Instituto Nacional de Pesquisas da Amazônia – INPA
Programa de Pós-Graduação em Ecologia

ALLOBATES FEMORALIS COMO MODELO PARA CARACTERIZAR GRADIENTES GEOGRÁFICOS SUTIS E NÍTIDOS NA BACIA AMAZÔNICA

ANTHONY SANTANA FERREIRA

Manaus, Amazonas
Dezembro 2018

ANTHONY SANTANA FERREIRA

ALLOBATES FEMORALIS COMO MODELO PARA CARACTERIZAR GRADIENTES GEOGRÁFICOS SUTIS E NÍTIDOS NA BACIA AMAZÔNICA

Orientadora: Dra. Albertina Pimentel Lima (INPA)
Co-Orientador: Dr. Robert Jehle (University of Salford)

Tese apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Doutor em Biologia (Ecologia).

Manaus, Amazonas
Dezembro 2018

PG·ECO·INPA
PÓS-GRADUAÇÃO EM ECOLOGIA

MINISTÉRIO DA
CIÊNCIA, TECNOLOGIA,
INOVAÇÕES E COMUNICAÇÕES

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Aos 03 dias do mês de dezembro do ano de 2018, às 14:00 horas, no Auditório dos PPG's ATU/CFT/ECO, Campus III, INPA/V8. Reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Daniel Oliveira Mesquita**, da Universidade Federal da Paraíba - UFPB, o(a) Prof(a). Dr(a). **Christine Strüssmann,** da Universidade Federal de Mato Grosso - UFMT, o(a) Prof(a). Dr(a). **Igor Luis Kaefer**, da Universidade Federal do Amazonas - UFAM, o(a) Prof(a). Dr(a). **Rafael do Nascimento Leite**, do Instituto Nacional de Pesquisas da Amazônia - INPA, o(a) Prof(a). Dr(a). **Alexandre Camargo Martensen,** da Universidade Federal de São Carlos - UFSCar, tendo como suplentes o(a) Prof(a). Dr(a). Mateus Ferreira, do Instituto Nacional de Pesquisas da Amazônia - INPA, e o(a) Prof(a). Dr(a). Adrian Paul Ashton Barnett, do Instituto Nacional de Pesquisas da Amazônia - INPA, sob a presidência do(a) primeiro(a), a fim de proceder a arguição pública do trabalho de **TESE DE DOUTORADO** de **ANTHONY SANTANA FERREIRA**, intitulado ***"ALLOBATES FEMORALIS*** **COMO MODELO PARA CARACTERIZAR CLINES GEOGRÁFICOS NÍTIDOS NA BACIA AMAZÔNICA"**, orientado pelo(a) Prof(a). Dr(a). Albertina Pimentel Lima, do Instituto Nacional de Pesquisas da Amazônia – INPA e coorientado pelo(a) Prof(a). Dr(a). Robert Jehle, University of Salford.

Após a exposição, o(a) discente foi argüido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

☒ APROVADO(A) ☐ REPROVADO(A)
☒ POR UNANIMIDADE ☐ POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a).Dr(a). Daniel Oliveira Mesquita ____________________
Prof(a).Dr(a). Christine Strüssmann Strüssman
Prof(a). Dr(a). Igor Luis Kaefer Igor Luis Kaefer
Prof(a) Dr(a). Rafael do Nascimento Leite ____________________
Prof(a). Dr(a). Alexandre Camargo Martensen ____________________
Prof(a). Dr(a). Mateus Ferreira ____________________
Prof(a). Dr(a). Adrian Paul Ashton Barnett ____________________

____
Coordenação PPG-ECO/INPA

INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA – PPG ECO
Av. André Araújo, nº 2936, Bairro – Petrópolis, Manaus-AM, CEP: 69.067-375
Site: http://pg.inpa.gov.br e-mail: pgecologia@gmail.com

AGRADECIMENTOS

Agradeço ao **INPA** e ao Programa de Pós-Graduação em Ecologia (**PG-ECO**) por todo apoio oferecido durante os quatro anos do meu doutorado.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico – **CNPq** pela concessão da minha bolsa, incluindo a taxa de bancada, que foi fundamental para o desenvolvimento do projeto de Doutorado. Parte desta tese foi financiada através do Programa Ciência sem Fronteiras (CNPq/processo: 401327/2012-4) e pela fundação de Amparo à Pesquisado Estado do Amazonas (FAPEAM/CNPq PRONEX processo: 653/2009). A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - **CAPES** pela bolsa concedida do Programa de Doutorado Sanduíche no Exterior (processo: 88881.133683/2016-01).

A minha querida orientadora, **Albertina P. Lima**, pela amizade e confiança. Mesmo sem me conhecer, aceitou me orientar me dando a oportunidade de realizar meu sonho de conhecer e pesquisar na Amazônia. Meu muito obrigado pela honra, por transmitir seus conhecimentos e por todo seu apoio, principalmente nos momentos mais difíceis dessa trajetória.

Aos amigos e companheiros de laboratório da herpeto/INPA, **Miquéias Ferrão**, **Rafael de Fraga**, **Rafael Jorge**, **Randolpho Terceiro**, **Jussara Dayrell**, **André Barros**, **Diana Rojas-Ahumada**, **Diego Ortiz**, **Romildo Souza**, **Douglas Lacerda** e **Kamila Xavier**, pelo companheirismo nessa jornada, ótima convivência, amizade, boas troca de ideias e ajuda no que fosse possível.

Ao meu co-orientador, **Robert Jehle** por não ter poupado esforços para me orientar a distância, estando sempre disposto a contribuir com o projeto e pelas excelentes revisões nos capítulos desta tese.

Ao meu supervisor no doutorado sanduíche, **Adam Stow** por ter me recebido de braços abertos na Macquarie University e pacientemente ter me ajudado a compreender as análises genômicas. Além de ser um ótimo anfitrião me levando para seu sítio, onde pude conhecer belíssimas praias australianas. Obrigado também a Dra. **Rachael**

Dudaniec da Macquarie University pelas explicações e script sobre genética de paisagens.

Aos colegas do laboratório de evolução da Macquarie University, **Sonu Yadav**, **Alexander Carey**, **Jessica Thompson**, **Kayt** e **Sarah Reid-Anderson** pela ótima recepção e por ter tornado o curto período de muito trabalho na Austrália mais agradável e divertido.

Aos amigos internacionais que fiz durante minha estadia em Sydney/Austrália, **Kazuki Tachibana**, **Jaya Surya**, **Armand Gauvrit**, **John Haiti**, **Thomas Toucheboeuf**, **Qiran Shao**, **Hassam Al-Kshei**, **Heeju Jung**, **Hyunjii Lee**, **Evelyn Jing Ling** e **Afrin Shairaj** que mesmo sabendo que eu não tinha muito “tempo a perder” sempre estavam dispostos a me tirar do trabalho e fazer turismo nos finais de semana. Foi um prazer ter conhecido as diversas culturas e a história de vida de cada um e pelos ótimos momentos vividos com vocês!

Ao pessoal do Laboratório Temático de Biologia Molecular (**LBTM**) do INPA, especialmente **Mateus Ferreira**, pelo treinamento das atividades laboratoriais, o que me ajudou a entender todo o processo.

Aos pesquisadores **Fernanda Werneck**, **Paulo Bobrowiec**, **Igor Kaefer**, **Rafael Leite** e **Elizabeth Franklin** pela participação na qualificação do projeto de tese e pelas contribuições feitas.

Aos professores **Bill Magnusson**, **Camila Ribas**, **Rafael Leite**, **Fernando Figueiredo**, **Marina Anciães**, **Arley Camargo**, **Albertina Lima** e **Fabrício Baccaro** pelos ensinamentos nas disciplinas que cursei durante o doutorado.

As **secretárias do PG-Ecologia do INPA** e do **CENBAM** pelo ótimo trabalho e prontidão em resolver questões burocráticas da Pós-Graduação e de outros assuntos.

Ao **PPBio-CENBAM** e toda a equipe pelo apoio logístico e financeiro na coleta de dados no interflúvio Purus-Madeira.

Aos ajudantes de campo **Pinduca** e **João Souza** (Joãozinho) por terem me acompanhado e auxiliado nas coletas de *Allobates femoralis* na "selva amazônica". As experiências de campo que vivenciamos, especialmente na BR-319, foram perigosas, porém incríveis.

Aos amigos e colegas manauaras (muitos!), e também aos de fora (assim como eu) que conheci ao longo desses quatro anos, pela amizade e por todos os momentos de descontração. Vocês me ajudaram a sobreviver ao stress da pós-graduação e foram fundamentais para eu manter a sanidade mental.

Minha gratidão ao meu modelo de estudo ***Allobates femoralis*** que no início das coletas me deixou com muita raiva por ser muito arisco e difícil de coletar, mas com prática e paciência se tornou fácil e encantador trabalhar com essa espécie.

Muito obrigado a minha família, aos meus amados pais **Antônio** e **Neuza** e irmãs **Tahinara** e **Taheana**, por todo amor, pelo apoio incondicional nas minhas escolhas e por terem me incentivado ao longo da minha formação acadêmica, mesmo minhas irmãs zombarem por eu ter escolhido essa vida!

Por fim, e mais importante, agradeço a **Deus** pelo dom da vida e ser minha base sólida de espiritualidade e paz.

Esta tese é resultado da colaboração de muitas pessoas, que me ajudaram direta ou indiretamente de diversas formas (não citadas aqui). Meus mais sinceros agradecimentos a todos vocês!!

F383 Ferreira, Anthony Santana
Allobates femoralis como modelo para caracterizar gradientes geográficos sutis e nítidos na bacia amazônica / Anthony Santana Ferreira. – 2018.
132p.

Tese (Doutorado) - INPA, Manaus, 2018.
Orientadora: Albertina Pimentel Lima
Coorientador: Robert Jehle
Área de concentração: Ecologia

1-*Allobates femoralis* 2-Interflúvio Purus-Madeira 3-Heterogeneidade ambiental 4-Hipótese dos Gradientes 5-Genética de paisagens 6-SNPs 7-Amazônia

CDD 597.80415

Sinopse

Nesta tese investigamos o papel de gradientes ambientais na distribuição, abundância, variabilidade genética e fluxo gênico de *A. femoralis* ao longo de 880 Km de floresta de terra-firme no interflúvio Purus-Madeira (IPM). Testamos se as características do solo e da floresta estão associadas com a distribuição e abundância relativa de *A. femoralis* usando um sistema de amostragens padronizadas. Foi aplicado uma abordagem de modelagem multinível baseada em dados genômicos para testar se o gradiente geográfico e ambiental ao longo da paisagem heterogênea do IPM influencia a variabilidade genética e o fluxo gênico dessa espécie.

