonally dry tropical forest), the Cerrado (tropical savanna) and the Gran Chaco (Pennington et al., 2006; Werneck et al., 2011). Among the main hypotheses for the diversification of the DOF biota are the Tertiary compartmentalization of the Central Brazilian Plateau (CBP) and the Quaternary climatic oscillations (Colli, 2005; Werneck, 2011; Werneck et al., 2012b). The compartmentalization of the CBP was driven by epeirogenic movements, resulting from the convergence of the Nazca and South American plates and the Andean orogeny, erosion and sedimentation that created ample depressions (valleys) among ancient plateaus (Assine et al., 2015; Cogné et al., 2012; Ross, 2016; Zalán, 2004). This promoted the diversification of several groups by acting as a soft or hard barrier to gene flow between populations (Faria et al., 2013; Fonseca et al., 2018; Oliveira et al., 2018a; Prado et al., 2012).

Quaternary paleoclimatic oscillations impacted DOF species by influencing their historical demography or by structuring populations through habitat fragmentation and formation of refugia. Distribution models for South American biomes predicted contraction and fragmentation of savannas and seasonally dry forests during the LGM (Costa et al., 2018). These oscillations promoted conspicuous demographic responses in several DOF species (Brusquetti et al., 2019; Gehara et al., 2017; Vasconcellos et al., 2019). Areas of climatic instability in the DOF also reduced gene flow among climatically stable areas (isolation by instability), promoting population divergence (Vasconcellos et al., 2019). Climate oscillations also structured DOF populations by affecting other landscape features, such as rivers (Oliveira et al., 2018b).

Life-history traits can affect species dispersal ability and gene flow; consequently, they can determine how landscape evolution will affect the distribution of individuals and genes (Paz et al., 2015). Landscape resistance attributed to elevation, hydrology and habitat suitability are among the main drivers of genetic differentiation in amphibians (Barratt et al., 2018; McCartney-Melstad and Shaffer, 2015). Furthermore, because of their low individual dispersion and tendency to philopatry, anurans are expected to show marked phylogeographic structure (Beebee, 2005; Gehara et al., 2014; Reading et al., 1991). Among DOF anurans, the treefrog *Boana raniceps* (Cope 1862) is an ideal target for testing the effects of past and contemporary landscape features on spatiotemporal patterns of genetic diversity at a continental scale. *Boana raniceps* has a widespread distribution in South America, mainly associated with the DOF and Amazonian savannas, generally occurring in lowlands, below 800 m of elevation, in almost all major river basins of South America (except those that drain in the Pacific). Despite being broadly distributed, which would suggest a potentially high hidden genetic diversity, previous works unexpectedly found low levels of genetic divergence between widely separated populations (Fouquet et al., 2007). As a lowland species, we expect that the compartmentalization of the CBP played a major role on the evolution of *B. raniceps*. Further, hydrology should also have structured its populations because both adults and larvae can be carried by water bodies. Finally, paleoclimatic changes in the DOF should also have impacted its distribution and demography, once its biomes historically responded to climatic oscillations.

Here, we used multilocus datasets to investigate the role of historical and contemporary processes of landscape evolution in the diversification of *Boana raniceps*. We tested four non-mutually exclusive hypothesis: (1) genetic divergence follows the isolation by distance model (IBD); (2) the CBP compartmentalization resulted in lineages (at least two) either associated with valleys or plateaus; (3) genetic divergence is associated with connectivity among major river basins, according to an isolation by environment resistance model (IBE); (4) Quaternary climatic fluctuations affected demographic history by promoting population expansion and contraction through time and genetic differentiation among climatically stable areas (refugia). These hypotheses plus the null hypothesis of panmixia, along with their predictions, are summarized in Table 1.

2. Material and Methods

2.1 Data collection and sample sequencing

We obtained 288 tissues samples of *B. raniceps* from 115 localities (Fig. 1), covering most of its geographic distribution, and two samples of *B. albopunctata* (Spix 1824) used as outgroup. Samples were collected by the authors and through loans from herpetological collections (Table S1 in Supporting Information).

We extracted total genomic DNA from liver or muscle using a standard salt extraction protocol (Bruford et al., 1992). Through polymerase chain reaction, we amplified fragments of two mitochondrial genes: 16S rRNA (16S, 478 aligned bp) and NADH dehydrogenase subunit 1 (ND1, 848 bp). Additionally, we collected data of four nuclear genes: β-fibrinogen intron 7 (Fib, 445 bp), proopiomelanocortin (POMC, 448 bp), ribosomal protein L3 intron 5 (RPL3, 521 bp), and tyrosinase (Tyr, 427 bp). Detailed information about amplification and sequencing protocols are in Table S2 (Supporting Information). Sequencing and purification were performed by Macrogen Inc. First, we sequenced 283 individuals for 16S, from which we selected a subset based on unique haplotypes, comprising 123 samples from 75 localities. From this subset, we obtained sequences of the remaining genes.

We edited chromatograms, assembled, and aligned sequences in GENEIOUS 9.1.6 (https://www.geneious.com), using the MUSCLE algorithm (Edgar, 2004). Gaps found in 16S, Fib and RPL3 genes were removed using GBLOCKS 0.91b (Castresana, 2000), using default options. We defined the most probable allele pairs of each nuclear gene sequence with the Phase algorithm (Stephens et al., 2001) in DNASP 5.10 (Librado and Rozas, 2009), using default options and keeping allele pairs with reconstruction probabilities higher than 60%. Due to weakly resolved gametic phases, we excluded from posterior analyses samples of Fib (n = 9), RPL3 (n = 4), and Tyr (n = 3). We tested the presence of recombination in nuDNA using the PHI test implemented in SPLITSTREE4 (Huson and Bryant, 2006). We found no evidence for recombination in nuclear genes. Lastly, we estimated the best substitution model for each gene fragment using Bayesian Information Criterion (BIC) in JMODELTEST v2.1.7 (Darriba et al., 2012).

2.2 Population assignment analysis

Our second, third and fourth hypotheses claim that landscape features acted by imposing restrictions to gene flow and, consequently, structuring populations. To assess their predictions, we estimated population boundaries with ‘geneland’ R package (Guillot et al., 2005a, 2005b). GENELAND allows using both haploid and diploid data in the same analysis and incorporates a spatial model, linking genetic data to points of occurrence of each sample. We performed the analysis with 15 repetitions, 5 x 106 iterations each, sampling at every 5 x 103 iterations. We used a range of the number of populations from 1 to 6, using both mtDNA and nuDNA. We used the term lineage to the recovered populations/clusters resulted from GENELAND.

For each lineage and locus, we estimated the following summary statistics with DNASP: number of polymorphic sites (S), haplotype diversity (Hd), number of haplotypes (h), nucleotide diversity (π), and Tajima's D. Next, we performed a hierarchical analysis of molecular variance (AMOVA) to calculate the genetic differentiation between lineages and gene fragments with 104 permutations with ARLEQUIN v.3.5 (Excoffier and Lischer, 2010).

2.3 Environmental suitability

Species distribution models (SDM) are commonly used in phylogeographic studies because it is assumed that part of genetic variation can be related by both past and present-day climate (Alvarado-Serrano and Knowles, 2014). We used environmental data to build SDMs to predict climatically stable areas during the Late Quaternary (last 130 thousand years) and matrix of environmental resistances (see below). Environmental data were downloaded from Worldclim (19 bioclimatic variables; available at http://www.wordclim.org) at a spatial resolution of 2.5 arc-minutes. To identify stable areas, we constructed four SDMs, for the following periods: current, Holocene (past 6 kya), Last Glacial Maximum (LGM; 21 kya), and Last Interglacial (LIG; 120 kya). We used 374 geographic coordinates of the species obtained from our sampling plus occurrence data available in digital databases (SpeciesLink, http://splink.cria.org.br/; Global Biodiversity Information Facility, http://www.gbif.org/; July 2016). To avoid sampling bias, we filtered geographic points at a spatial distance of 30 km with 'spThin' package (Aiello-Lammens et al., 2015).

To build SDMs we used nine bioclimatic variables (highly correlated variables were excluded, retained variables biologically relevant; Pearson correlation coefficient > 0.8), as following: mean diurnal range (BIO2), temp. seasonality (BIO4), max. temp. of warmest month (BIO5), min. temp. of coldest month (BIO6), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18), precipitation of coldest quarter (BIO19). Models were generated with the maximum entropy algorithm MaxEnt (Phillips et al., 2006). To tuning and evaluate MaxEnt models, we used ENMval package (Muscarella et al., 2014) with 10,000 background points and six feature classes (FC) combinations (L, H, LQ, LQH, LQHP and LQHPT). The area under the curve (AUC) was used to check model performance. Binary maps for each period (current, Holocene, LGM and LIG) were constructed with a threshold obtained from "Equal training sensitivity and specificity logistic threshold" value. After that we superimposed and summed the maps, showing likely stable areas over time. We repeated those procedures for each lineage (see Results).

To test whether environmental niches of lineages are divergent, we used an ordination approach PCA-env (Broennimann et al., 2012) taking SDMs as background areas for each lineage with ecospat package (Di Cola et al., 2017). Niche overlap was estimated by Schoener's D metrics, that varies from 0 to 1, indicating no to complete overlap. The observed overlap was compared to a null distribution, obtained from 100 random points at the background. Statistical significance of the niche overlap ($P \leq 0.05$) were obtained with niche equivalency and similarity tests, with the following settings: alternative = "lower" and rand.type = 1.

2.4 Demography

We assessed changes in population size over time for the two lineages (northeast and southwest) using Bayesian Skyline Plots (BSP) in BEAST 1.8.4 (Drummond et al., 2012). To evaluate clock model fit, we used an uncorrelated lognormal relaxed clock for. Our results showed that the coefficient of variation (CV) was close to 0; because of this result, we choose the strict clock instead. We used the mtDNA with a uniform prior with a substitution rate of 7.35 x 10-9 /site/year for 16S (Gehara et al., 2014). We linked 16S and ND1 fragments, trees and clock rate to estimate the final tree. We ran three independent chains, each for 5x107 steps, sampling every 5x103 steps, with a 10% burn-in. We used Tracer 1.5 (Rambaut and Drummond, 2007) to check the chain stationary, lack of convergence of the parameters (ESS ≥ 200), and to generate the plots. We also estimated the effective population size for ancestral and both northeast (NE) and southwest (SW) lineages (see below).

2.5 Divergence through space and time

We used a coalescent-based analysis that uses isolation with migration model (IM) to estimate the migration rate and divergence time between lineages, implemented in IMa2 (Hey, 2010; Hey and Nielsen, 2007). We applied for 16S and nuDNA (phased) the Hasegawa-Kishino-Yano (HKY) model of nucleotide substitution (Hasegawa et al., 1985). We set the upper bounds for the priors of divergence time, maximum migration (m), and maximum population size as: t = 5.0, m = 0.8 and q = 20.0, respectively (following the manual). We set the inheritance scalars to 0.25 for 16S and 1.0 for nuDNA and assumed a generation time of one year. As suggested in the user's manual we converted the substitution rate used in BEAST to years. Firstly, we performed the M-mode run, and assessed the stationarity of the three independent runs with the trend plots and controlled the length of burn-in and recording phase with the IMburn and IMrun files. Our final run had a burn-in of approximately 1.56 x 106 steps and was performed for 1.02 x 107 steps. Lastly, we ran the L-mode to evaluate a total of 25 nested models using 100,000 genealogies. We compared the log-likelihood ratio tests among nested models and accessed the best model through AIC.

To test our hypotheses that Quaternary climatic fluctuations affected the diversification and/or demography of *Boana raniceps*, we used the approximate Bayesian computation (ABC) approach to test four alternative scenarios of diversification (Fig. 2). We recovered two geographically structured lineages, one in the northeast, another in the southwest portion of the range. Results indicated an increase in population size only in the northeast lineages, while the southwest lineages remained constant over time (see Results). We also detected significant migration between the two lineages. Thus, we used all available information to assess the following diversification scenarios: (a) divergence with gene flow and recent population expansion in northeast lineage from the Last Glacial Maximum (LGM) – Model 1; (b) divergence with recent gene flow from LGM and recent population expansion in northeast lineage from the LGM – Model 2; (c) divergence with gene flow until LGM and recent population expansion in northeast lineage from the LGM – Model 3; (d) divergence with gene flow from LGM with recent population expansion in northeast lineage – Model 4. For the first three diversification scenarios, we incorporated divergence time recovered in IMA2. We also hypothesized a fourth scenario of divergence from LGM. We estimated population sizes with IMA2 and changes in population sizes through time using Bayesian skyline analysis in BEAST. Finally, we used migration rates estimated with IMA2.

We used the R package 'PipeMaster' (Gehara et al., in review.; www.github.com/gehara/PipeMaster) to simulate 100,000 data points under each model. Our simulated datasets were identical to our observed dataset, i.e., same number of genetic markers, individuals per lineage, genetic inheritance, sequence length. We assumed a uniform prior distribution with minimum and maximum values extracted from previous analyses (Beast, IMa, Skyline plots). From each simulation we calculated six summary statistics for each lineage and for the whole species (totalizing 18 summary statistics): number of polymorphic sites, nucleotide diversity, haplotype diversity, Tajima's D, and Fu and Li's D and F. We estimated posterior probabilities and model support for each model using the postpr function in R package 'abc' (Csilléry et al., 2012). We set the tolerance value to 0.01 and used the multinomial logistic regression method to compare models. To evaluate model accuracy, we used cross-validation and built misclassification bar plots with the cv4abc function of the 'abc' package.

2.6 Isolation by distance and isolation by environmental resistance

Our first hypothesis of isolation by distance (IBE) postulate that gene flow among populations assumes a positive correlation between spatial distance and genetic distance. To estimate the pairwise distance between localities we used Geographic distance Matrix Generator 1.2.3 (Ersts, 2011) and obtained a matrix of geographic distances.

Our second, third and fourth hypotheses posit that landscape heterogeneity can shape patterns of genetic differentiation in *B. raniceps*. Because of that, we tested hypotheses of genetic differentiation driven by isolation by environmental resistance (IBE). We built IBE models based on six predictors: climate suitability (current, LGM and refuges), rivers and tributaries, and topographic complexity (altitude and slope). We used circuit theory to predict spatial resistance among pairs of population for each of the six predictors of the IBE hypotheses. To calculate pairwise resistance distance, we built environmental resistance surfaces with CIRCUITSCAPE v.5.0 Julia package (Anantharaman et al., 2019). For scenarios of genetic differentiation related to climate we used current, LGM, and stability SDM maps. For the stability map, we scaled values to a range from 0 to 1 (i.e. 0.25 for presence in one period, 0.50 in two periods, 0.75 in three periods and 1 presence in all for periods). Because lower values of suitability correspond to higher costs to population connectivity, we calculated the resistance due to current and historical unsuitability habitats (LGM and refuges), inverting the SDMs' raster values (1-suitability). In the same way we used main perennial rivers as drivers of spatial connectivity while its absence was considered as barriers to dispersion. Our river network raster contains the presence of main rivers from South America (stream lines at 1:10m scale; https://www.naturalearthdata.com/). We obtained the elevation raster from NASA Jet Propulsion Laboratory (https://landscape.jpl.nasa.gov/). We used the elevation map to derive a slope raster in ARCMAP v.10.3 (ESRI). We assumed that higher elevations or steeper slopes meant a higher cost for gene flow. All rasters were at a spatial resolution of 2.5 arc-minutes. CIRCUITSCAPE reads zeros as hard barriers and because of that we changed all 0 by 0.0001.

We obtained our pairwise matrix of genetic differentiation among localities (ϕ_{st}) with an analysis of molecular variance (AMOVA) in ARLEQUIN v.3.5.2 (Excoffier and Lischer, 2010). The significance of pairwise comparisons was assessed by 10000 permutations. Because the number of missing data in nuDNA fragments and the different heritage of markers we used solely the 16S fragment from 237 individuals from 61 sites. We replaced negative ϕst values (n = 74) with 0.0001.

To test whether different landscape features promoted genetic differentiation in *B. raniceps*, we conducted a generalized dissimilarity modeling (GDM). This analysis is a matrix regression tool that takes into account non-linear rather than linear relationships with the use of I-spline basis functions (Ferrier et al., 2007). I-splines can modeling turnover of the response variable (i.e. genetic data) in response to each environmental predictor (Fitzpatrick and Keller, 2015). For each predictor, GDM calculates the dissimilarity between site pairs and fit the coefficients to the I-spline basis functions. The maximum height of the I-splines indicates the amount of genetic differentiation, for example, along the gradient of each predictor of the landscape. Thus, we accessed the relationship between genetic differentiation (response variable) and seven environmental distances (predictors): matrix of geographic distance, current, LGM, refugia, rivers, slope, and altitude. We performed GDM with the ‘gdm’ R package (Manion et al., 2018). First, we assessed the importance of each predictor with non-zero coefficients by summing the three I-spline coefficients of the I-splines functions. Then, to find the best predictors of ϕ_{st} turnover we applied a stepwise matrix permutation (Ferrier et al., 2007). Thereby, we used the gdm.varImp function in ‘gdm’ R package with 1000 random permutations with backward elimination, removing at each step the predictor with the least significant contribution.

2.7 Phenotype comparison between lineages

We were also interested in testing whether other factors of the life history of the species, such as behavioral traits, could vary along its history and distribution. Advertisement calls are primarily used by males to attract mates for reproduction, and are an important character for species recognition (Duellman and Trueb, 1994). For acoustical analyses we measured 962 calls from 65 individuals from 32 sites. Those calls were obtained from public sound collections and recorded by us (deposited at ASUFRN). Comparisons were made using three datasets: entire advertisement calls, Note A and Note B. For the former we measured seven acoustic parameters: dominant frequency (DF; Hz), call duration (CD; s), note duration (ND; s), number of notes (NN), number of pulses (NP), note rate (NR = NN/ CD; notes/s) and pulse rate (PR = NP/ND or CD; pulses/s). For A and B notes (N = 406 and N = 1217, respectively) we measured just the ND, NP and NR. Spectrograms were produced with fast Fourier transform set at a resolution of 256 points and overlap 50% in RAVEN 1.4 software. To test if advertisement calls and notes of lineages obtained from population assignment test are different, we performed MANOVAs. Multivariate outliers and variables highly correlated (Pearson correlation coefficient > 0.7) were excluded from analyses.

3. Results

3.1 Population assignment and sequence information

After excluding gaps, we obtained a final alignment of 1306 bp for mtDNA and 1838 bp for nuDNA. Highest levels of genetic diversity were found for ND1 and RPL3. The northeastern lineage had in general higher nucleotide diversity, except for Bfib and Tyr. Tajima's *D* suggested a rapid and significant population expansion only for 16S in the NE lineage (Table 2). Most of the genetic variation was observed within populations for all genes, with low and significant F_{ST} (from 3% to 25%; Table 3).

GENELAND recovered two geographically structured populations (Fig. 1; Fig. S1 Supplementary material): one ranging in Caatinga and northern Cerrado (northeast lineage), the other in southern Cerrado, Chaco and Amazonian savannas (southwest lineage).

3.2 Environmental suitability

The Maxent model had a good performance in predicting the current occurrence of *B. raniceps* (AUC = 0.93). The model predicted areas of suitability along most of the DOF and in Amazonian savannas, with some over-prediction in Venezuela and Colombia, a region with disjunct savannas on the Guyana shield. We inferred complex distribution dynamics during the Pleistocene climatic oscillations. Current and LGM suitability showed a marked discontinuity in central Brazil that closely matches the boundaries between the two genetic lineages (Fig. 3a, c), while this discontinuity is not apparent during the Holocene and LIG (Fig. 3b, d); a major contraction in climatically suitable areas occurred during the LGM (Fig. 3c). During the periods modelled, there were areas of climatic stability for both lineages: north Cerrado and Caatinga for the northeastern lineage and the Chaco region for the southwestern lineage (Fig. 3e, f).

Boana raniceps lineages showed very limited niche overlap (Schoener's D = 0.07). Furthermore, their niches were not equivalent ($P < 0.05$) and background similarity was not significant ($P > 0.05$; Fig. 3g), suggesting that lineages occupy different environmental niches. Along the environmental space, lineages segregated mainly in PC2, related to temperature seasonality, precipitation seasonality and precipitation of driest and warmest quarter (Fig. 3g).

3.3 Demography and divergence through space and time

Bayesian skyline plots showed different demographic patterns for each lineage. While the southwest lineage had no signal of abrupt demographic changes through time, considering the confidence interval, the northeast lineage exhibited population expansion from 100 kya, corresponding to the Late Pleistocene (Fig. 4).

According to the Isolation with Migration model there was significant bidirectional migration between northeast and southwest lineages, with a higher rate of migrants per generation from Northeast to Southwest (Table 4). Northeastern lineage had its effective population size (Ne) two to three times larger than Southwest (Ne- Northeast ≈ 1.8 million individuals; Ne- Southwest ≈ 0.49 million), which is slightly larger than the ancestral population (1.58 million individuals). Divergence between lineages occurred about 340 kya (95% of HPD; 257–522), during the middle Pleistocene. Among all nested models, lowest ΔAIC account for equal population sizes and migration rates between northeast and southwest lineages (Table 5).

Our ABC analysis recovered the highest posterior probability for model 3 (0.93; Fig. S2 in Supplementary material), which indicates divergence with gene flow until the LGM and recent population expansion in the northeastern lineage since the LGM (Fig. 2).

3.4 Isolation by distance and isolation by environmental resistance

The GDM analysis indicated a major role of environmental complexity on the genetic differentiation between *B. raniceps* lineages. The full GDM model explained 6.82% of the total observed genetic variation. Of the variables used, five predictors contributed to explain the total deviance (Fig. 5). The main predictors for genetic differentiation were associated to environmental resistance due to: topographic complexity through differences in slope of the terrain (summing of the coefficient of the *I*-spline basis function = 1.20) and higher altitudes (0.64); followed by resistance from unsuitable climate through instability areas (0.32) and current climate (0.27); and then geographic distance (0.16). Only slope had a significant contribution ($P \leq 0.05$) for the observed genetic differentiation.

3.5 Phenotype comparison between lineages

Advertisement calls were different between lineages ($F_{1,56} = 3.92$; $P < 0.005$), with males from southwest calling at faster pulse rate ($F = 13.53$: $P < 0.001$: Table 6). The two notes that can compose the advertisement call also differed between lineages, with highest number of pulses and pulse rate in both notes for males from southwest (Note 1 – $F_{1,55} = 3.16$; $P < 0.05$: Note 2 – $F_{1,60} = 8.73$; $P < 0.001$).

4. Discussion

We found strong support for the isolation by environment hypothesis, that steeper slopes in the CBP should acted as a soft vicariant barrier permeable to gene flow. *Boana raniceps* is spatially structured in two lineages: a northern Cerrado and Caatinga lineage (northeastern) and a southern Cerrado, Chaco, and Amazonian savannas lineage (southwestern). Genetic break among these lineages matches a highly complex topographic region in the CBP within the

Cerrado. These lineages diverged during the middle Pleistocene and present a shallow genetic structure with bidirectional gene flow, likely resulting from a scenario of isolation with migration from the middle Pleistocene until the LGM, followed by recent population expansion in the northeastern lineage since the LGM. Genetic differentiation was mainly explained by isolation by environmental resistance through topographic complexity, but periods of biome expansions during Quaternary climatic oscillations seem to have contributed to gene flow between lineages across time. This result is congruent with the scenario of gene flow breaks and reconnection through time, in which current and LGM climates could reduce migration of populations. Furthermore, northeastern and southwestern lineages occupy different environmental niches that, along with topography and differences in advertisement calls, could potentially reduce gene flow between them. Our findings diverge from phylogeographic studies on widely distributed lineages from the South American DOF that showed deeply structured lineages with ancient divergences (Fonseca et al., 2018; Lanna et al., 2018; Oliveira et al., 2018a; Recoder et al., 2014; Werneck et al., 2012a). Conversely, diversification processes associated with Pleistocene climatic shifts apparently are pervasive for several plant (Bonatelli et al., 2014; Correa Ribeiro et al., 2016; Diniz-Filho et al., 2016) and animal species (Bartoleti et al., 2017; Prado et al., 2012).