Palavras chave: *Allobates femoralis*, interflúvio Purus-Madeira, heterogeneidade ambiental, hipótese dos gradientes, genética de paisagens, SNPs, Amazônia.

“O pensamento, quando apoiado por um forte desejo, tem a tendência de se transformar em seu equivalente físico”

Napoleon Hill

RESUMO

Está bem estabelecido que a ocorrência das espécies depende das suas necessidades biológicas em determinados ambientes, e que as espécies estão distribuídas em manchas de habitats favoráveis que são separadas umas das outras por habitats inadequados. No entanto, determinar como a heterogeneidade do habitat interage com a distribuição e a densidade das espécies em várias escalas geográficas e como os processos históricos e atuais do ambiente influenciam o fluxo gênico ao longo da paisagem continua sendo um dos maiores desafios da ecologia e biologia evolutiva. Esse tipo de abordagem ainda é raro na Amazônia, especialmente quando se trata de anuros. O objetivo geral desta tese foi investigar o papel de gradientes ambientais na distribuição, abundância, variabilidade genética e fluxo gênico de *Allobates femoralis* ao longo de 880 km de paisagens heterogêneas que ocorrem no interflúvio entre os rios Purus e Madeira (IPM). No **primeiro capítulo**, testamos se as características do solo e da floresta estão associadas com a distribuição e abundância relativa de *A. femoralis* através do IPM. Nós mostramos que *A. femoralis* é mais abundante em florestas abertas e ausente ou raro em florestas densas e positivamente associado com solos ricos em argila. Nossos dados sugerem que a distribuição e abundância relativa dessa espécie é moldada por gradientes ecológicos graduais. No **segundo capítulo**, aplicamos uma abordagem de modelagem multinível baseada em dados genômicos e teoria dos circuitos para testar se a distância geográfica e o gradiente ambiental ao longo da paisagem heterogênea do IPM influenciam a variabilidade genética e o fluxo gênico de *A. femoralis*. Nós mostramos que os padrões espaciais de variabilidade genética e o fluxo gênico em *A. femoralis* são influenciados tanto pela distância geográfica quanto pelos gradientes ambientais, suportando assim a hipótese dos gradientes para diversificação. Nós também identificamos quatro grupos genéticos que foram associados com variações fenotípicas (cor da mancha femoral), forte divergência na parte nordeste do IPM que está relacionada a processos históricos e com a presença de diferentes tipos de floresta. Sugerimos que investigando o papel da heterogeneidade ambiental em outras espécies co-distribuídas irá fornecer dados que podem ser usados para melhor entender o papel de gradientes ambientais na diversificação parapátrica de espécies na Amazônia.

ABSTRACT

It is well established that the occurrence of species depends on their biological needs in given environments, and that species are distributed to favorable habitat patches which are separated from each other by unsuitable habitats. However, determining how habitat heterogeneity interacts with the distribution and density of species at various geographic scales and how historical processes and the contemporary environment has influenced gene flow across landscapes remains one of the major challenges in ecology and evolutionary biology. This type of approach is still rare in Amazonia, especially when it comes to anurans. The main goals of this thesis were to investigate the role of environmental gradients on the distribution, abundance, genetic variability and gene flow of *Allobates femoralis* along 880 km of heterogeneous landscape in the interfluve between the Purus and Madeira rivers (PMI). In the **first chapter**, we tested whether soil and forest characteristics are associated with the distribution and relative abundance of *A. femoralis* along PMI. We show that *A. femoralis* is more abundant in open forests and absent or rare in dense forests and positively associated with clay-rich soils. Our findings suggest that the distribution and relative abundance of this species is shaped by gradual ecological clines. In the **second chapter**, we apply a multilevel modeling approach based on genomic data and circuit theory to test whether geographic distance and environmental gradients across the heterogeneous landscape of PMI influences the genetic variability and gene flow of *A. femoralis*. We show that spatial patterns of genetic variability and gene flow in *A. femoralis* are influenced by both geographic distance and environmental gradients, thus supporting the gradient hypothesis for diversification. We also identified four genetic clusters which partly paralleled phenotypic variation (femoral spot colour), strong divergence in the northeastern part of the PMI that is likely due to historical processes, and with the presence of different forest types. We suggest that investigating the role of environmental heterogeneity in other codistributed species will provide data that can be used to better understand the role of environmental gradients in the parapatric diversification of species in the Amazon.

SUMÁRIO

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

Table 4. Summary of model selection, maximum likelihood population effects mixed effects models (MLPE) and distance-based redundancy analyses (dbRDA) showing the effects of isolation by resistance (IBR) on gene flow in *Allobates femoralis* along the Purus-Madeira interfluve.

LISTA DE FIGURAS

CAPÍTULO 1

Figure 1. Purus-Madeira interfluve showing module locations (M1–M14), and the two main types of forest phytophysiognomy: lowland dense rainforest to the northeast (dark green) and lowland open rainforest to the southwest (light green). Red circles indicate the presence of *A. femoralis* with sizes representing relative abundances; blue circles indicate absence of *A. femoralis*. The scale of the symbols represents the range of means of the relative abundance of *A. femoralis* in the modules when present. The modules are approximately 50–60 km apart, with the exception of the distance between M11 and M12 which are separated by open transition forest and savanna. Map created in QGis 2.14 Essen (http://qgis.osgeo.org).

Figure 2. Median, quartiles and maximum and minimum values of *A. femoralis* relative abundance (sum of individuals per plot across all samplings taking detectability into account) in the research modules along the Purus-Madeira interfluve. Green bars (M1–M10) represent modules in the lowland dense rainforest, and orange bars (M11–M14) represent modules in the lowland open rainforest.

Figure 3. Median (black vertical line), quartiles and maximum and minimum values of (A) basal area; (B) number of trees; (C) silt content and (D) clay content across 14 research modules along the Purus-Madeira interfluve.

Figure 4. Partial regressions derived from generalized linear mixed-effects models (GLMMs) investigating the effects of (A) silt content, (B) basal area, (C) clay content and (D) number of trees per plot on *A. femoralis* relative abundance taking detectability into account in forests along the Purus-Madeira interfluve. Fitted lines indicate fixed-effect probabilities < 0.05 in the linear mixed-model analyses.

Figure 5. Relationship between mean *A. femoralis* relative abundance and (A) silt content, (B) clay content, (C) basal area and (D) number of trees per module, taking detectability into account and excluding the outlier M11 (open circles; see the main text for more details). The relationships were statistically significant (percentage of silt: r^2=

0.69, $p < 0.001$; percentage of clay: $r^2 = 0.66$, $p < 0.001$; basal area: $r^2 = 0.63$, $p < 0.001$ and number of trees: $r^2 = 0.47$, $p < 0.01$).

CAPÍTULO 2

Figure 1. Map of sites at which samples of *Allobates femoralis* were collected in the central-southern Amazonia, Brazil. For results from population structure analyses see Figure 3 and sample sizes see Table 3.

Figure 2. Shapes of the isolation-by-resistance (IBR) relationships tested for the effect of temperature seasonality and vegetation-cover type on genetic distance $F_{ST}/(1-F_{ST})$ using seven values of γ (0.01, 0.1, 0.5, 1, 5, 10, 100). The different slopes are not shown (α values) and is set at 5 here for simplicity. The curves show decreasing landscape resistances from right to left for vegetation-cover type (A) and left to right for temperature seasonality (B).

Figure 3. Barplots of population structure analyses conducted for *Allobates femoralis* along Purus-Madeira interfluve in central-southern Amazonia, resulting from SNPs. A) ADMIXTURE, B) sNMF and C) DAPC. Sampling locations and the most probable number of genetic clusters (k) is provided. Each genetic cluster is assigned to a different color, and each bar represent an individual with the proportion of their admixed genome ($p < 0.05$), except to DAPC.

Figure 4. Results of the DAPC analysis among geographic populations of *Allobates femoralis* using 10 275 SNPs. Custer A (green circles), samples from M6-M8; cluster B (yellow circles), samples from B8_9-M11 and Cluster C (blue circles), samples from M12-M14.

Figure 5. Patterns of gene flow based on correlation between genetic distance $F_{ST}/(1-F_{ST})$ and geographic distance, indicating isolation by geographic distance in *A. femoralis* across Purus-Madeira interfluve.

Figure 6. Spatial autocorrelation of Mantel calculated for seven classes of geographic distance (km) over genetic distances *F*st/(1-*F*st) in *Allobates femoralis* across Purus-Madeira interfluve. Black squares indicate significant distance classes ($p \leq 0.05$).

INTRODUÇÃO GERAL

Ao longo das décadas, diversas hipóteses foram propostas para tentar explicar a diversificação e bioregionalização de organismos amazônicos, entre eles, hipótese dos rios como barreiras (Wallace 1852; Gascon *et al.* 2000), hipótese dos refúgios pleistocênicos (Haffer 1969; 1974), gradientes ecológicos (Endle 1977; 1982), arcos tectônicos (Bush, 1994), dinâmica geológica e hidrológica da bacia (Hoorn 1994; Hoorn & Wesselingh 2010), ilhas e incursões marítimas do Terciário Superior (Rasanen *et al.* 1995; Lovejoy *et al.* 1998; Nores 1999) e estocasticidade demográfica (Cohn-Haft 2000). Dentre estas, as hipóteses alternativas mais exploradas envolviam especiação alopátrica por vicariância, ou seja, barreiras físicas teriam isolados espacialmente as populações e o fluxo gênico entre elas, dando origem a novas espécies (como a hipótese dos rios como barreiras; Wallace 1852). Embora alguns estudos tenham encontrado concordância com o padrão de distribuição e diferenciação genética de vários táxons associado às porções de terras separadas pelos grandes rios Amazônicos (Hayes & Sewlal 2004; Funk *et al.* 2007; Ribas *et al.* 2012; Fernandes *et al.* 2014; Boubli *et al.* 2015; Dias-Terceiro *et al.* 2015; Ortiz *et al.* 2018; Ribas *et al.* 2018), esse padrão não foi verificado para todos os rios, ou para todas as espécies (Gascon *et al.* 1996; 1998; Lougheed *et al.* 1999; Gascon *et al.* 2000; Symula *et al.* 2003; Aleixo 2004; Hughes *et al.* 2013). Sendo que as hipóteses que envolve especiação alopátrica ainda é objeto de debate (ver Collinvaux *et al.* 2000; Oliveira *et al.* 2017; Santorelli *et al.* 2018).