Landscape features may limit or potentialize dispersal, causing genetic differentiation to be more related to environmental resistance/connectivity than to geographic distances (Lawson, 2013; McRae, 2006). Accordingly, genetic differentiation in *B. raniceps* is best explained by landscape features rather than by geographic distances among populations. The current genetic break coincides with a complex topographic region in the CBP that may have acted as a soft barrier to gene flow between lineages. Fouquet et al. (2007) suggested that *B. raniceps* could be a single, widespread anuran species by comparing mtDNA samples from extreme locations across its latitudinal distribution (French Guiana and Argentina). Indeed, although this species seems to fit the definition of a single evolving lineage (de Queiroz, 2007), the two individuals used by Fouquet et al. (2007) likely belong to the same lineage (southwestern), what may mask the total within species diversity according to our data.

During the Late Miocene, the CBP compartmentalization promoted the diversification of different organisms along the DOF. However, the strength of this factor as a driver of diversification varies according to each species' biology. For lowland species, high plateaus could have acted as topographic barriers, promoting isolation between lineages occurring in the valleys (Oliveira et al., 2018a; Werneck et al., 2012a). For the gecko *Phyllopezus pollicaris* (Spix 1825) and the frogs *Dermatonotus muelleri* (Boettger 1885) and *Physalaemus cuvieri* Fitzinger 1826, the CBP acted as the primary source of genetic structure (Miranda et al., 2019; Oliveira et al., 2018a; Prado et al., 2012; Werneck et al., 2012a). Our analysis testing IBE identified slope as a key predictor of environmental resistance to gene flow for *B. raniceps*. The erosion of plateau surfaces during the Quaternary promoted the expansion of vast depressions between plateaus, increasing fragmentation and landscape complexity in Cerrado (Ab'Sáber, 1998; Colli, 2005). Hence, although not being influenced by the uplift itself, the genetic diversity and differentiation within *B. raniceps* seems influenced by the topography resulting from the compartmentalization due to CBP uplift.

It is expected that phylogeography structure could also be driven by intrinsic features such as dispersal ability, reproductive mode, body size, habitat of occurrence and physiology (Fouquet et al., 2012; Papadopoulou and Knowles, 2016; Zamudio et al., 2016). Anurans have a tendency to show higher phylogeographic structure in topographically complex habitats, but open-area species usually have lower intraspecific genetic divergences (Rodríguez et al., 2015). Accordingly, *Boana albopunctata*, a species closely related to *B. raniceps* and also associated to open areas, occurs in altitudes from 0 to 2000 m (Aquino et al., 2010). Although it occupies a smaller geographic area, *B. albopunctata* presents a more complex genetic structure than *B.*

raniceps with three known lineages (Prado et al., 2012). At lower elevations, *B. raniceps* seems to replace *B. albopunctata*, and both species rarely occur syntopically (Prado et al., 2012), suggesting that biotic interactions between these closely related species can also influence geographic structure. Because *B. albopunctata* occurs throughout a wider altitudinal range than *B. raniceps*, the observed differences in geographic structure of the species can be expected.

The middle Pleistocene witnessed the split among several South American plant lineages, vertebrates, and invertebrates (Turchetto-Zolet et al., 2013). Accordingly, divergences among lineages of *B. raniceps* dated from 0.26–0.52 Ma (Table 4). Likewise, South American treefrogs from open habitats like *B. albopunctata* and *Boana lundii* (Burmeister 1856) present genetic clusters also formed during middle Pleistocene (Prado et al., 2012; Vasconcellos et al., 2019). Climatic changes during the Pleistocene altered the extension of biomes (Costa et al., 2018; Werneck et al., 2012b, 2011) and demographic histories of populations and communities. In the Caatinga biome, for example, climatic changes promoted synchronous demographic responses of squamates and frogs in which most populations expanded during the late Pleistocene (Gehara et al., 2017). We also detected a recent demographic expansion for the northeastern lineage, albeit more recently (100 kya). Because we detected a substantial expansion of suitable areas for the southwestern lineage after the LGM, we expected to find a concordant population expansion, but we did not observe such tendency. Parts of the Chaco biome were flooded during Middle-Late Miocene (Werneck, 2011) and past evolutionary signals in these areas could be lost. Recurring cycles of marine incursions that lasted until 800 kya (Haq et al., 1987) could have extinct lowland populations in the south and west species' distribution, with a posterior recolonization event.

Recent climatic models suggested that Neotropical forests expanded during the LGM, promoting the fragmentation and contraction of open and dry biomes (Costa et al., 2018; Ledo and Colli, 2017). Indeed, our data indicates that during LGM gene flow between *B. raniceps* lineages halted, and SDMs accordingly indicate a contraction of climatically suitable areas for *B. raniceps* during this period. The Amazon and Atlantic forests limit the distribution of *B. raniceps* to the west and to the east, respectively. Additionally, as a lowland species, a complex topographic gradient in central Brazil also reduces the chances of panmixia. Thus, it is possible that different current and historical landscape features have affected genetic structure of *B. raniceps* in opposite ways. Genetic structure in *B. raniceps* was related to topographic resistance in central Brazil, but paleoclimatic changes either reinforced this pattern (e.g. LGM) or maximized gene flow during warmer periods (e.g. LIG and Holocene). Moreover, lineages occupied different environmental niches and showed dissimilar advertisement calls.

Advertisement calls differed between lineages, mainly regarding pulse rate. This acoustic parameter is commonly used to discriminate species, once it is less variable and should be used for species recognition by females (Gerhardt, 1991). Advertisement calls of anurans serves primarily for mate attraction and secondarily for delimiting territories among males, being an important pre-zygotic reproductive barrier (Duellman and Trueb, 1994). Several mechanisms can lead to differences in acoustic signals of lineages, such as genetic drift and natural and sexual selection (Köhler et al., 2017). Population differences in phenotypes may respond to genetic divergence and phylogeographic structure in general, by means of isolation by distance, pleiotropic effects, local adaptations in response to different habitats, or by biotic interactions (Funk et al., 2009; Köhler et al., 2017; Zamudio et al., 2016). In anurans, given that they are ectothermic organisms, temperature has an effect on temporal calling parameters, such as pulse rate, once it affects muscular contraction (McLister, 2001; Wells, 2007). Still, it's important to test whether *B. raniceps* females prefer calls from males of its own lineage or if they can mate indiscriminately. For some frogs, divergent sexual selection can have promoted speciation through behavioral isolation. In these cases, there were evidences for genetic divergence, with reduced gene flow among different populations, and incipient speciation in which males from different populations produced different calls and females preferred calls from native males (Boul et al., 2007; Guerra and Ron, 2008). If females of *B. raniceps* strongly prefer males from its respective lineages, incipient speciation reinforced by sexual selection is possible to happen, in which future lineages can be sympatric but do not interbreed anymore.

Geographic structure in *Boana raniceps* is possible related to a landscape complexity that involves both climatic suitability and topography. As a lowland species, a complex topographic gradient in the Central Brazilian also reduces the chances of populations to becoming uniform. Many unsolved questions remain opened regarding the patterns and processes of diversification of the South American open biomes (Turchetto-Zolet et al., 2013). Due to its continental distribution, occurring in most open biomes, *Boana raniceps* still can be used to test several biogeographical hypotheses. It is also interesting to compare genetic diversity between stables and unstable areas as already done for some lizards in the open diagonal (Oliveira et al., 2018b; Santos et al., 2014). Under a comparative phylogeographic approach, using several co-distributed taxa it is possible to test alternative diversification scenarios simultaneously (Hickerson et al., 2010). Therefore, a next step should be using all genetic data already generated for organisms distributed across Diagonal of Open Formations, to test if divergence occurred at a community level at the same time and if Pleistocene climatic changes affected similarly their population size.

5. Conclusions

Our results show a complex and recent genetic divergence in a continentally distributed species in South America during the middle Pleistocene. *Boana raniceps* corresponds to a single and widespread species, composed of two geographically structured lineages that occupy different niches in the environmental space. Northeastern and southwestern lineages divergence was mediated by a semipermeable barrier formed by an environmental constrain related to topography and climate. Climatic changes throughout the Late Pleistocene apparently affected the northeastern lineage once expansion started during this period. Our results reinforce the need for more studies in this region since other species may reveal distinct patterns and processes of diversification. However, we hypothesize that the history of instability in southwestern DOF areas can have impacted lowland communities in a similar fashion.

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Table 1. Hypotheses of genetic differentiation in the treefrog *Boana raniceps*. For each mechanism involved in the processes of species diversification, considering the effects of landscape and natural history, a prediction stating the expected pattern in the data.

Hypothesis	**Mechanism**	**Prediction**
Isolation by distance (IBD)	Dispersal limitation restricts gene flow among distant populations	Positive correlation between spatial distance and genetic distance
Central Brazilian Plateau compartmentalization	For lowland species the CBP was a strong vicariant barrier segregating at least two lineages	Deep phylogeographic structure and absence of gene flow between populations. Divergence time between lineages should matches with the final uplift period (7–5 Ma)
	For large and highly dispersive species, the CBP can be a soft vicariant barrier to gene flow	Shallow genetic structure with gene flow between populations. Permeability of CBP drives to isolation with migration model
River connectivity	For amphibians highly dependent of water bodies, rivers should favor the connectivity among populations	Negative correlation between presence of rivers and genetic distance
Quaternary climatic fluctuations	Climatic shifts during Quaternary affected demographic parameters promoting population expansion and contraction through time	Changes of effective population size and species paleoclimatic distribution models
	Climatic instability areas should be resistance to gene flow among populations from climatic stable areas (refuges)	Positive correlation between presence of climatically unsuitable areas and genetic differentiation
Panmixia (= null hypothesis)	*B. raniceps* is a highly dispersive species and tolerant to habitat fragmentation	A single and widely distributed lineage

Table 2. Summary statistics for each locus and lineage of the long-legged treefrog *Boana raniceps*. Length in base pairs; N – number of samples; S – polymorphic sites; H – number of haplotypes; Hd – haplotype diversity; π – nucleotide diversity; P-values of the Tajima's D test. Nuclear markers Beta-fibrinogen, POMC, RPL3 and Tyrosinase are phased.

Locus	Lineage	Length	N	S	H	Hd	π	Tajima's D	*P*-value
16S	All	478	123	33	32	0.860	0.00402	-2.08736	<0.05
	Northeast		66	22	22	0.886	0.00387	-1.86806	<0.05
	Southwest		58	12	11	0.754	0.00302	1.31054	>0.10
ND1	All	828	82	76	50	0.981	0.01375	-1.00818	>0.10
	Northeast		38	54	26	0.972	0.01483	-0.29374	>0.10
	Southwest		44	42	26	0.965	0.00895	-0.90442	>0.10
Beta-fibrinogen	All	444	132	22	24	0.881	0.00473	-1.36134	>0.10
	Northeast		72	18	17	0.793	0.00365	-1.36134	0.10>*P*>0.05
	Southwest		66	12	15	0.882	0.00544	-1.36134	>0.10
POMC	All	447	222	14	21	0.717	0.00255	-1.28489	>0.10
	Northeast		116	8	14	0.800	0.00288	-0.35053	>0.10
	Southwest		106	11	12	0.555	0.00181	-1.59277	0.10>*P*>0.05
RPL3	All	521	182	67	71	0.961	0.01745	-0.76453	>0.10
	Northeast		94	54	34	0.916	0.02396	0.38730	>0.10
	Southwest		88	31	41	0.936	0.00814	-0.95864	>0.10
Tyrosinase	All	426	224	16	40	0.800	0.00492	-0.55114	>0.10
	Northeast		118	9	16	0.757	0.00469	0.45095	>0.10
	Southwest		106	14	30	0.829	0.00500	-0.55097	>0.10

Table 3. Results of the analyses of molecular variance (AMOVAs) with variance percentages for each gene, considering northeast and southwest lineages of the long-legged tree frog *Boana raniceps*. All P-values < 0.0001.

Locus	Source of variation		
	Between lineages	Within lineages	F_{ST}
16S	20.82	79.18	0.20821
ND1	25.47	74.53	0.25472
Beta-fibrinogen	10.61	89.39	0.10607
POMC	12.51	87.49	0.12508
RPL3	12.23	87.77	0.12230
Tyrosinase	3.22	96.78	0.03216

Table 4. Results from Isolation with Migration analysis (IMa2). NE size – effective population size of northeast lineage; SW size – effective population size of southwest lineage; effective population size of ancestral population; divergence time between lineages in years; migration rates for each lineage in coalescent direction. For both migrations rates *P* < 0.01.

Value	NE size	SW size	Ancestral size	Divergence time	NE>SW	SW>NE
HiPt	1,807,402	492,634	1,578,045	340,806	1.274	1.911
Mean	1,892,659	534,447	1,629,153	370,779	1.654	2.201
HPD95Lo	1,355,148	308,502	1,177,477	256,816	0.3094	0.4623
HPD95Hi	2,595,617	835,055	2,188,588	521,708	3.645	4.519

Table 5. Results of the isolation with migration model with IMa2 L-mode (nested models) for northeast and southwest lineages. *K* - Number of demographic parameters; log(P) - log of the posterior density function; 2LLR - log-likelihood ratio statistics; df - degrees of freedom; $^{\#}$test distribution of 2LLR is a mixture; *not rejected by the 2LLR test in favor of the full model with *P*<0.05; AIC - Akaike Information Criterion. In bold models with ΔAIC < 3.

Model	*K*	log(P)	2LLR	df	*P*	AIC	ΔAIC
Θ_1 Θ_2 Θ_A m_1 m_2	5	-1.351	-	-	-	12.702	3.368
Θ_2 Θ_1=Θ_A m_1 m_2	4	-1.434	0.1646	1	0,70*	10.868	**1.534**
Θ_1=Θ_2 Θ_A m_1 m_2	4	-3.237	3.772	1#	>0,05*	14.474	5.14
Θ_1 Θ_2=Θ_A m_1 m_2	4	-3.518	4.333	1#	<0.03	15.036	5.702
Θ_1 Θ_2 Θ_A m_1=m_2	3	-104	205.3	2#	<0,001	214	204.666
Θ_1 Θ_2 Θ_A m_1=0 m_2	4	-1.494	0.2844	1	0,60*	10.988	**1.654**
Θ_1 Θ_2 Θ_A m_1 m_2=0	3	-1.667	0.6322	2	0,70*	9.334	**0**
Θ_2 Θ_1=Θ_A m_1=m_2	3	-3.273	3.843	2#	0,15*	12.546	3.212
Θ_2 Θ_1=Θ_A m_1=0 m_2	3	-3.888	5.074	2#	0,08*	13.776	4.442
Θ_2 Θ_1=Θ_A m_1 m_2=0	2	-109	215.2	3#	<0,001	222	212.666
Θ_1=Θ_2=Θ_A m_1 m_2	4	-7.795	12.89	1	<0,001	23.59	14.256
Θ_1=Θ_2 Θ_A m_1=m_2	3	-8.308	13.91	2	<0,001	22.616	13.282
Θ_1=Θ_2 Θ_A m_1=0 m_2	3	-17.9	33.1	2#	<0,001	41.8	32.466
Θ_1=Θ_2 Θ_A m_1 m_2=0	3	-8.749	14.8	2#	<0,001	23.498	14.164
Θ_1 Θ_2=Θ_A m_1=m_2	2	-135.2	267.6	3#	<0,001	274.4	265.066
Θ_1 Θ_2=Θ_A m_1=0 m_2	4	-10.28	17.86	1	<0,001	28.56	19.226

Θ_1 $\Theta_2=\Theta_A$ m_1 $m_2=0$	3	-11.75	20.8	2	<0,001	29.5	20.166
Θ_1 Θ_2 Θ_A $m_1=0$ $m_2=0$	3	-23.28	43.87	2#	<0,001	52.56	43.226
Θ_2 $\Theta_1=\Theta_A$ $m_1=0$ $m_2=0$	3	-22.44	42.18	2#	<0,001	50.88	41.546
$\Theta_1=\Theta_2=\Theta_A$ $m_1=m_2$	2	-141.5	280.2	3#	<0,001	287	277.666
$\Theta_1=\Theta_2=\Theta_A$ $m_1=0$ m_2	3	-11.78	20.87	2	<0,001	29.56	20.226
$\Theta_1=\Theta_2=\Theta_A$ m_1 $m_2=0$	2	-11.81	20.92	3	<0,001	27.62	18.286
$\Theta_1=\Theta_2$ Θ_A $m_1=0$ $m_2=0$	2	-28.62	54.54	3#	<0,001	61.24	51.906
Θ_1 $\Theta_2=\Theta_A$ $m_1=0$ $m_2=0$	2	-35.21	67.71	3#	<0,001	74.42	65.086
$\Theta_1=\Theta_2=\Theta_A$ $m_1=0$ $m_2=0$	1	-141.9	281.1	4#	<0,001	285.8	276.466

Table 5. *Cont.*

Table 6. Advertisement call parameters of the long-legged tree frog *Boana raniceps* and northeast and southwest lineages. In parenthesis is the number of calls analyzed. Cdur – call duration, DFreq – dominant frequency, NN – number of notes, Ndur – note duration, NP – number of pulses, NR – note rate, and PR – pulse rate. Note1 and Note2 are the note type.

	Total (65)	Northeastern (51)	Southwest (14)
N calls	962	745	217
Cdur (s)	1.25± 2.06	1.38± 2.30	0.77± 0.52
Dfreq (Hz)	1732± 545	1766± 490	1605± 719
NN	3.62± 0.82	4.86± 9.56	2.64± 1.46
Ndur (s)	0.17± 0.04	0.17± 0.87	0.18± 0.05
NP	7± 1.73	6.83± 1.72	7.63± 1.70
Nrate (NN/s)	3.62± 0.82	3.62± 0.87	3.65± 0.63
Prate (NP/s)	40.29± 4.35	39.31± 4.15	43.87± 3.05
Cdur-Note1	0.26± 0.09	0.26± 0.08	0.26± 0.08
NP-Note1	10.00± 3.30	10.24± 3.35	11.11± 3.47
NR-Note1	39.35± 12.46	40.07± 12.34	42.62± 11.76
Cdur-Note2	0.13± 0.02	0.13± 0.03	0.14± 0.05
NP-Note2	5.12± 0.69	5.31± 1.05	6.07± 1.84
NR-Note2	39.93± 4.73	40.69± 6.88	43.69± 12.12

FIGURES

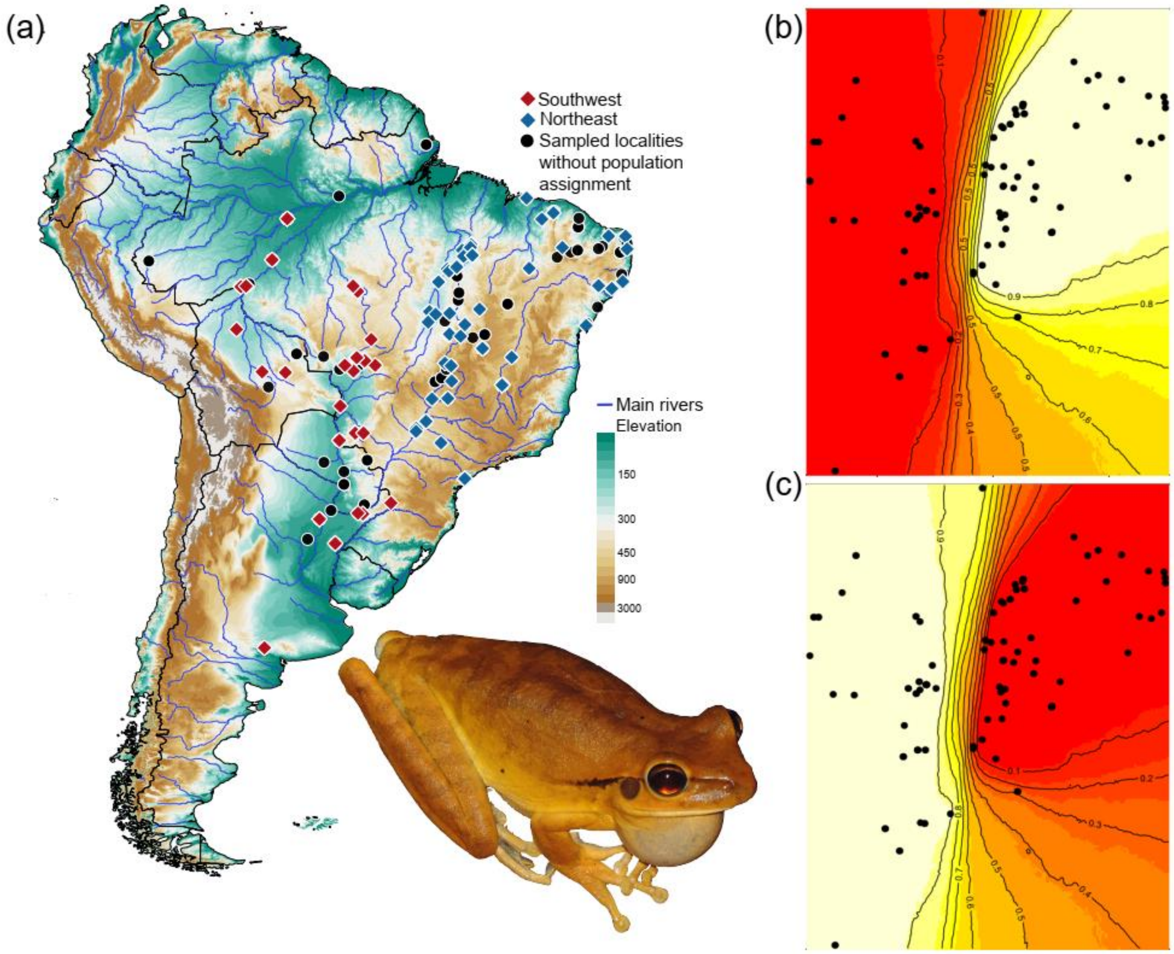

Figure 1. (a) Geographic distribution of samples of the long-legged *Boana raniceps* used in this study. Results of GENELAND population assignment analysis, depicting (b) northeastern and (c) southwestern lineages; colors and isoclines indicate posterior probabilities of assignment to each lineage.

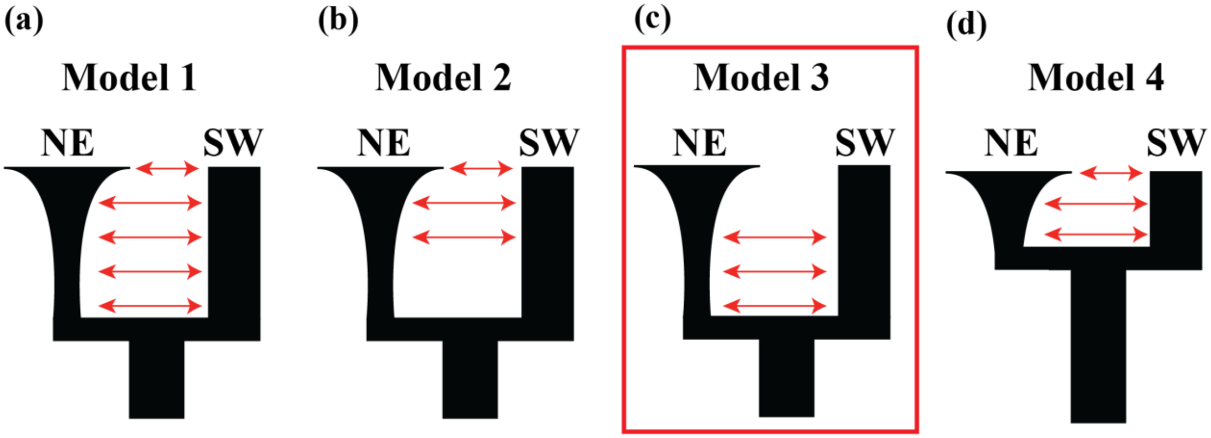

Figure 2. Divergence scenarios tested using ABC for northeastern (NE) and southwestern (SW) lineages of *Boana raniceps*. All scenarios consider recent population expansion in the northeast (NE) lineage. Scenarios assume (a) constant gene flow through time, (b) gene flow after LGM, (c) gene flow until LGM or (d) constant gene flow from LGM. Divergence time of lineages in models 1, 2, and 3 was estimated with IMa2 (during middle Pleistocene) and in model 4 was set at the Last Glacial Maximum (LGM). The best model is highlighted by a red box.