Por outro lado, outros estudos mostraram padrões de distribuição e variabilidade genética heterogêneas entre populações ao longo de paisagens contínuas ou clines geográficos (hipótese dos gradientes; Endle 1977). Nesse cenário, as populações diferenciam-se seguindo um modelo de isolamento por distância, muitas vezes facilitado pela adaptação ecológica a ambientes adjacentes, mas diferentes e ainda podendo ocorrer fluxo gênico entre populações divergentes (Moritz *et al.* 2000; Nosil 2012; Leite & Rogers 2013; Ortiz *et al.* 2018). Desta forma, a capacidade de migração das espécies depende de processos de dispersão, que por sua vez depende de características das espécies, por exemplo, modos de dispersão e reprodução e características espaciais do habitat, como conectividade e grau de resistência ambiental (Opdam & Wascher 2004). Essas ideias influenciaram novas e mais complexas subdivisões ecológicas da Amazônia (Sombroek 2000; Fine *et al.* 2005; Baker *et al.* 2014; Smith *et al.* 2014; Dexter *et al.* 2017) e a extensiva coleta de dados bióticos e abióticos que vem acontecendo nas últimas décadas têm confirmado a existência de uma grande heterogeneidade ambiental (Quesada *et al.* 2010; Lewis *et al*. 2011; Cintra *et al*. 2013; Martins *et al*. 2014; Schietti *et al*. 2016) e reforçado a importância de filtros ecológicos para a estruturação espacial da diversidade e composição da biota amazônica.

Desta forma, a hipótese da heterogeneidade de habitat se estabeleceu como uma das pedras angulares da ecologia (MacArthur & Wilson 1967; Lack 1969; Pinto *et al*. 2003; Tews *et al*. 2004), tendo sido responsável pela mudança da aceitação da homogeneidade para o reconhecimento da heterogeneidade como chave para a compreensão da complexidade da natureza (Wiens 1989). Segundo Legendre & Fortin (1989), na natureza os organismos não se distribuem nem aleatória, nem uniformemente, mas em manchas ou gradientes, ou seja, heterogeneamente. A heterogeneidade espacial pode ser entendida como a expressão de atributos estruturais do ambiente, incluindo componentes horizontais, verticais e qualitativos que permitem a presença de micro-habitats diferenciados em uma área (Pianka 1966; Tews *et al*. 2004).

Atualmente o reconhecimento da influência da dimensão espacial nos padrões ecológicos e evolutivos é apontado como um novo paradigma da ecologia (Pinto *et al*. 2003), cujo tema central envolve a compreensão da influência do ambiente sobre processos biológicos em distintas escalas (Legendre 1993; Gaston & Blackburn 2003; Ricklefs 2004; Tuomisto 2007). As variações nas características ambientais produzem condições que influenciam a distribuição espacial e densidades dos organismos (Ricklefs 2003; Pough *et al*. 2003), de modo que os fatores que tornam um habitat favorável para determinada espécie precisam atuarem diretamente sobre as necessidades biológicas e fisiológicas das espécies. Isso é consequência do fato de que os processos ecológicos e evolutivos ocorrem em um contexto histórico, geográfico, através da adaptação, seleção e deriva genética (Endler 1977; Diniz-Filho *et al*. 2008). Os processos que geram a diversidade intra e interespecífica podem ser considerados em diferentes escalas temporais, caracterizando então os processos atuais ou ecológicos e históricos ou evolutivos (Avise 2000; Moritz *et al*. 2000; Tuomisto 2007; Morrone 2009).

Além das condições ambientais, os fenômenos biogeográficos históricos e as taxas diferenciais de especiação também podem influenciar o padrão de distribuição das espécies (Zimmerman & Simberloff 1996), separando as áreas de endemismo e propondo um padrão de distribuição não visto em nenhum outro lugar do mundo

(Cracraft 1985; Borges 2007; Borges & Silva 2012). As condições ambientais são fatores abióticos que influenciam a distribuição das espécies em macroescala (Duellman 1999). Em mesoescala, tanto fatores bióticos como abióticos podem influenciar os padrões observados (Duellman 1995; 1999; Menin *et al*. 2007; Drucker *et al*. 2008).

Na floresta amazônica fatores ambientais influenciam a ocorrência, distribuição, densidade e atividade local dos anuros (Duellman & Trub 1986; Zimmerman & Bierregaard 1986; Duellman 1988; 1995; Menin *et al*. 2007; Menin *et al*. 2011; Ribeiro *et al*. 2012; Rojas-Ahumada *et al*. 2012; Jorge *et al*. 2016; Ferrão *et al*. 2018). A partilha espacial inclui a exploração de uma grande diversidade de microambientes como sítios reprodutivos (Hödl 1990; Zimmerman & Rodrigues 1990). As espécies de anuros com reprodução terrestre podem apresentar uma distribuição mais ampla, sendo encontradas ao longo de todo o gradiente ambiental, com alguns fatores determinando uma maior abundância (Menin *et al*. 2007). Enquanto as espécies com reprodução aquática apresentam uma distribuição mais restrita, sendo encontradas principalmente em zonas ripárias, pois possuem maior dependência de corpos d'água (Duellman & Trub 1986; Hödl 1990; Haddad & Prado 2005; Rojas-Ahumada *et al*. 2012). Porém, esses estudos que relacionaram fatores ambientais à distribuição e densidade de anuros na Amazônia foram limitados a pequenas e médias escalas, e a maior parte deles foram realizados na Amazônia central.

Investigar as relações entre a estrutura genética das populações e os fatores ambientais em uma estrutura de distribuição espacial ao longo de gradientes ambientais é essencial para identificar as características de dispersão, extensão e escala que modulam as forças que influenciam o fluxo gênico e evidências de adaptação local de espécies particulares (Stow *et al*. 2001; Nielsen 2005; Lowe & Allendorf 2010; Savolainen *et al*. 2013; Wang & Bradburd 2014; Benestan *et al*. 2016). E para entender os processos que impulsionam a distribuição espacial da diversidade genética intraespecífica, os fatores que influenciam essa distribuição devem ser investigados em diferentes escalas, considerando a história recente e a configuração atual da paisagem, uma vez que os padrões regionais acabam sendo um resultado de processos locais (Wiens & Donoghue 2004; Rull 2011). Estudos abrangendo grandes escalas podem revelar associações espécies-habitat que não seria possível em escalas menores. Nesse contexto, as terras baixas amazônicas são especialmente desafiadoras, com uma história geológica recente e dinâmica associada à transição de um ecossistema pantanoso dominado por um sistema fluvial, e drenado por rios historicamente dinâmicos, com registros de grandes mudanças espaciais recentes em seus cursos (Fittkau *et al.* 1975; Sombroek 2000; Rossetti *et al*. 2005; Hoorn *et al*. 2010; Smith *et al*. 2014), e devido a heterogeneidade ambiental ao longo de sua extensão (Cintra *et al*. 2013; Martins *et al*. 2014; Schietti *et al*. 2016), se mostra ideal para investigar as influencias de gradientes ambientais na diferenciação genética de organismos.

Como resultado da importância dessas questões, a última década viu uma proliferação de métodos quantitativos que combinam modelagem de paisagem com dados genéticos que são mais sensíveis para avaliar como os fatores da paisagem influenciam a estrutura genética espacial, dispersão e fluxo gênico dentro e entre populações (Dudaniec *et al*. 2013; Peterman *et al*. 2014; Marrote *et al*. 2014; Manthey & Moyle 2015; Dudaniec *et al*. 2016; Row *et al*. 2017). Assim como o potencial poder explicativo de tal investigação foi substancialmente aprimorado pelo desenvolvimento de ferramentas genômicas cada vez mais acessíveis para sequenciamento de próxima geração (*NGS – Next Generation Sequencing*), baseado em milhares de polimorfismos de nucleotídeo único (SNPs), que permitem estimativas confiáveis de estrutura genética usando poucos indivíduos (Willing *et al*. 2012; Lemmon & Lemmon 2013; Willette *et al*. 2014; McKinney *et al*. 2016), no entanto, esta abordagem tem sido raramente aplicada a táxons de floresta tropical (Radespiel & Bruford 2014; Ruiz-Lopez *et al*. 2016; Fraga *et al*. 2017).

Desta forma, *Allobates femoralis* como seu comportamento sedentário e movimento aparentemente restrito, com indivíduos distribuídos em manchas, aliadas a grande abundância e fácil amostragem, é um excelente modelo para investigar como os gradientes ambientais interferem na distribuição, densidade, estruturação genética e troca de fluxo gênico em diferentes escalas espaciais. *Allobates femoralis* apresenta uma ampla distribuição, mas até o momento não havia sido estudado o que determina a presença da espécie nas manchas de paisagem e nem que fatores locais determinam a abundância da espécie nos ambientes onde está presente. A ausência de uma base de dados moleculares e de amplas amostragens também haviam impossibilitado inferências mais expressivas sobre as relações evolutivas e fluxo gênico das linhagens que compõe esse sistema. Assim, estudos abrangendo grandes extensões da paisagem e em diferentes escalas espaciais (ampla, média e local), podem revelar padrões inéditos, e o delineamento amostral com sítios de coleta padronizados ao longo de 880 km no interflúvio Purus-Madeira se mostra ideal para entender o papel de gradientes ambientais na distribuição, abundância, variabilidade genética e fluxo gênico de espécies de anuros. Portanto, além de contribuir para o progresso intelectual no tema, os resultados dessa tese podem subsidiar melhores ações para a conservação da biodiversidade Amazônica.

OBJETIVOS

O principal objetivo desta tese foi investigar o papel de gradientes ambientais na distribuição, abundância, variabilidade genética e fluxo gênico de *Allobates femoralis* ao longo de paisagens heterogêneas que ocorrem no interflúvio entre os rios Purus e Madeira (IPM).

Capítulo 1: testar se as características do solo e da floresta estão associadas com a distribuição e abundância relativa de *Allobates femoralis* em um gradiente ambiental de 880 km através do interflúvio Purus-Madeira.

Capítulo 2: Aplicar uma abordagem de modelagem multinível baseada em dados genômicos para testar se o gradiente geográfico e ambiental ao longo da paisagem heterogênea do IPM influencia a variabilidade genética e o fluxo gênico de *Allobates femoralis*.