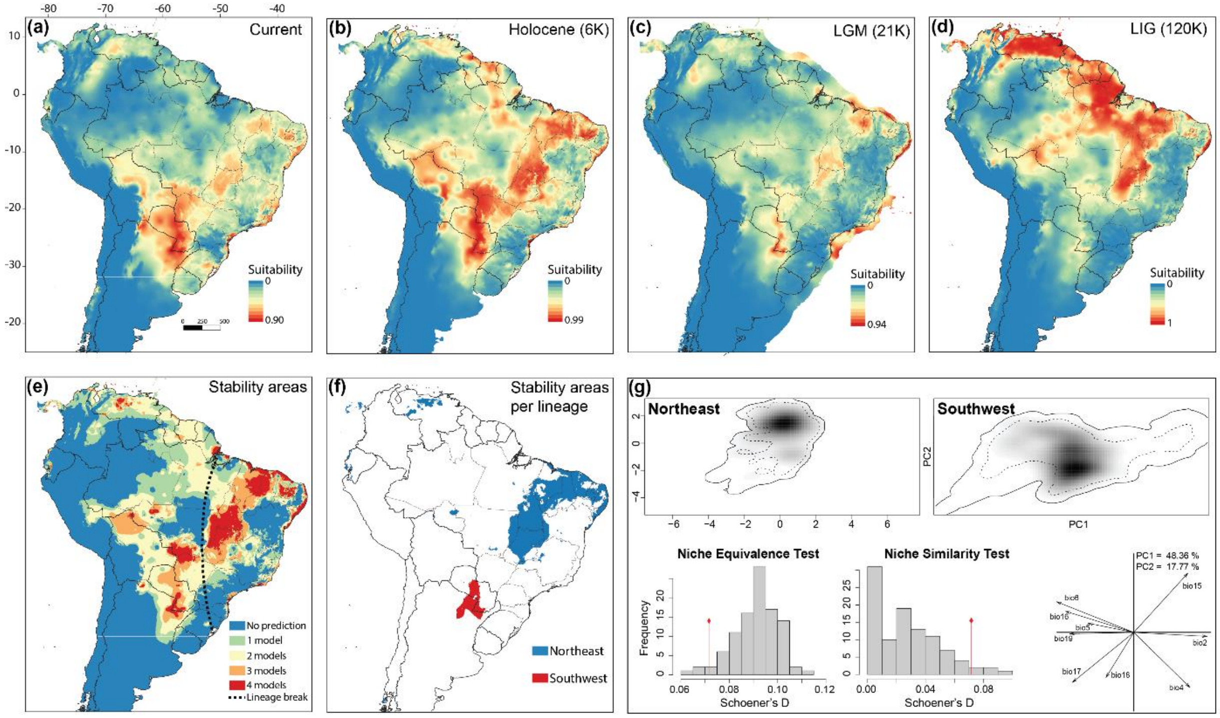

Figure 3. Species distribution models and niche comparison of northeast and southwest lineages of the *Boana raniceps* treefrog. Projection of potential suitable areas during the Late Quaternary are shown in panels *a-d*. Warmer colors indicate higher probabilities of occurrence based solely on environmental aspects, which do not account for barriers or biotic interactions. The map of stability areas represents the sum of maps in the four periods for *B. raniceps* (panel *e*) and for each lineage (panel f). In the panel *g*, upper graphs represent the environmental space occupied by both lineages, with darker cells showing the highest density of occurrences and available background in lines. In the lower left corner, histograms of niche equivalence (P = 0.04) and niche similarity tests (P = 0.95), with observed overlap in red (D = 0.07); in the lower right corner, contribution of each variable in the environmental space.

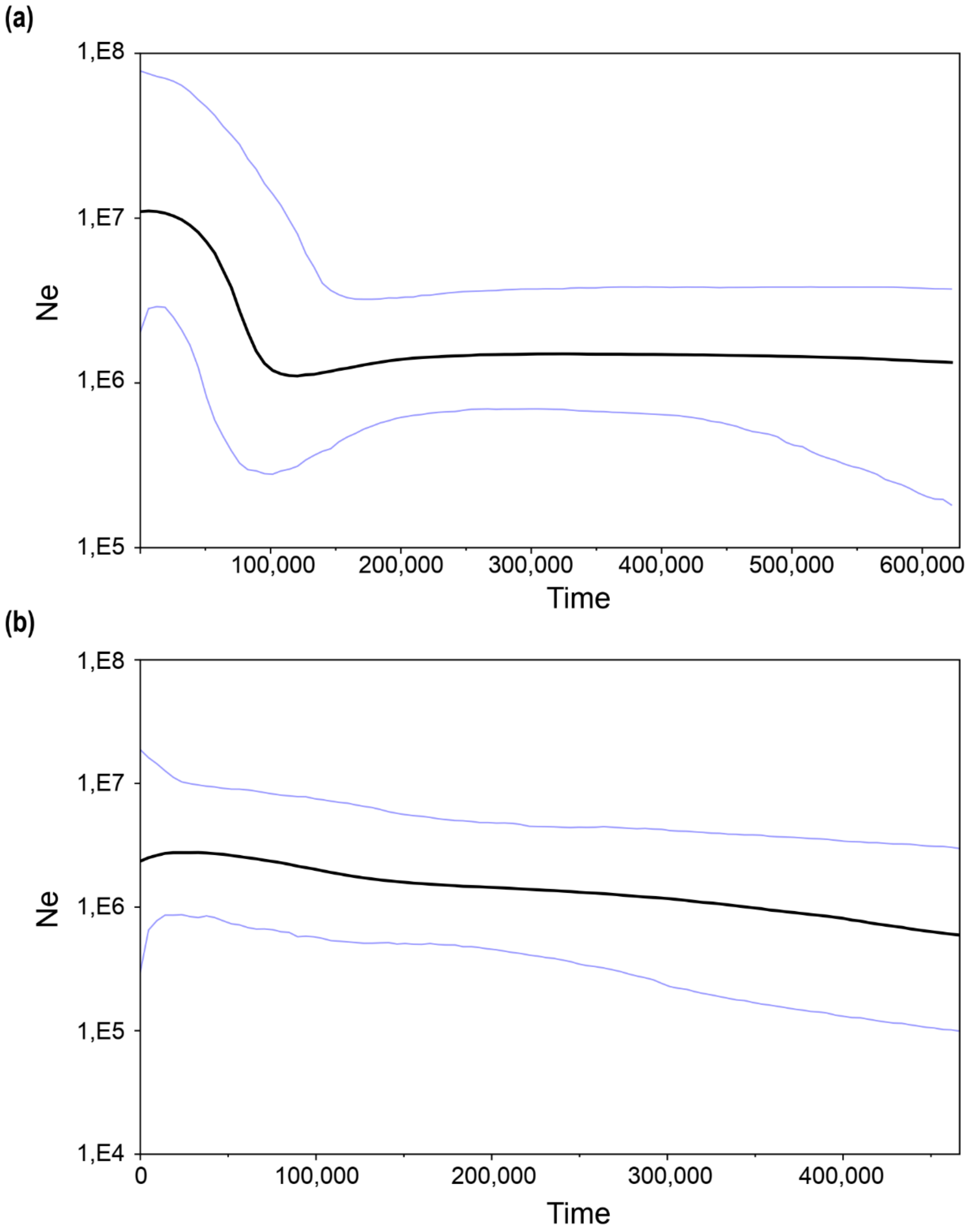

Figure 4. Bayesian Skyline Plots (BSP) performed for each lineage of the long-legged treefrog *Boana raniceps*. Ne means effective population size through time (in years). (a) northeast lineage and (b) southwest lineage. Black line shows the median population size with 95% higher posterior probability in blue lines.

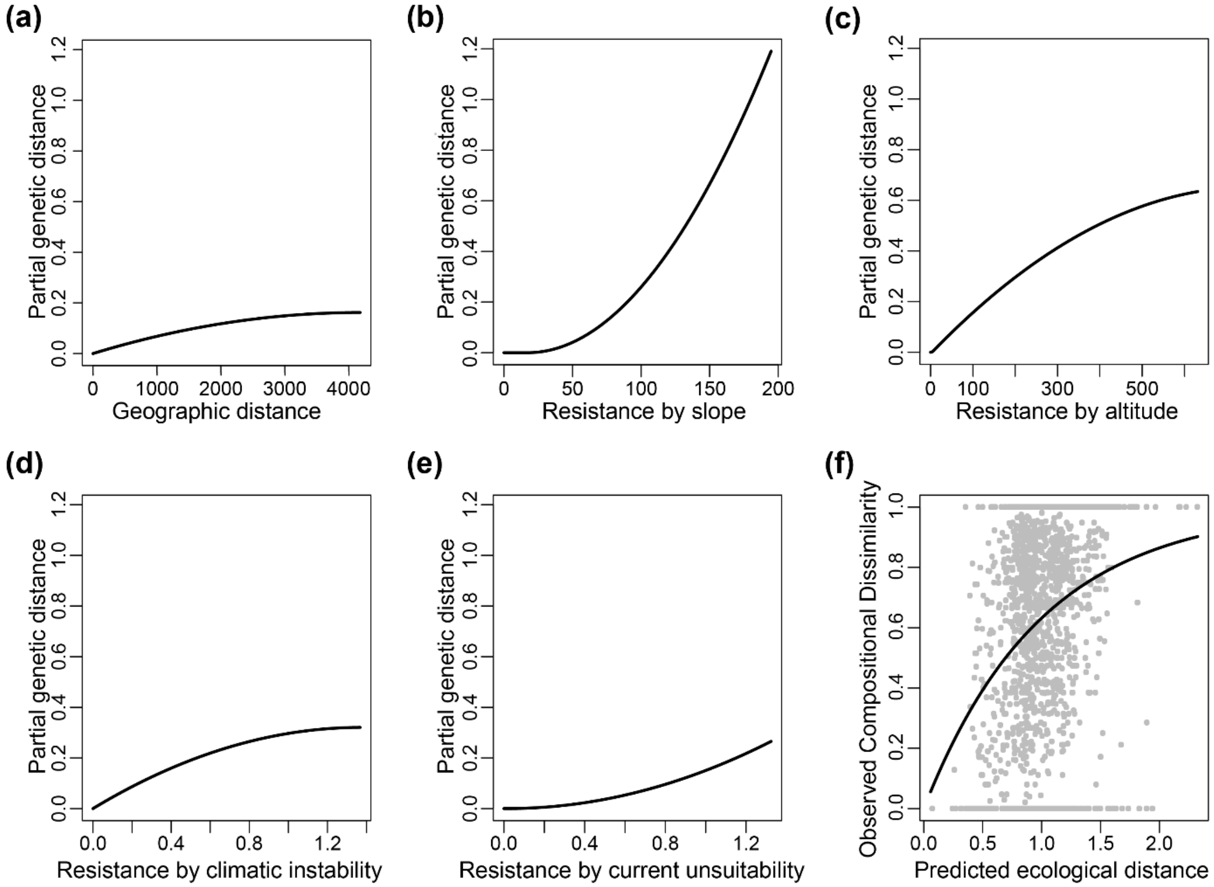

Figure 5. Generalized dissimilarity model-fitted I-splines (partial regression fit) of geographic distance (a) and environmental resistance by: slope (b), altitude (c), historical climatic instability (d) and current climatic unsuitability (e), as predictors of genetic differentiation in *Boana raniceps*. In panel *f* the relationship between observed pairwise genetic dissimilarity and the linear predictor of the GDM (predicted pairwise between-cell ecological distance).

SUPPORTING INFORMATION

Appendix S1. Details on sampled individuals, genes and primers information, and PCR profiles.

Table S1. Tissue samples from *Boana raniceps* and *B. albopunctata* (outgroup) used in our analyses, including respective institutions and voucher numbers from tissues, localities and geographic coordinates. *Museum Senckenberg Gesellschaft fur Naturforschung. Acronyms of Brazilian states are: AC = Acre, AL = Alagoas, AM = Amazonas, AP = Amapá, BA = Bahia, CE = Ceará, DF = Distrito Federal, ES = Espírito Santo, GO = Goiás, MA = Maranhão, MG = Minas Gerais, MS = Mato Grosso do Sul, MT = Mato Grosso, PA = Pará, PB = Paraíba, PE = Pernambuco, PI = Piauí, RN = Rio Grande do Norte, RO = Roraima, SE = Sergipe, SP = São Paulo, TO = Tocantins. Ar = Argentina, Bo = Bolivia, Py = Paraguay.

Species	**Institution**	**Voucher**	**Locality**	**Lat**	**Long**
B. raniceps	UFRN	AAGARDA10638	Ubajara, CE	-3.7370	-40.9029
B. raniceps	UFRN	AAGARDA10639	Ubajara, CE	-3.7370	-40.9029
B. raniceps	UFRN	AAGARDA10640	Ubajara, CE	-3.7370	-40.9029
B. raniceps	UFRN	AAGARDA11598	Quixadá, CE	-4.9634	-38.9889
B. raniceps	UFRN	AAGARDA11869	Quixadá, CE	-4.9634	-38.9889
B. raniceps	UFRN	AAGARDA11870	Quixadá, CE	-4.9634	-38.9889
B. raniceps	UFRN	AAGARDA11871	Quixadá, CE	-4.9634	-38.9889
B. raniceps	UFRN	AAGARDA11887	Quixadá, CE	-4.9634	-38.9889
B. raniceps	UFRN	AAGARDA1315	Macaiba, RN	-5.8574	-35.3607
B. raniceps	UFRN	AAGARDA3257	Caicó, RN	-6.4695	-37.0825
B. raniceps	UFRN	AAGARDA3926	Areia, PB	-6.9429	-35.6979
B. raniceps	UFRN	AAGARDA3927	Areia, PB	-6.94294	-35.6979
B. raniceps	UFRN	AAGARDA3928	Areia, PB	-6.94294	-35.6979
B. raniceps	UFRN	AAGARDA4001	Areia, PB	-6.94294	-35.6979
B. raniceps	UFRN	AAGARDA4038	Macaiba, RN	-5.85737	-35.3607
B. raniceps	UFRN	AAGARDA5075	Extremoz, RN	-5.68847	-35.2729
B. raniceps	UFRN	AAGARDA5076	Extremoz, RN	-5.68847	-35.2729
B. raniceps	UFRN	AAGARDA5078	Extremoz, RN	-5.68847	-35.2729
B. raniceps	UFRN	AAGARDA6570	Lavras, CE	-6.78217	-39.0294
B. raniceps	UFRN	AAGARDA780	Itumbiara, GO	-18.4097	-49.2163
B. raniceps	UFRN	AAGARDA783	Itumbiara, GO	-18.4097	-49.2163
B. raniceps	UFRN	AAGARDA9052	São José da Tapera, AL	-9.55907	-37.3905

B. raniceps	UFRN	AAGARDA9053	São José da Tapera, AL	-9.55907	-37.3905
B. raniceps	UFRN	AAGARDA9155	Estância, SE	-11.2458	-37.4636
B. raniceps	UFRN	AAGARDA9213	Mamanguape, PB	-6.68146	-35.1491
B. raniceps	UFRN	AAGARDA9214	Mamanguape, PB	-6.68146	-35.1491
B. raniceps	UFRN	AAGARDA9215	Mamanguape, PB	-6.68146	-35.1491
B. raniceps	UFRN	AAGARDA9216	Mamanguape, PB	-6.68146	-35.1491
B. raniceps	*M. Jansen	AS0169	Bolivia	-16.3754	-61.6554
B. raniceps	*M. Jansen	AS0218	Bolivia	-14.9569	-61.0863
B. raniceps	*M. Jansen	AS0304	Bolivia	-14.9569	-61.0863
B. raniceps	*M. Jansen	AS0468	Bolivia	-12.9922	-65.7937
B. raniceps	*M. Jansen	AS0729	Bolivia	-16.3232	-63.7845
B. raniceps	*M. Jansen	AS0739	Bolivia	-16.3754	-61.9555
B. raniceps	GeneBank	AY843657	Santa Fe, Ar	-29.4593	-60.2149
B. raniceps	UNESP	CFBH-T10014	Alta Floresta, MT	-10.0097	-56.2941
B. raniceps	UNESP	CFBH-T10015	Alta Floresta, MT	-10.0097	-56.2941
B. raniceps	UNESP	CFBH-T10748	Santa Rita Trivelato, MT	-13.784	-55.2259
B. raniceps	UNESP	CFBH-T108	Pariquera Açu, SP	-24.7098	-47.8844
B. raniceps	UNESP	CFBH-T1089	Paraíso, MS	-26.6182	-53.6735
B. raniceps	UNESP	CFBH-T109	Pariquera Açu, SP	-24.7098	-47.8844
B. raniceps	UNESP	CFBH-T110	Pariquera Açu, SP	-24.7098	-47.8844
B. raniceps	UNESP	CFBH-T11279	Gurupi, TO	-11.7293	-49.0713
B. raniceps	UNESP	CFBH-T1138	Passo de Camarajibe, AL	-9.24124	-35.4914
B. raniceps	UNESP	CFBH-T11409	Porangatu, GO	-13.4328	-49.1424
B. raniceps	UNESP	CFBH-T11419	Porangatu, GO	-13.4328	-49.1424
B. raniceps	UNESP	CFBH-T11487	Figueirópolis, TO	-12.3412	-49.4466
B. raniceps	UNESP	CFBH-T11516	Quirinópolis, GO	-18.4476	-50.4552
B. raniceps	UNESP	CFBH-T11528	Jandaia, GO	-17.1016	-50.2241
B. raniceps	UNESP	CFBH-T11537	Jandaia, GO	-17.1016	-50.2241
B. raniceps	UNESP	CFBH-T11540	Campestre de Goiás, GO	-16.8007	-49.7117
B. raniceps	UNESP	CFBH-T11545	Campestre de Goiás, GO	-16.8007	-49.7117
B. raniceps	UNESP	CFBH-T12426	Tibau do Sul, RN	-6.23417	-35.1567
B. raniceps	UNESP	CFBH-T13865	Porto Nacional, TO	-10.7028	-48.4075
B. raniceps	UNESP	CFBH-T14020	Guaraí, TO	-8.81389	-48.4934
B. raniceps	UNESP	CFBH-T14025	Darcinópolis, TO	-6.79305	-47.7243
B. raniceps	UNESP	CFBH-T14030	Darcinópolis, TO	-6.79305	-47.7243
B. raniceps	UNESP	CFBH-T14043	Wanderlândia, TO	-6.88519	-47.9268
B. raniceps	UNESP	CFBH-T14251	Porto Nacional, TO	-10.7028	-48.4075
B. raniceps	UNESP	CFBH-T14263	Gurupi/Peixe, TO	-11.7293	-49.0713
B. raniceps	UNESP	CFBH-T14451	Macauba, GO	-17.0581	-48.9105
B. raniceps	UNESP	CFBH-T14459	Porangatu, GO	-13.3346	-49.0966
B. raniceps	UNESP	CFBH-T14463	Porangatu, GO	-13.3624	-49.0884

B. raniceps	UNESP	CFBH-T14522	Porangatu, GO	-13.4976	-49.0979
B. raniceps	UNESP	CFBH-T15137	Porto Murtinho, MS	-21.7101	-57.7213
B. raniceps	UNESP	CFBH-T16295	Três Lagoas, MS	-20.7883	-51.703
B. raniceps	UNESP	CFBH-T16618	Teresina de Goiás, GO	-13.6396	-47.2533
B. raniceps	UNESP	CFBH-T1973	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T1974	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T1975	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T1980	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T2000	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T2001	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T2002	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T2004	Porto Franco, MA	-6.34141	-47.4012
B. raniceps	UNESP	CFBH-T2066	Pirapora, MG	-17.3354	-44.8981
B. raniceps	UNESP	CFBH-T2087	Angicos, RN	-5.67165	-36.6046
B. raniceps	UNESP	CFBH-T2353	Cafelândia, SP	-21.8996	-49.7499
B. raniceps	UNESP	CFBH-T2814	Dois Irmãos, TO	-6.78337	-48.0834
B. raniceps	UNESP	CFBH-T2815	Dois Irmãos, TO	-6.78337	-48.0834
B. raniceps	UNESP	CFBH-T3500	Araguaína, TO	-7.25798	-47.9855
B. raniceps	UNESP	CFBH-T3501	Araguaína, TO	-7.25798	-47.9855
B. raniceps	UNESP	CFBH-T4231	Três Lagoas, MS	-20.75985	-51.6951
B. raniceps	UNESP	CFBH-T4260	Brejo do Piauí, PI	-8.19713	-42.8331
B. raniceps	UNESP	CFBH-T4261	Brejo do Piauí, PI	-8.19713	-42.8331
B. raniceps	UNESP	CFBH-T4263	Brejo do Piauí, PI	-8.19713	-42.8331
B. raniceps	UNESP	CFBH-T4264	Brejo do Piauí, PI	-8.19713	-42.8331
B. raniceps	UNESP	CFBH-T4532	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4533	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4534	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4535	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4536	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4537	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4538	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4539	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4541	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4542	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4543	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4544	Bonito, MS	-21.1313	-56.4801
B. raniceps	UNESP	CFBH-T4588	Nioaque, MS	-21.1270	-55.8318
B. raniceps	UNESP	CFBH-T4589	Nioaque, MS	-21.1270	-55.8318
B. raniceps	UNESP	CFBH-T4590	Nioaque, MS	-21.1270	-55.8318
B. raniceps	UNESP	CFBH-T4591	Nioaque, MS	-21.1270	-55.8318
B. raniceps	UNESP	CFBH-T4592	Nioaque, MS	-21.1270	-55.8318

B. raniceps	UNESP	CFBH-T4626	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4627	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4628	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4629	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4630	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4631	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4632	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4633	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4634	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4635	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4637	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4640	Dom Aquino, MT	-15.8056	-54.9158
B. raniceps	UNESP	CFBH-T4641	Dom Aquino, MT	-15.8056	-54.9158
B. raniceps	UNESP	CFBH-T4644	Dom Aquino, MT	-15.8056	-54.9158
B. raniceps	UNESP	CFBH-T4669	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4670	Chapada dos Guimarães, MT	-15.4529	-55.7390
B. raniceps	UNESP	CFBH-T4745	Acorizal, MT	-15.2083	-56.3585
B. raniceps	UNESP	CFBH-T4746	Acorizal, MT	-15.2083	-56.3585
B. raniceps	UNESP	CFBH-T4747	Acorizal, MT	-15.2083	-56.3585
B. raniceps	UNESP	CFBH-T4748	Acorizal, MT	-15.2083	-56.3585
B. raniceps	UNESP	CFBH-T4749	Acorizal, MT	-15.2083	-56.3585
B. raniceps	UNESP	CFBH-T4778	Poconé, MT	-16.2573	-56.6221
B. raniceps	UNESP	CFBH-T4779	Poconé, MT	-16.2573	-56.6221
B. raniceps	UNESP	CFBH-T4780	Poconé, MT	-16.2573	-56.6221
B. raniceps	UNESP	CFBH-T4804	Poconé, MT	-16.2573	-56.6221
B. raniceps	UNESP	CFBH-T4991	Pariquera Açu, SP	-24.7098	-47.8844
B. raniceps	UNESP	CFBH-T4992	Pariquera Açu, SP	-24.7098	-47.8844
B. raniceps	UNESP	CFBH-T5409	Ubajara, CE	-3.8534	-40.9292
B. raniceps	UNESP	CFBH-T5410	Ubajara, CE	-3.8534	-40.9292
B. raniceps	UNESP	CFBH-T5434	Ubajara, CE	-3.8534	-40.9292
B. raniceps	UNESP	CFBH-T7598	Cruzeiro do Sul, AC	-7.6280	-72.6762
B. raniceps	UNESP	CFBH-T7599	Cruzeiro do Sul, AC	-7.6280	-72.6762
B. raniceps	UNESP	CFBH-T7600	Cruzeiro do Sul, AC	-7.6280	-72.6762
B. raniceps	UNESP	CFBH-T7827	Campo Alegre, AL	-9.7831	-36.3508
B. raniceps	UNESP	CFBH-T8213	São Félix do Araguaia, GO	-11.5946	-50.6611
B. raniceps	UNESP	CFBH-T8214	São Félix do Araguaia, GO	-11.5946	-50.6611
B. raniceps	UNESP	CFBH-T8215	São Félix do Araguaia, GO	-11.5946	-50.6611
B. raniceps	UNESP	CFBH-T8216	São Félix do Araguaia, GO	-11.5946	-50.6611
B. raniceps	UNESP	CFBH-T8219	São Félix do Araguaia, GO	-11.5946	-50.6611
B. raniceps	UNESP	CFBH-T940	Santa Fé do Sul, SP	-20.2115	-50.9272
B. raniceps	UNESP	CFBH-T941	Santa Fé do Sul, SP	-20.2115	-50.9272