CAPÍTULO 1

Ferreira, A.S.; Jehle, R.; Stow, A.J.; Lima, A.P. 2018. Soil and forest structure predicts large-scale patterns of occurrence and local abundance of a widespread Amazonian frog. *Peerj*, 6, e5424. DOI: 10.7717/peerj.5424

Soil and forest structure predicts large-scale patterns of occurrence and local abundance of a widespread Amazonian frog

Anthony S. Ferreira[1], Robert Jehle[2,*], Adam J. Stow[3,*] and Albertina P. Lima[4,*]

[1] Programa de Pós-Graduação em Ecologia, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil
[2] School of Environment and Life Sciences, University of Salford, Salford, UK
[3] Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia
[4] Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil
* These authors contributed equally to this work.

Submitted 29 December 2017
Accepted 21 July 2018
Published 9 August 2018

Corresponding author
Anthony S. Ferreira,
anthony.ferreira@inpa.gov.br

Academic editor
Leonardo Montagnani

Additional Information and Declarations can be found on page 15

DOI **10.7717/peerj.5424**

ABSTRACT

The distribution of biodiversity within the Amazon basin is often structured by sharp environmental boundaries, such as large rivers. The Amazon region is also characterized by subtle environmental clines, but how they might affect the distributions and abundance of organisms has so far received less attention. Here, we test whether soil and forest characteristics are associated with the occurrence and relative abundance of the forest-floor dwelling Aromobatid frog, *Allobates femoralis*. We applied a structured sampling regime along an 880 km long transect through forest of different density. High detection probabilities were estimated for *A. femoralis* in each of the sampling modules. Using generalized linear mixed-effects models and simple linear regressions that take detectability into account, we show that *A. femoralis* is more abundant in open forests than in dense forests. The presence and relative abundance of *A. femoralis* is also positively associated with clay-rich soils, which are poorly drained and therefore likely support the standing water bodies required for reproduction. Taken together, we demonstrate that relatively easy-to-measure environmental features can explain the distribution and abundance of a widespread species at different spatial scales. Such proxies are of clear value to ecologists and conservation managers working in large inaccessible areas such as the Amazon basin.

Subjects Biogeography, Conservation Biology, Ecology, Environmental Impacts, Population Biology
Keywords Amazonia, Ecology, *Allobates femoralis*, Environmental heterogeneity, Ecological limiting factors, Tropical forest

INTRODUCTION

The distribution of species is often fragmented, with favorable habitats being separated from each other by unsuitable habitats (Krebs, 1972; Hanski, 1999). Nevertheless, determining how habitat heterogeneity influences the distribution and abundance of

How to cite this article Ferreira et al. (2018), Soil and forest structure predicts large-scale patterns of occurrence and local abundance of a widespread Amazonian frog. **PeerJ 6:e5424; DOI 10.7717/peerj.5424**

species at various geographic scales remains one of the major challenges in ecology and conservation biology (Leibold et al., 2004; Fraterrigo, Wagner & Warren, 2004; Tews et al., 2004; McGarigal et al., 2016). Species interact with each other at fine scales, and habitat heterogeneity within a landscape moderates the broad-scale consistency of such interactions, producing variation in environmental effects at intermediate scales (Lawton, 1999). A persistent problem is explaining how abiotic and biotic factors affect the distributions of species across spatial scales which are hierarchical in nature (Fraterrigo, Wagner & Warren, 2004). Recent work has disentangled historical and environmental determinants for the spatial turnover of species assemblage compositions (Ricklefs & Schluter, 1993; Holyoak, Leibold & Holt, 2005; Bitar et al., 2017). However, for single species, the main focus has been on predicting entire ranges by extrapolating from local data on habitat requirements (e.g., using species distribution models, see Elith & Leathwick, 2009; Zurell et al., 2016), for which an understanding of the potential hierarchy of controls on species' niches is critically important (Araujo & Luoto, 2007; Fraterrigo, Wagner & Warren, 2004). Studies that use empirical data to quantify habitat associations for single species across biogeographic scales, however, are surprisingly rare.

In the Amazon basin, biogeographic and large-scale ecological studies are particularly challenging, due to difficulties of access and a general lack of baseline knowledge (Tuomisto et al., 2003; Laurance et al., 2004; Betts, Malhi & Roberts, 2008; Gardner et al., 2008; De Fraga et al., 2014). To explain patterns of endemism, marked ecological barriers such as large rivers have been found to predict the distribution of many taxa (Cracraft, 1985; Aleixo, 2006; Araripe et al., 2008; Ribas et al., 2012; Dias-Terceiro et al., 2015; Nazareno, Dick & Lohmann, 2017; Oliveira, Vasconcelos & Santos, 2017). While sharp environmental boundaries clearly influence the abundance and occurrence of organisms, the effect of more gradual clines of biotic and abiotic features are less appreciated (Quesada et al., 2012; Cintra et al., 2013; Emilio et al., 2013; Schietti et al., 2016). Under such a scenario, the abundance of organisms and patterns of local adaptation are shaped by continuous environmental changes across the landscape (Endler, 1977; Leite & Rogers, 2013; Dias-Terceiro et al., 2015; Bitar et al., 2017).

Anurans are useful models to evaluate biogeographic and ecological determinants of species assemblages in tropical ecosystems owing to their high diversity, low vagility and specific environmental requirements (Zimmerman & Bierregaard, 1986; Ernst & Rödel, 2008; Menin et al., 2007; Keller et al., 2009). Furthermore, based on their life histories, groups of species can be assigned to specific guilds (e.g., lotic and lentic aquatic breeders, forest-floor dwellers and canopy species; for case studies from Amazonia see Zimmerman & Simberloff, 1996; Menin et al., 2007; Rojas-Ahumada, Landeiro & Menin, 2012; Landeiro, Waldez & Menin, 2014; Dias-Terceiro et al., 2015; Bitar et al., 2017). Related species may share behavioral, physiological, and morphological traits because of common ancestry, rather than as a result of being exposed to similar selection pressures and convergent evolution (Huey, 1987; Losos, 1990; Cadle & Greene, 1993; Zimmerman & Simberloff, 1996). Given that some habitat requirements are therefore likely to be shared by all individual species of a guild, it is remarkable that relatively little is known about habitat associations of particular species across significant parts of their range (but see e.g., Jorge et al., 2016).

Many anurans associate with standing water, so their populations are often patchily distributed across the landscape (*Smith & Green, 2005*). Therefore, a population size estimate is often unattainable as a metric for monitoring population status across large scales, due to its variability and work involved in collecting the data at each patch (*Smith et al., 2014*). Documenting patch (site) occupancy is a more practical option because it can be measured using presence/absence surveys, utilizing each site as a sampling unit (*MacKenzie et al., 2002*). Moreover, the identification of accessible and stable environmental features that reflect specific habitat requirements would circumvent the problem of directly measuring standing water bodies, which are often ephemeral, and therefore difficult to record.

The present study identifies and characterizes important environmental parameters linked to the distribution and relative abundance of a widespread Amazonian forest-floor anuran, the Aromobatid frog *Allobates femoralis*, using a structured sampling regime spanning an 880 km environmental gradient across an interfluvial landscape. We focus on structural forest features and soil characteristics as surrogates for the species' microhabitat requirements, and show that these features are able to predict both its regional occurrence as well as its large-scale relative abundance. Our findings suggest that the distribution and relative abundance of *A. femoralis* is shaped by gradual ecological clines.

MATERIAL AND METHODS

Study species

The brilliant-thighed poison frog *A. femoralis* (*Boulenger, 1883*; Anura: Aromobatidae *Grant et al., 2017*; ♂ snout-vent length = 28–33 mm; ♀ snout-vent length = 33–35 mm) is widely distributed in non-flooded primary forests of the Amazon Basin and Guiana Shield in Brazil, Bolivia, Peru, Ecuador, Colombia, Guyana, Suriname, French Guiana and Venezuela (*Lescure & Marty, 2000*; *Lima et al., 2006*; *Amézquita et al., 2009*; *Barrio-Amorós & Santos, 2010*), although phylogeographic and taxonomic studies suggest that it comprises a suite of cryptic species (*Grant et al., 2006*, *2017*; *Fouquet et al., 2007*; *Santos et al., 2009*; *Simões, Lima & Farias, 2010*). *A. femoralis* is active in leaf litter or on fallen tree trunks on the forest floor, with males exhibiting territorial behavior (*Roithmair, 1992*; *Montanarin, Kaefer & Lima, 2011*). Females lay eggs on dead leaves in male territories during the rainy season, and males use water bodies ranging from shed palm bracts, Brazil-nut capsules, puddles on fallen tree trunks, peccary wallows and temporary puddles on the forest floor to deposit tadpoles after hatching (*Roithmair, 1994*; *Gascon, 1995*; *Ringler, Ursprung & Hödl, 2009*; *Beck, Thebpanya & Filiaggi, 2010*; *Ringler, Hödl & Ringler, 2015*; *Pašukonis et al., 2016*, *2017*). The availability and location of sites for tadpole deposition influences year-to-year displacement of individuals that survive more than one breeding season (*Ringler, Ursprung & Hödl, 2009*). The ephemeral occurrence of suitable bodies of water also sometimes forces male *A. femoralis* to deposit tadpoles more than 180 m away from their territories (*Ringler et al., 2013*), to which they reliably return (*Pašukonis et al., 2013*, *2014*). Over the last two decades, *A. femoralis* has been used as a model species to address questions on diversification (e.g., *Lougheed et al., 1999*; *Simões et al., 2008*; *Amézquita et al., 2009*), sexual selection and

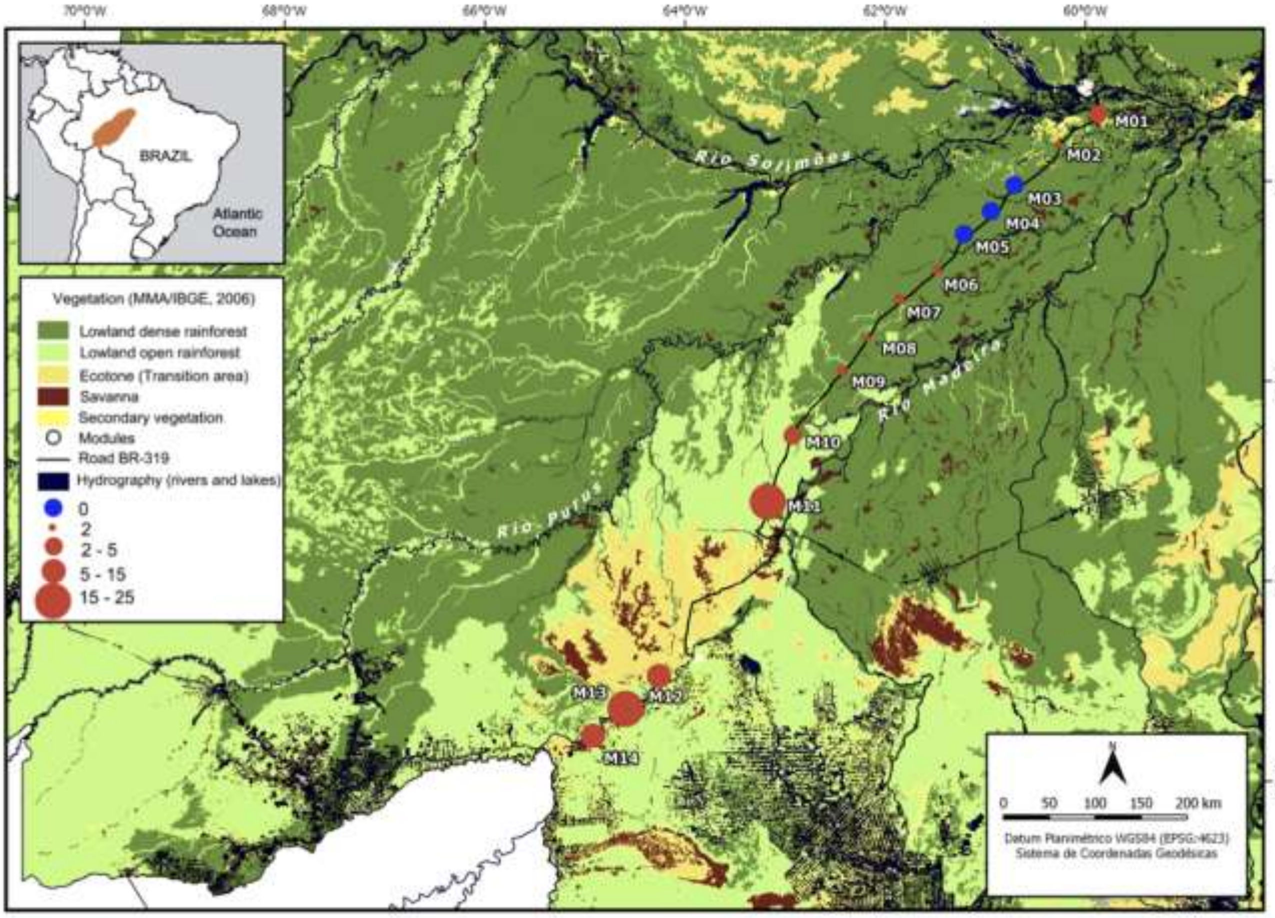


Figure 1 **Purus-Madeira interfluve showing modules locations with the two main types of phytophysiognomy and the range of means of the relative abundance of *A. femoralis*.** Purus-Madeira interfluve showing module locations (M1–M14), and the two main types of forest phytophysiognomy: lowland dense rainforest to the northeast (dark green) and lowland open rainforest to the southwest (light green). Red circles indicate the presence of *A. femoralis* with sizes representing relative abundances; blue circles indicate absence of *A. femoralis*. The scale of the symbols represents the range of means of the relative abundance of *A. femoralis* in the modules when present. The modules are approximately 50–60 km apart, with the exception of the distance between M11 and M12 which are separated by open transition forest and savanna. Map created in QGis 2.14 Essen (http://qgis.osgeo.org).

Full-size DOI: 10.7717/peerj.5424/fig-1

parental care (Ringler et al., 2015, 2016, 2017a; Ursprung et al., 2011; Pašukonis et al., 2016, 2017), movement ecology and spatial cognition (Pašukonis et al., 2016; Beck et al., 2017), and communication (Hödl, Amézquita & Narins, 2004; Amézquita, Castellanos & Hödl, 2005; Amézquita et al., 2006; Narins et al., 2005; Betancourth-Cundar et al., 2016; Ringler et al., 2017b).

Study area

The Purus-Madeira interfluve (PMI) is located on the eastern boundary of the Inambari area of endemism in central-southern Amazonia, delimited by the Amazon, Purus and Madeira rivers and covering about 15.4 million hectares (Fearnside et al., 2009; Fig. 1).

It is of sedimentary origin in its northeastern parts (Late Pleistocene-Early Holocene see Sombroek, 2000; Rossetti, Toledo & Góes, 2005), where the water table is closer to the surface and large areas are waterlogged by temporary small streams during the rainy season (Fan & Miguez-Macho, 2010; Schietti et al., 2016). Soils are mainly plinthosols characterized by poor drainage; the predominant texture is silt and fine sand in the northeast (Cintra et al., 2013; Martins et al., 2014) and podzolic soils with a predominant texture of clay and sand in the southwest (IBGE, 1997). The vegetation is classified as humid tropical lowland rainforest, composed of lowland dense rainforest in the northeast, with an about 40 m high canopy and frequent occurrence of palms in the understory, and lowland open rainforest with an about 40 m high canopy in the southeast (IBGE, 1997; Sousa, 2007). Considerable areas of savanna and transition between lowland open forest-savanna are present in the extreme southwest (Fig. 1). In the northeast of the PMI, forests are characterized by a lower basal area, lower canopy heights and lower mean wood density (lowland dense forests) compared to the central and southeast sites (lowland open forests), associated with an increase in rainfall seasonality and a change in soil structure (Sombroek, 2000; Cintra et al., 2013; Schietti et al., 2016). Rainfall is seasonal and heaviest between November and May. The mean annual precipitation ranges from 2,100 mm in the southwest to 2,800 mm in the northeast (Cintra et al., 2013; Alvares et al., 2014). Elevation ranges from 27 to 80 m above sea level (Sombroek, 2000). Temporary ponds occur in lower areas during the rainy season and are formed by undulating terrain (Rossetti, Toledo & Góes, 2005; Ferrão et al., 2018). A more detailed description of the study area is presented by Cintra et al. (2013) and Schietti et al. (2016).

Sampling design

The BR-319 Highway has not been maintained as a thoroughfare since 1998, but still allows access to a large section of the PMI. The implementation of standardized sampling sites along this highway through the RAPELD (Rapid Assessment for Long Duration Ecological Projects; Magnusson et al., 2013) system has generated a large amount of environmental and biotic information (Levis et al., 2012; Baccaro et al., 2013; Emilio et al., 2013; Cintra et al., 2013; Martins et al., 2014; Schietti et al., 2016; Ferrão et al., 2016; 2018; De Abreu, Schietti & Anciães, 2018). For the present study, we collected data in 152 plots at 14 RAPELD research sites (modules, M) which are spread along a 880-km-long transect (Fig. 1; Table S1, see Magnusson et al., 2013 for more details). Standard RAPELD modules consist of two straight parallel five km long trails starting at one km distance from each other, with five plots of 250 × 60 m (~1.5 ha) that follow altitudinal isoclines to minimize within-plot environmental heterogeneity installed on each trail, at distances of one km (Fig. S1). A total of 11 modules were installed along the BR-319 Highway (M01–M11), with plots established at least one km from the road to avoid secondary forests. Three other modules are near the left bank of the Madeira River (M12–M14), with seven 250 m plots resulting in a total of 14 plots per module and installed in the same way as those along the BR-319 Highway. Detailed descriptions of RAPELD sampling units throughout the Amazon basin are available at https://ppbio.inpa.gov.br.

Allobates femoralis sampling

We used time- and space-limited visual sampling (adapted from Crump & Scott, 1994; 2 min searches every 10 m along the 250 m long plots) and auditory searches (using playback to stimulate male responses) to quantify the relative abundance of *A. femoralis*. Each sampling session lasted about one hour along the 250 m long plot central line, and was undertaken by two experienced observers. The presence or absence of *A. femoralis* was recorded in segments of 10 m. As only a single record per segment was made, the maximum number of records was 25 per plot. We were careful not to record the same individual more than once on the same segment or in neighboring segments. Sampling was carried out during the breeding season, which coincides with the regional rainy season (Kaefer et al., 2012; Ferrão et al., 2018), between December and February 2010–2015.

Data were collected during the daily periods of peak vocalization for the species (7:00–10:00 a.m. and 14:00–18:00 p.m., Kaefer et al., 2012). To determine if *A. femoralis* was present, we recorded data on calling males following the audio strip transect method outlined by Zimmerman (1994). The *A. femoralis* advertisement call is one of the best studied anuran vocalizations (Narins, Hödl & Grabul, 2003; Hödl, Amézquita & Narins, 2004; Amézquita, Castellanos & Hödl, 2005; Amézquita et al., 2006, 2009; Göd, Franz & Hödl, 2007; Simões et al., 2008; Ringler et al., 2017b), and in the study area is a trill composed of four whistle-like notes with ascending frequency modulation. Calling males of *A. femoralis* could be heard from a distance of 30 m, and are easily recognized. Each of the 152 plots was sampled once per field season, giving a total of four surveys per site in total. To avoid temporal bias, two sampling sessions followed the directions M1–M14 and M14–M1, respectively, with the remaining two sampling sessions not undertaken in consecutive order. The research project was approved by the appropriate governmental bodies: Ministério do Meio ambiente (MMA), the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio license 13777) and the Sistema de Autorização e Informação em Biodiversidade (SISBIO license 7836-1) for the sampling of *A. femoralis*. All sampling procedures were approved by the ethics animal welfare committees of the Instituto Nacional de Pesquisas da Amazônia (CEUA/INPA: 041/2015) in accordance with established scientific practice guidelines and current Brazilian legislation.

Environmental variables

To represent environmental effects on the distribution and relative abundance of *A. femoralis* along the PMI, we used physical soil parameters (sand, clay and silt contents) and forest structure (basal area and number of trees, see Table S2). In all plots, soil samples were extracted with an auger every 50 m along the central 250 m long transect to a depth of 10 cm (a total of six samples per plot). Samples were kept in sealed plastic bags for 2–5 days, air dried at ambient temperature, and mixed to form one composite sample per plot (Cintra et al., 2013). Soil physical structure was analyzed following a standard protocol of total dispersion, using sodium pyrophosphate to obtain relative clay, sand and silt contents (Donagema et al., 2011). Percentage of sand was determined with a 0.053 mm mesh sieve (tensile bolting cloth 16), dividing the remaining fraction into silt and clay

(Donagema et al., 2011). The proportion of clay was determined by separating particles of 20 μm from other particles, and the proportion of silt was determined by the difference between clay and sand values; for a full description of the methodology see Quesada et al. (2010) and Donagema et al. (2011).