B. raniceps	UNESP	CFBH-T9417	Palmares, PE	-8.67648	-35.5822
B. raniceps	UNESP	CFBH-T9748	Paranaíta, MT	-9.58973	-56.6503
B. raniceps	UNESP	CFBH-T9749	Paranaíta, MT	-9.58973	-56.6503
B. raniceps	UNESP	CFBH-T9944	E. E. Serra das Araras, MT	-15.8054	-57.2936
B. raniceps	UNESP	CFBH-T9946	E. E. Serra das Araras, MT	-15.8054	-57.2936
B. raniceps	UNESP	CFBH-T9947	E. E. Serra das Araras, MT	-15.8054	-57.2936
B. raniceps	UNESP	CFBH-T9960	Paranaíta, MT	-9.58973	-56.6503
B. raniceps	UNESP	CFBH-T9962	Cáceres, MT	-16.126	-57.715
B. raniceps	UNESP	CFBH-T9975	Alta Floresta, MT	-10.0097	-56.2941
B. raniceps	UNESP	CFBH-T9976	Alta Floresta, MT	-10.0097	-56.2941
B. raniceps	Cornell Univ./ LGE	DB 2076	General San Martín, Ar	-37.9794	-63.6023
B. raniceps	Cornell Univ./ LGE	DB 2096	General San Martín, Ar	-37.9794	-63.6023
B. raniceps	Cornell Univ./ LGE	DB 2470	Curuzú Cuatiá, Ar	-29.7915	-58.0499
B. raniceps	Cornell Univ./ LGE	DB 2472	Curuzú Cuatiá, Ar	-29.7915	-58.0499
B. raniceps	Cornell Univ./ LGE	DB 3374	San Fernando, Ar	-27.8839	-59.2833
B. raniceps	Cornell Univ./ LGE	DB 3403	San Fernando, Ar	-27.8839	-59.2833
B. raniceps	Cornell Univ./ LGE	DB 3468	Capital, Ar	-27.4733	-55.8913
B. raniceps	Cornell Univ./ LGE	DB 3469	Capital, Ar	-27.4733	-55.8913
B. raniceps	Cornell Univ./ LGE	DB 3471	Capital, Ar	-27.4733	-55.8913
B. raniceps	Cornell Univ./ LGE	DB 3497	Capital, Ar	-27.4733	-55.8913
B. raniceps	Cornell Univ./ LGE	DB 3498	Capital, Ar	-27.4733	-55.8913
B. raniceps	Cornell Univ./ LGE	DB 4373	Ituzaingó, Ar	-27.4145	-56.2655
B. raniceps	Cornell Univ./ LGE	DB 5373	San Fernando, Ar	-27.8839	-59.2833
B. raniceps	Cornell Univ./ LGE	DB 5635	San Fernando, Ar	-27.8839	-59.2833
B. raniceps	UFRN	FEPE369	Castilho, SP	-20.9935	-51.7074
B. raniceps	UFRN	FEPE397	Castilho, SP	-20.9935	-51.7074
B. raniceps	UFRN	FEPE400	Castilho, SP	-20.9935	-51.7074
B. raniceps	UFRN	FEPE401	Castilho, SP	-20.9935	-51.7074
B. raniceps	UFRN	FEPE403	Castilho, SP	-20.9935	-51.7074
B. raniceps	Cornell Univ.	IIBP1348	Paraguay	-26.7556	-55.7664
B. raniceps	Cornell Univ.	IIBP1349	Paraguay	-26.7556	-55.7664
B. raniceps	Cornell Univ.	IIBP161	Mariano Roque Alonso, Py	-25.1573	-57.3647
B. raniceps	Cornell Univ.	IIBP162	Mariano Roque Alonso, Py	-25.1573	-57.3647
B. raniceps	Cornell Univ.	IIBP163	Mariano Roque Alonso, Py	-25.1573	-57.3647
B. raniceps	Cornell Univ.	IIBP164	Mariano Roque Alonso, Py	-25.1573	-57.3647
B. raniceps	Cornell Univ.	IIBP324	Neembucú, Py	-27.2023	-58.3783
B. raniceps	Cornell Univ.	IIBP536	Capitán Bado, Py	-23.2602	-55.5402
B. raniceps	Cornell Univ.	IIBP538	Capitán Bado, Py	-23.2602	-55.5402
B. raniceps	Cornell Univ.	IIBP539	Capitán Bado, Py	-23.2602	-55.5402
B. raniceps	Cornell Univ.	IIBP711	Pozo Colorado, Py	-23.4225	-58.9339
B. raniceps	GeneBank	JF790129	Bolivia	-14.9121	-61.0825

B. raniceps	GeneBank	JF790134	Bolivia	-16.3596	-62.0001
B. raniceps	GeneBank	KF723065	Bolivia	-16.3596	-62.0001
B. raniceps	GeneBank	KF723066	Bolivia	-16.3596	-62.0001
B. raniceps	GeneBank	KF723067	Bolivia	-17.5172	-63.29
B. raniceps	GeneBank	KF723068	Bolivia	-16.3596	-62.0001
B. raniceps	Cornell Univ.	LM690	Lençois Maranhenses, MA	-2.68940	-43.0369
B. raniceps	*M. Jansen	MJ 1675	Department Santa Cruz, Bo	-16.3754	-61.9555
B. raniceps	*M. Jansen	MJ 1676	Department Santa Cruz, Bo	-16.3754	-61.9555
B. raniceps	*M. Jansen	MJ 1677	Department Santa Cruz, Bo	-16.3754	-61.9555
B. raniceps	MPEG	MPEG32925	Urucará, AM	-2.5306	-57.7543
B. raniceps	USP	MTR14409	Serra Geral do Tocantins, TO	-11.4063	-46.7252
B. raniceps	USP	HJ0159	Jirau, RO	-9.59805	-65.0478
B. raniceps	USP	H1653	UHE Jirau Abuna, RO	-9.64211	-65.4471
B. raniceps	USP	H1654	UHE Jirau Abuna, RO	-9.64211	-65.4471
B. raniceps	USP	HJ0183	Jirau, RO	-9.59805	-65.0478
B. raniceps	USP	MTR18581	Lago Chaviana/Itapuru, AM	-4.30917	-61.8152
B. raniceps	USP	MTR18584	Lago Chaviana/Itapuru, AM	-4.30917	-61.8152
B. raniceps	USP	MTR18601	Lago Chaviana/Itapuru, AM	-4.30917	-61.8152
B. raniceps	USP	MTR18664	Lago Chaviana/Itapuru, AM	-4.30917	-61.8152
B. raniceps	USP	MTR18776	Lago Chaviana/Itapuru, AM	-4.30917	-61.8152
B. raniceps	USP	MTR18965	Lago Chaviana/Itapuru, AM	-4.30917	-61.8152
B. raniceps	USP	MTR2104	UHE Jirau Mutum, RO	-9.59805	-65.0478
B. raniceps	USP	H214	Jirau, RO	-9.59805	-65.0478
B. raniceps	USP	ESTR2160	Estreito, MA	-6.56126	-47.4436
B. raniceps	USP	H2638	UHE Jirau Mutum, RO	-9.59805	-65.0478
B. raniceps	USP	H3018	UHE Jirau Mutum, RO	-9.59805	-65.0478
B. raniceps	USP	MTJ0382	Januaria, MG	-15.1811	-44.2036
B. raniceps	USP	MTJ0383	Januaria, MG	-15.1811	-44.2036
B. raniceps	USP	MTR4005	Paranã, TO	-12.6164	-47.8759
B. raniceps	USP	MTR4265	Paranã, TO	-12.6164	-47.8759
B. raniceps	USP	HJ0555	UHE Jirau Mutum, RO	-9.5981	-65.0478
B. raniceps	USP	RGA5745	UHE Guaporé, MT	-15.1167	-58.9667
B. raniceps	USP	MTR6703	UHE Lajeado, TO	-10.1168	-48.3435
B. raniceps	USP	MTR6735	UHE Lajeado, TO	-10.1168	-48.3435
B. raniceps	USP	HJ0718	UHE Jirau Abuna, RO	-9.64211	-65.4471
B. raniceps	USP	MTR7579	Guaraí, TO	-8.8139	-48.4934
B. raniceps	USP	MTR7581	Guaraí, TO	-8.8139	-48.4934
B. raniceps	USP	H803	UHE Jirau Caicara, RO	-9.4332	-64.7951
B. raniceps	USP	MTR8274	Petrolina, GO	-16.1157	-49.3247
B. raniceps	USP	H911	UHE Jirau Mutum, RO	-9.5981	-65.0478
B. raniceps	UFBA	T378	Mata de São João, BA	-12.5311	-38.3012

B. raniceps	UFBA	T417	Santa Rita de Cássia, BA	-11.0002	-44.5214
B. raniceps	UFBA	T419	Santa Rita de Cássia, BA	-11.0002	-44.5214
B. raniceps	UFBA	T420	Santa Rita de Cássia, BA	-11.0002	-44.5214
B. raniceps	UFBA	T421	Santa Rita de Cássia, BA	-11.0002	-44.5214
B. raniceps	UFBA	T557	Mata de São João, BA	-12.5311	-38.3012
B. raniceps	UFBA	T664	Camaçari, BA	-12.6937	-38.363
B. raniceps	UFBA	T665	Camaçari, BA	-12.6937	-38.363
B. raniceps	UFMS	UFMS236	Porto Murtinho, MS	-21.7019	-57.7208
B. raniceps	UFMS	UFMS237	Porto Murtinho, MS	-21.7019	-57.7208
B. raniceps	UFMS	UFMS240	Porto Murtinho, MS	-21.7019	-57.7208
B. raniceps	UFMS	UFMS241	Porto Murtinho, MS	-21.7019	-57.7208
B. raniceps	UFPB	FSCHUFPB3072	Aiuaba, CE	-6.5651	-40.2271
B. raniceps	UFPB	FSCHUFPB5723	Serra Negra do Norte, RN	-6.6607	-37.4
B. raniceps	UnB	CHUNB32540	Humaitá, AM	-7.5315	-63.0004
B. raniceps	UnB	CHUNB32545	Humaitá, AM	-7.5315	-63.0004
B. raniceps	UnB	CHUNB32547	Humaitá, AM	-7.5315	-63.0004
B. raniceps	UnB	CHUNB32550	Humaitá, AM	-7.5315	-63.0004
B. raniceps	UnB	CHUNB33669	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33672	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33673	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33676	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33682	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33684	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33695	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33724	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB33727	Alvorada do Norte, GO	-14.4861	-46.4945
B. raniceps	UnB	CHUNB42831	Corumbá, MS	-19.0098	-57.6548
B. raniceps	UnB	CHUNB42986	Tartarugalzinho, AP	1.5060	-50.9114
B. raniceps	UnB	CHUNB42987	Tartarugalzinho, AP	1.5060	-50.9114
B. raniceps	UnB	CHUNB43145	Formoso do Araguaia, TO	-11.8087	-50.1744
B. raniceps	UnB	CHUNB43148	Conceição do Araguaia, PA	-8.2648	-49.2648
B. raniceps	UnB	CHUNB43157	Couto de Magalhães, TO	-8.4159	-49.1222
B. raniceps	UnB	CHUNB43459	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB43464	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB43465	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB43466	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB43467	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB43468	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB43469	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB43948	São Domingos, GO	-13.4004	-46.3222
B. raniceps	UnB	CHUNB43950	São Domingos, GO	-13.4004	-46.3222