Forest structure was represented by the basal area and the hierarchical sum of the number of trees and palms in three size classes: (1) in a band (left side of the center line) of 250 × 1 m (0.025 ha), counting all stems with diameter at breast height (DBH) $\geq$ 1 cm; (2) in a band of 250 × 20 m (0.5 ha), counting all stems with DBH $\geq$ 10 cm; and (3) in a band of 250 × 40 m (one ha), counting all stems with DBH $\geq$ 30 cm (Magnusson et al., 2005). Tree diameters were measured with a diametric tape to mm precision (Schietti et al., 2016). Total plot basal area was calculated by the sum of the transverse areas of all trees as $\pi(\text{DBH})^2/4$. Data for modules M1–11 were obtained from Schietti et al. (2016), whereas data for modules M12–14 were previously unpublished. Forest structure has previously been identified as an important factor affecting the distribution and abundance of frogs in Amazonia (Menin et al., 2007; Menin, Waldez & Lima, 2011; Landeiro, Waldez & Menin, 2014; Ferrão et al., 2018).

Data analysis

To estimate occupancy and detection probabilities of *A. femoralis* for each module, we used a multi-season occupancy model based on four seasons of sampling and presence-absence data without covariates in the program PRESENCE v.12.10 (MacKenzie et al., 2003). Models developed for estimating occupancy can account for imperfect detection by using data from repeat surveys to discriminate between a species being either truly absent, or present but undetected (MacKenzie et al., 2003; 2006; Tyre et al., 2003). The probability of occupancy is only estimated for the first season in a multi-season analysis, with occupancy parameters for the subsequent seasons being derived using a recursive equation (MacKenzie et al., 2003). Detection probabilities might vary slightly among areas within each module as a function of change in habitat features. However, because we were interested in estimating detectability at the scale of tens of kilometres for each module, we used a model without covariates. We excluded from the model the three modules where *A. femoralis* has never been found (M3–M5).

Taking detection probabilities per module into account, we used Generalized Linear Mixed-Effects Models (GLMMs) to investigate the variation in relative abundance along gradients with the forest-structure components (basal area and number of trees) and soil texture (sand, clay and silt) as fixed effects. Modules were included in the model as a random effect to account for the nested design (plots within modules, Zuur et al., 2009).

We also used the detectability-corrected data per module to ran simple linear regressions to investigate relationships between the relative abundance of *A. femoralis* with each predictor environmental variable. We used Shapiro–Wilk analyses to test for significant deviation from normality, and Spearman's coefficient to verify correlations between environmental variables. As M11 is characterized by high variation in silt across plots and a high abundance of *A. femoralis* in three plots appearing as an outlier, separate analyses with and without M11 were undertaken. As the number of records was low in most plots, we used the sum of recorded individuals (instead of the mean) for the four samplings to represent the relative abundance of *A. femoralis* in each plot (following Bueno et al., 2012; Ferrão et al., 2018). To test for spatial autocorrelation among the modules, we used a Moran's correlogram of geographical distance between pairs of modules and on the residuals of linear regression analysis of dependent variable between pairs of modules.

Statistical analyses were carried out in the statistical platform R 3.2.3 (R Core Team, 2018). GLMM analyses were conducted with the packages lme4 (Bates, Maechler & Bolker, 2015), and the DHARMa package was used for the creation and simulation of scaled (quantile) residuals (Hartig, 2017). The marginal and conditional GLMMs r^2 were calculated using the package MuMIn (Bartoń, 2018) and figures were compiled using the package visreg (Breheny & Burchett, 2017). We used the APE package to test for spatial auto-correlation (Paradis, Claude & Strimmer, 2004). We only show data in figures when the simple linear regressions model was significant at the 0.05 level. Maps were prepared with *QGis 2.14 Essen* (QGIS Development Team, 2016).

RESULTS

We found *A. femoralis* in 11 of 14 modules. The average number of segments per module in which we detected *A. femoralis* varied from 1.5 to 10 in lowland dense rainforest modules when present, while in lowland open rainforest the mean the respective number ranged from 12 to 25 per module (Fig. 1). At the level of plots, the encountered number of *A. femoralis* was on average 80% higher in lowland open rainforest (mean = 15.67; SD = 17.21, Fig. 2) than in lowland dense rainforest (mean = 3; SD = 5.01, Fig. 2).

Estimates from the multi-season model showed that the median proportion of plots predicted to be occupied per module was 0.44 (0.14–0.78), and that median *A. femoralis* detection probabilities were 0.78 (0.48–0.96) across the four surveys (Table 1). There was no correlation between the geographical distance among modules and their dissimilarity in soil structure (sand, clay and silt contents; Moran tests, $p = 0.12$, 0.39, and 0.80, respectively) or forest structure (basal area and number of trees; Moran tests, $p = 0.70$ and 0.40, respectively).

The 880 km transect across the PMI was characterized by marked environmental gradients. Tree basal area ranged from 9.73 to 38.90 m^2 ha^{-1}. The number of trees per ha^{-1} varied from 1,335 to 11,475 considering all individuals with dbh $\geq$ 1 cm (Figs. 3A and 3B), with more trees in the central area of the PMI and fewer trees towards marginal areas in the southwest (Fig. 3B). To the northeast of the PMI, the soil had high levels of silt (average 30–77%), while the soil to the southwest had high clay content (average 50–77%, Figs. 3C and 3D). Module 11 had the highest variation in silt between plots (22–70%, Fig. 3C), and a high relative abundance of *A. femoralis*. Exclusion of this module from the analyses greatly changed the slope, but not the direction, of the curve and masked the relationships with environmental variables. Therefore, we excluded this module from the simple linear regressions (module level), but not from the GLMMs (plot level).

Four environmental variables were highly correlated ($r > 0.6$) with another variable: clay content with silt content, silt content and clay content with basal area, and basal

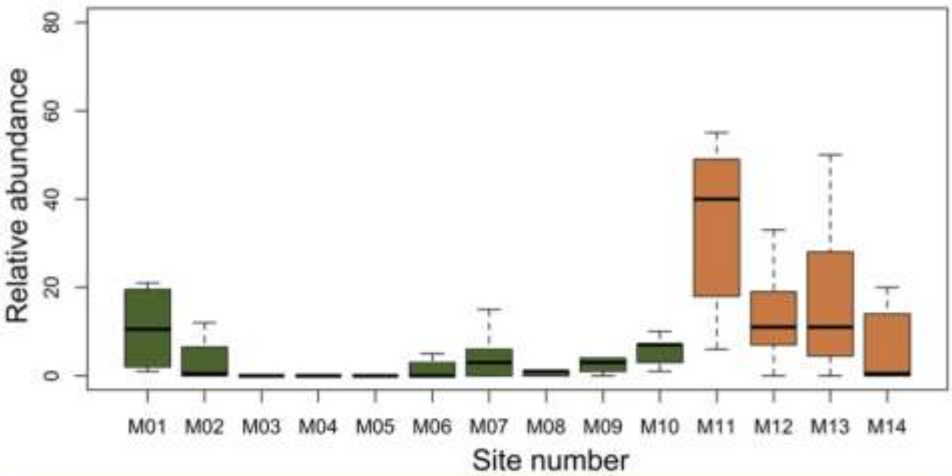


Figure 2 **Median of *A. femoralis* relative abundance in the research modules along the Purus-Madeira interfluve.** Median, quartiles and maximum and minimum values of *A. femoralis* relative abundance (sum of individuals per plot across all samplings taking detectability into account) in the research modules along the Purus-Madeira interfluve. Green bars (M1–M10) represent modules in the lowland dense rainforest, and orange bars (M11–M14) represent modules in the lowland open rainforest.

Full-size DOI: 10.7717/peerj.5424/fig-2

Table 1 **Occupancy probabilities and detection of *A. femoralis*.**

Modules	O.M.	SE_{OM}	p	SE_p
M1	0.53 (0.00–1.00)	0.42	0.61 (0.00–1.00)	0.32
M2	0.28 (0.00–0.85)	0.29	0.48 (0.06–0.89)	0.21
M6	0.14 (0.00–0.41)	0.14	0.83 (0.52–1.00)	0.15
M7	0.21 (0.00–0.64)	0.20	0.56 (0.24–0.89)	0.17
M8	0.34 (0.01–0.68)	0.17	0.75 (0.51–1.00)	0.12
M9	0.44 (0.06–0.82)	0.19	0.81 (0.31–1.00)	0.25
M10	0.57 (0.14–1.00)	0.22	0.90 (0.61–1.00)	0.15
M11	0.70 (0.42–0.99)	0.15	0.96 (0.89–1.00)	0.04
M12	0.78 (0.57–1.00)	0.11	0.91 (0.82–0.99)	0.04
M13	0.44 (0.10–0.78)	0.17	0.89 (0.74–1.00)	0.07
M14	0.42 (0.17–0.69)	0.13	0.87 (0.74–1.00)	0.07

Notes:

Occupancy probabilities and detection of *A. femoralis* in 11 research sites which consist of two straight parallel 5 km long trails in central-southern Amazonia. O.M., proportion of plots predicted to be occupied in each module with confidence intervals (± 95%); SE, standard error; p, probability of detection for *A. femoralis* with confidence intervals (± 95%) for the four samplings in the Purus-Madeira interfluve.

area with number of trees (Table 2). Soils with more silt coincided with low relative abundance of *A. femoralis* ($p = 0.006$; Fig. 4A), and areas with lower tree basal area had a high relative abundance of *A. femoralis* ($p = 0.05$; Fig. 4B). No interaction was found between clay content and number of trees to determine the relative abundance of *A. femoralis* in the GLMMs (Figs. 4C and 4D). Detailed results of the GLMMs are presented in Table 3.

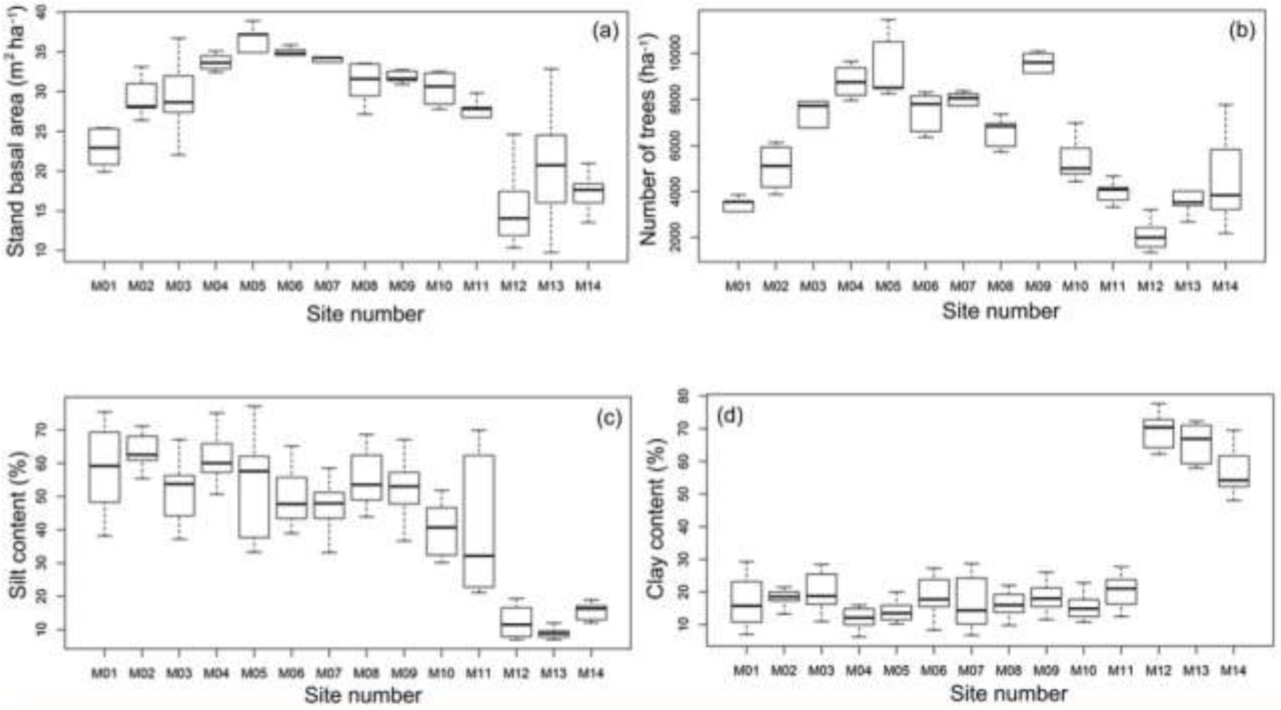


Figure 3 Median, quartiles and maximum and minimum values of environmental variables along the Purus-Madeira Interfluve. Median (black vertical line), quartiles and maximum and minimum values of (A) basal area; (B) number of trees; (C) silt content and (D) clay content across 14 research modules along the Purus-Madeira Interfluve.

Full-size DOI: 10.7717/peerj.5424/fig-3

Table 2 Pearson's correlation coefficients between environmental variables along the Purus-Madeira interfluve.

Variables	Sand	Silt	Clay	Basal area	Number of trees
Sand		–0.30	–0.30	0.38	0.25
Silt	–0.11		**–0.92**	**0.77**	0.54
Clay	–0.39	**–0.82**		**–0.86**	–0.59
Basal area	0.30	**0.64**	**–0.72**		**0.81**
Number of trees	0.37	0.53	–0.54	**0.74**	

Notes:

Pearson's correlation coefficients between environmental variables at the module level (upper right) and plot level (lower left) along the Purus-Madeira interfluve. Bold values correspond to correlated variables ($r > 0.60$, $p < 0.05$).

Simple linear regressions at the module level showed that the relative abundance of *A. femoralis* was negatively related to silt content ($F_{1,11} = 27.28$, $r^2 = 0.69$, $p < 0.001$; Fig. 5A), basal area ($F_{1,11} = 21.55$, $r^2 = 0.63$, $p < 0.001$; Fig. 5C) and number of trees ($F_{1,11} = 11.77$, $r^2 = 0.47$, $p < 0.01$; Fig. 5D), and positively related to clay content ($F_{1,11} = 24.78$, $r^2 = 0.66$, $p < 0.001$; Fig. 5B). Soil structure (silt and clay contents) explained up to 69% and 66%, and forest structure (basal area and number of trees) explained up to 63% and 47% of the variance in relative abundance of *A. femoralis*, respectively.

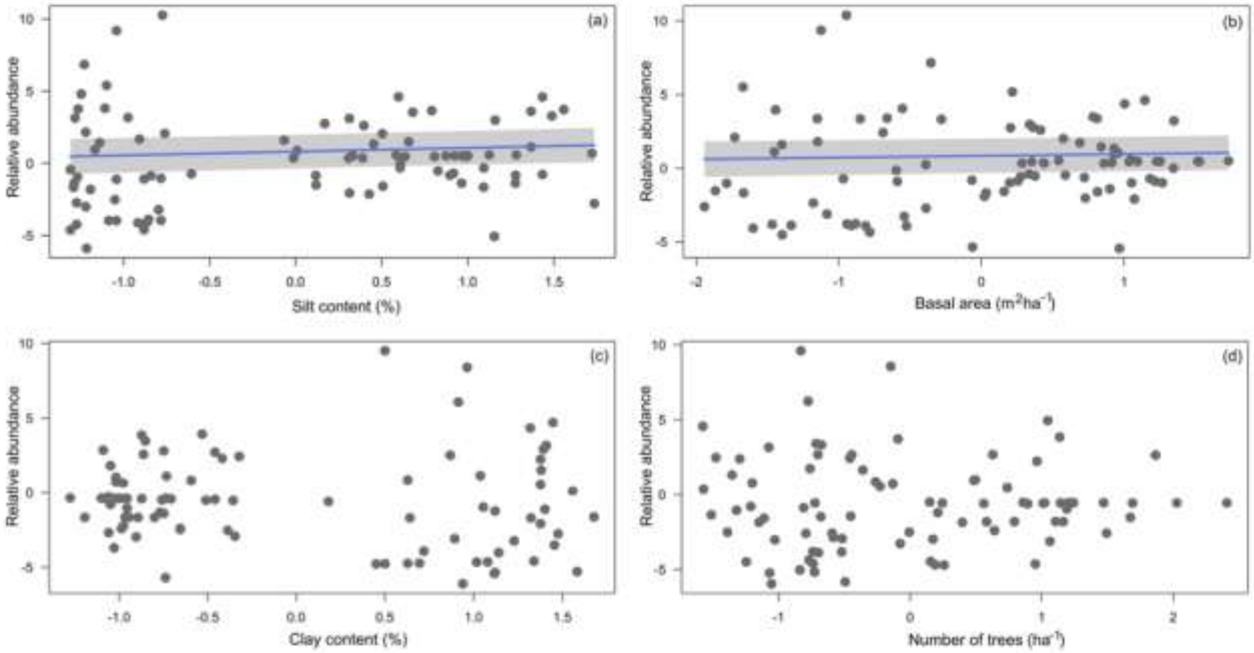


Figure 4 Partial regressions derived from generalized linear mixed-effects models. Partial regressions derived from generalized linear mixed-effects models (GLMMs) investigating the effects of (A) silt content, (B) basal area, (C) clay content and (D) number of trees per plot on *A. femoralis* relative abundance taking detectability into account in forests along the Purus-Madeira interfluve. Fitted lines indicate fixed-effect probabilities < 0.05 in the linear mixed-model analyses.
Full-size DOI: 10.7717/peerj.5424/fig-4

Table 3 Results of generalized linear mixed-effects models—GLMMs.

Dependent variable	Fixed effects	*df*	AIC	BIC	logLik	r^2_{marg}	r^2_{cond}	*p*
Relative abundance	Silt + basal area	86	1,138.6	1,148.6	−565.3	0.06	0.47	**0.006 0.05**
	Clay + trees	86	1,147.9	1,157.9	−569.9	0.21	0.44	0.28 0.99
	Sand + basal area	86	1,141.5	1,151.5	−566.7	0.02	0.49	0.09 **0.05**

Notes:
Results of generalized linear mixed-effects models (GLMMs) for the relative abundance of *A. femoralis* taking detectability into account as a function of sand, clay and silt contents, basal area and number of trees (fixed effects). Modules was considered a random effect in all model. Marginal r^2 values are for the models adjusted only considering fixed effects and the conditional r^2 corresponds to the full model, including the random effect. The relative contribution of predictors is given by the standardized coefficients of the GLMMs. The probability for each predictor is shown in the sequence as they appear in the models. Standardized coefficients in bold have $p < 0.05$. The outlier module M11 is included in all models.

DISCUSSION

We show that soil characteristics and forest structure can predict the distribution and relative abundance of the widespread forest-dwelling Amazonian frog *A. femoralis* at a range of spatial scales. The species is generally patchily distributed, and its occurrence and relative abundance is associated with gradual variation in environmental features. At a large geographic scale, we revealed that *A. femoralis* is more abundant in open forests and in areas with clay-rich soils. Due to the lack of RAPELD modules in the savanna-open

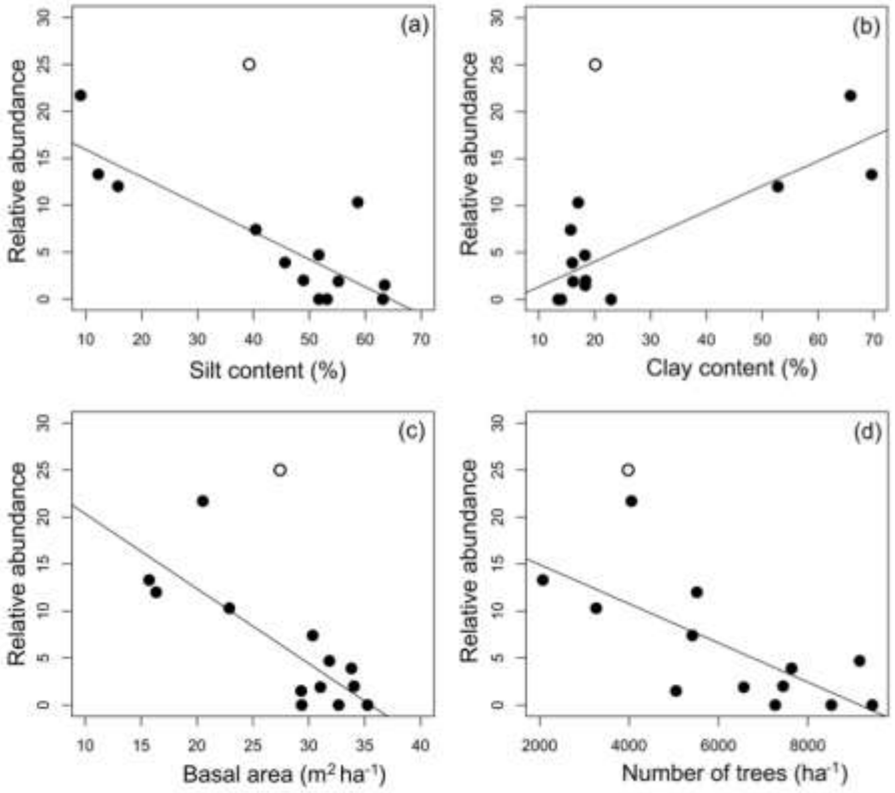


Figure 5 **Relationship between mean *A. femoralis* relative abundance and silt and clay contents, basal area and number of trees.** Relationship between mean *A. femoralis* relative abundance and (A) silt content, (B) clay content, (C) basal area and (D) number of trees per module, taking detectability into account and excluding the outlier M11 (open circles; see the main text for more details). The relationships were statistically significant (percentage of silt: $r^2 = 0.69$, $p < 0.001$; percentage of clay: $r^2 = 0.66$, $p < 0.001$; basal area: $r^2 = 0.63$, $p < 0.001$ and number of trees: $r^2 = 0.47$, $p < 0.01$).