B. raniceps	UnB	CHUNB43954	São Domingos, GO	-13.4004	-46.3222
B. raniceps	UnB	CHUNB44293	Buritizeiro, MG	-17.3625	-44.9610
B. raniceps	UnB	CHUNB45711	Caseara, TO	-9.2709	-49.9466
B. raniceps	UnB	CHUNB45717	Caseara, TO	-9.2709	-49.9466
B. raniceps	UnB	CHUNB45721	Caseara, TO	-9.2709	-49.9466
B. raniceps	UnB	CHUNB45723	Caseara, TO	-9.2709	-49.9466
B. raniceps	UnB	CHUNB45744	Caseara, TO	-9.2709	-49.9466
B. raniceps	UnB	CHUNB45745	Caseara, TO	-9.2709	-49.9466
B. raniceps	UnB	CHUNB49471	Jaraguá, GO	-15.646	-49.4481
B. raniceps	UnB	CHUNB50920	Colinas do Tocantins, TO	-8.0990	-48.5187
B. raniceps	UnB	CHUNB50921	Minaçu, GO	-13.5304	-48.2149
B. raniceps	UnB	CHUNB51814	Carolina, MA	-7.1765	-47.2924
B. raniceps	UnB	CHUNB51815	Carolina, MA	-7.1765	-47.2924
B. raniceps	UnB	CHUNB51816	Carolina, MA	-7.1765	-47.2924
B. raniceps	UnB	CHUNB51817	Carolina, MA	-7.1765	-47.2924
B. raniceps	UnB	CHUNB52778	Colinas do Tocantins, TO	-8.0990	-48.5187
B. raniceps	UnB	CHUNB57928	Novo Santo Antônio, MT	-12.4218	-50.7619
B. raniceps	UnB	CHUNB61221	Piripiri, PI	-4.3383	-41.8402
B. raniceps	UnB	CHUNB64405	Tocantinópolis, TO	-6.3249	-47.4228
B. raniceps	UnB	CHUNB65083	Nossa Senhora do Liv., MT	-15.9001	-56.2926
B. raniceps	UnB	CHUNB67413	Pirenópolis, GO	-15.8511	-48.9589
B. raniceps	UnB	CHUNB67414	Pirenópolis, GO	-15.8511	-48.9589
B. raniceps	URCA	URCA1548	Guaramiranga, CE	-4.2686	-38.9336
B. raniceps	URCA	URCA4939	Farias Brito, CE	-6.9261	-39.5733
B. raniceps	URCA	URCA5864	Caldeirão Grande do Piauí, PI	-7.3318	-40.637
B. raniceps	URCA	URCA6567	Lavras, CE	-6.7822	-39.0294
B. aff. albopunctata	UFRN	AAGARDA765	Pirenópolis, GO	-15.89	-48.96

Table S2. Genes and primers used for amplification and sequencing.

Gene	Primers	Sequence (5`-> 3`)	Annealing temperature	Citation
16S	16Sa-L	CGCCTGTTTATCAAAAACAT	50°C	(Palumbi et al., 1991)
	16Sb-H	CCGGTCTGAACTCAGATCACGT		
ND1	tMet-frog	TTGGGGTATGGGCCCAAAAGCT	57.4°C	(Wiens et al., 2005)
	L2507	CCCCGCCTGTTTACCAAAAA		(Macey et al., 2001)
	ND1-int-ACA	ACGTGATCTGAGTTCAGACCG		(Robertson and Zamudio, 2009)
β-Fib intron7	FIB-B17U	GGAGAAAACAGGACAATGACAATTCAC	56°C	(Prychitko and Moore, 1997)
	FIB-B17L	TCCCATATATCTGCCATTAGGGTT		
RPL3 intron5	RPL35F	AAGAAGTCYCACCTCATGGAGAT	55°C	(Pinho et al., 2010)
	RPL36RA	AGTTTCTTCTTTGTGTGCCAACGGCTAG		
POMC	POMC_DRV_F1	ATATGTCATGASCCAYTTYCGCTGGAA	57-58°C	(Vieites et al., 2007)
	POMC_DRV_R1	GGCRTTYTTGAAWAGAGTCATTAGWGG		
Tyrosinase	TyrC	GGCAGAGGAWCRTGCCAAGATGT	57-58°C	(Bossuyt and Milinkovitch, 2000)
	TyrG	TGCTGGCRTCTCTCCARTCCCA		

Appendix S2: Supplementary figures

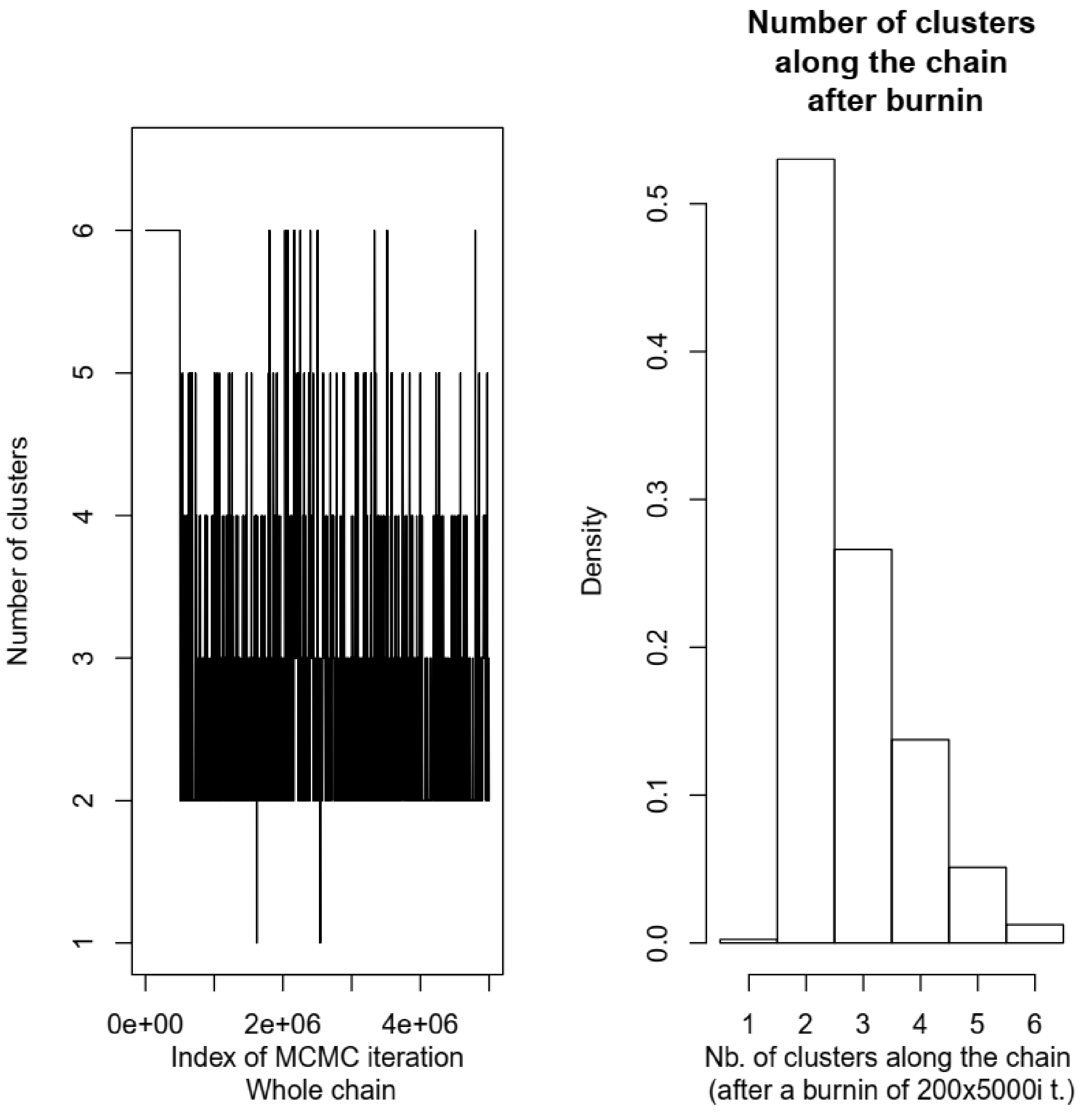

Figure S1. GENELAND results showing the number of clusters along the MCMC run (left) and number of clusters along the chain (right).

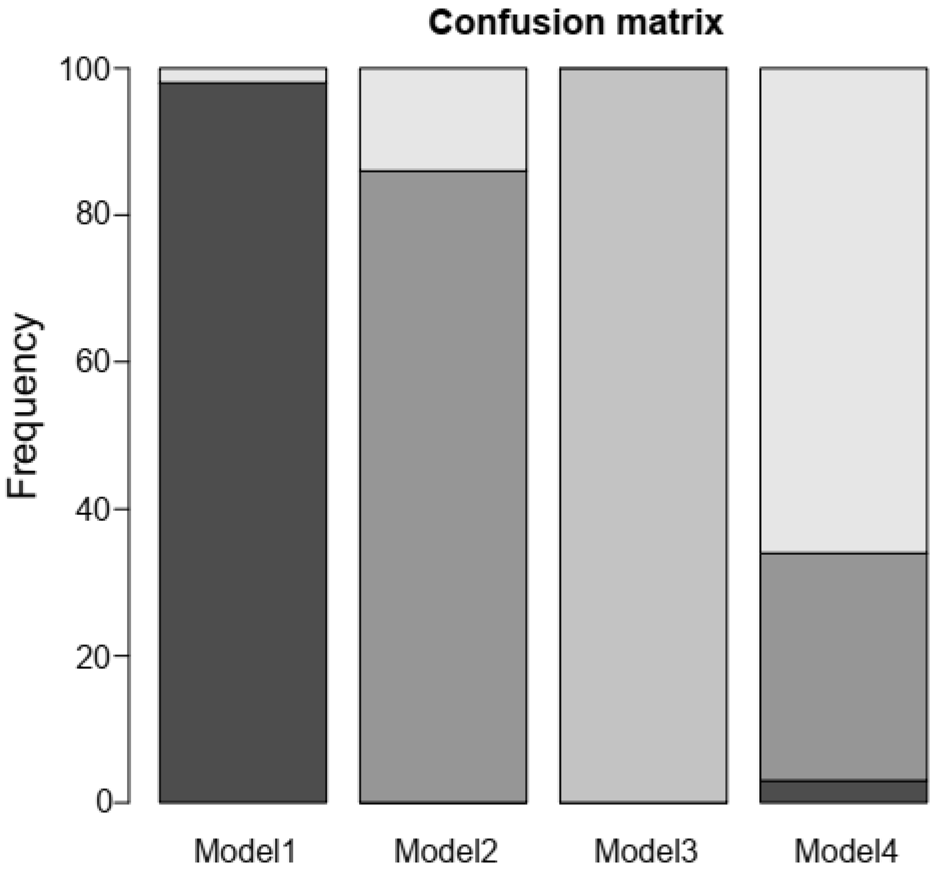

Figure S2. Misclassification proportions for models 1 to 4. The panel represents model accuracy using Mnlogistic. Tolerance rate = 0.01. Values for each model: Model 1 = 0, Model 2 = 0.075, Model 3 = 0.925 and Model 4 = 0.

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