Full-size DOI: 10.7717/peerj.5424/fig-5

lowland forest transition area in the southwest of the PMI, this region remained unstudied. Dense forests are likely to inhibit dispersal, but the edges appear to provide suitable habitat for *A. femoralis* to reproduce, and these habitats may therefore act as corridors. Soil characteristics predict both forest structure (*Cintra et al., 2013*; *Martins et al., 2014*) and the presence of surface water (*Woinarski, Fisher & Milne, 1999*; *Menin, Waldez & Lima, 2011*) a requirement for reproduction by anurans with aquatic tadpoles such as *A. femoralis*. The physical composition of the soil is thus fundamentally interlinked with the occurrence of Amazonian frog species that depend on small stagnant water bodies on the forest floor.

Our estimated detection probabilities demonstrate that multiple surveys of *A. femoralis* in the PMI are highly unlikely to result in false negatives (recorded absences when the species is actually present). We expected this result because *A. femoralis* is easily detectable even when it is scarce, especially because males respond reliably to playback calls (Amézquita, Castellanos & Hödl, 2005; Amézquita et al., 2006; Betancourth-Cundar et al., 2016). Although the modules in the northeast (M1–M2 and M6–M9) show a more dispersed occupation of *A. femoralis* across plots, the average detectability did not markedly differ from modules of the southwest region (M10–M14) that have higher occurrences and relative abundances. In the Amazon basin, the minimum number of surveys necessary to determine the presence or absence of an amphibian species is not specified through general guidelines. As a consequence, for example environmental consultants regularly conduct only a single survey per locations, which can result in detection failure and therefore generate erroneous predictions of species' responses to habitat factors (Gu & Swihart, 2003; Mazerolle et al., 2007). Our results suggest that the sampling regime applied for the present study (four surveys) is sufficient for the accurate detection of *A. femoralis*, although due to species-specific life histories this guide cannot be universally applied to other taxa (Smith et al., 2014).

The use of environmental proxies for estimating the occupancy of particular species has received some criticism, especially regarding the lack of consideration of behavioral interactions and the relevance of spatial scale (Stephens et al., 2015). The data used to evaluate species distributions in predictive models are often spatially biased and rarely include abundance (Warren, 2012), and the effects of biotic interactions are expected to be averaged out at broader scales (Fraterrigo, Wagner & Warren, 2004). Our data are not subject to these problems because sampling was carried out in a spatially stratified and consistent manner using the RAPELD research modules. Furthermore, data were obtained at large enough spatial scales in a hierarchical framework to test whether soil and forest characteristics measured in situ act as proxies for explaining the distribution and relative abundance of *A. femoralis* across a substantial part of its distribution. Due to the unavailability of module-specific precipitation data we are unable to include information on rainfall in our models, although they would be useful to predict whether soil ditches and other structures suitable for pond formation are actually filled with water.

Across the Amazon basin, differences in forest structure attributed to the physical characteristics of soil cause endogenous disturbances (Quesada et al., 2012; Cintra et al., 2013; Schietti et al., 2016), whereas edaphic or climatic factors can cause exogenous disturbances (Espírito-Santo et al., 2010, Cintra et al., 2013; Schietti et al., 2016). The two dominant forest phytophysiognomies in the PMI are spatially correlated with rainfall gradients (Sombroek, 2001), where forests in drier areas have lower stem densities and higher mass of individual trees compared to wetter forests, which have higher stem densities and lower individual tree mass (Cintra et al., 2013; Schietti et al., 2016). For *A. femoralis*, we show that these spatial differences in macro- and microhabitats shape its occurrence and relative abundance at both large and small scales, likely promoting its spatial differentiation. Parapatric segregation of populations associated with different forest formations has also recently been shown for an arboreal anuran occurring in the PMI (*Osteocephalus taurinus*, Ortiz, Lima & Werneck, 2018).

The two main vegetation types in the PMI are related to geomorphology and the establishment of the current Amazonian drainage system (Rossetti, Toledo & Góes, 2005;

Hoorn et al., 2010; *Latrubesse et al., 2010*; *Nogueira, Silveira & Guimarães, 2013*). Tributaries in the dense northern forest drain into the Madeira River depression, while southern tributaries located within open forests drain into the Purus river depression (Fig. 1). According to *Rossetti, Toledo & Góes (2005)*, seasonally flooded areas are linked to Holocene terrains, and the two major sedimentary units deposited 47,000 and 27,000 years ago are represented by lowland dense rainforest and lowland open rainforest. Drainage dynamics in each basin differ, and this coupled with different sedimentary loads and deposition age also influences vegetation establishment (*Cohen et al., 2014*). Old and well-drained soils are highly weathered (*Emilio et al., 2013*), whereas the soils in the northeast of the PMI are characterized by young and poorly drained sediments. Seasonally-flooded and poorly-structured soils provide a poorer substrate for root development and anchorage, resulting in frequent disturbances and high tree mortality rates, and consequently more dynamic, younger, and denser forests (*Castilho et al., 2006*; *Feldpausch et al., 2011*; *Quesada et al., 2012*; *Cintra et al., 2013*; *Schietti et al., 2016*). On the other hand, more structured soils with higher clay content are associated with older forests, where trees have higher individual mass and spacing between trees is larger (*Castilho et al., 2006*; *Feldpausch et al., 2011*; *Emilio et al., 2013*). Therefore, soil type can act as an environmental filter, selecting for different tree-growth strategies and partitioning the forest into patches of vegetation that are structurally distinct (*Cintra et al., 2013*; *Emilio et al., 2013*; *Schietti et al., 2016*).

Soil type also reflects other attributes of the environment relevant to anurans. A study conducted in central Amazonia found higher production of litter in plane areas with clay-rich soils (*Luizão et al., 2004*). Other studies found higher species richness and abundance of anurans at sites with clay-rich soil, presumably linked to higher surface water availability (*Hadden & Westbrooke, 1996*; *Woinarski, Fisher & Milne, 1999*; *Menin et al., 2007*). Rain can create ponds isolated from streams on soil rich in clay (*Menin, Waldez & Lima, 2011*). Consequently, such soil is directly related to the availability of small standing water bodies (*Menin, Waldez & Lima, 2011*), a prerequisite for the reproduction of *A. femoralis* (*Kaefer et al., 2012*; *Ringler, Hödl & Ringler, 2015*). *Gascon (1995)* described the general advantages of natural pools for frogs, and *Ringler, Hödl & Ringler (2015)* demonstrated the impact of simulated peccary presence on *A. femoralis* population size, where installing artificial pools almost doubled the density of frogs within two years. Because assessing small water bodies that may serve as tadpole deposition sites is difficult over large areas, using proxies to predict the distribution and abundance of *A. femoralis* in environments such as the Amazon basin can save time and money. Clay-rich soils are also associated with a higher variety and density of terrestrial arthropods (*Franklin, Magnusson & Luizão, 2005*; *Aguiar, Gualberto & Franklin, 2006*), which generally provide food for leaf litter frogs. However, the relationships between invertebrates and soil texture have not been investigated in the PMI, and additional data are necessary to establish a link between prey density and *A. femoralis* occurrence and relative abundance.

The influence of environmental heterogeneity on the distribution of frogs in the Amazonian lowlands generally depends on reproductive modes and breeding habitats

(*Zimmerman & Bierregaard, 1986*; *Menin et al., 2007*; *Menin, Waldez & Lima, 2011*; *Landeiro, Waldez & Menin, 2014*; *Ferrão et al., 2018*). *A. femoralis* is likely to have ecological requirements in common with other forest frogs with similar life histories. For example, the density of *A. sumtuosus* was positively related to the number of isolated pools on a local scale (*Jorge et al., 2016*), and the occurrence and relative abundance of tree frogs (*Scinax*), which use similar water bodies for reproduction, is also positively affected by soil silt content (*Ferrão et al., 2018*).

CONCLUSION

Frogs face a range of threats, including habitat loss and fragmentation, disease, and introduced species (*Kats & Ferrer, 2003*; *Stuart et al., 2004*; *Lips et al., 2006*; *Becker et al., 2007*). Given the pace of development in the Amazon Basin and the limited resources available for conservation, quick and cost-effective methods for predicting anthropogenic impacts are required. Our assessment of *A. femoralis* revealed its patchy distribution, with higher occupancy probabilities in areas with high, open vegetation and clay-rich soil. Identifying relatively easy-to-measure environmental features that reflect the distribution and abundance of suits of organisms, including species-specific detection probabilities, are of clear value to ecologists and conservation managers.

ACKNOWLEDGEMENTS

We thank Adrian A. Barnet for language editing of the first manuscript version, William E. Magnusson and Fabrício Baccaro for help with statistical procedures, the Programa de Grande Escala da Biosfera-Atmosfera na Amazônia (LBA) for providing the field assistant João Souza. We also like to thank Dr. Leonardo Montagnani and three anonymous referees for suggestions that have substantially improved the quality of the paper.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) who granted a PhD scholarship to Anthony S. Ferreira (number 161883/2014-1). Fieldwork and infrastructure were funded by the Instituto Nacional de Pesquisas da Amazônia (INPA), Programa de Suporte a Núcleos de Excelência (PRONEX, number 653/2009), CNPq—number 475559/2013-4, Programa de Pesquisa em Biodiversidade (PPBio/RAPELD), Centro Integrado de Estudos da Biodiversidade Amazônica (INCT—CENBAM) and Santo Antônio Energia S.A. This study was carried out in the modules along the Purus-Madeira interfluvial of Programa de Pesquisa em Longa Duração (PPBio/RAPELD) financed by FAPEAM and CNPq under grants conceded to Albertina P. Lima—number 653/2009. Collection of soil and forest structure data used in this study was funded by FAPESP/FAPEAM (465/2010) and additional funding was provided by PRONEX—FAPEAM (1600/2006) and PPBio Manaus (CNPq 558318/2009-6). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).
Instituto Nacional de Pesquisas da Amazônia (INPA).
Programa de Suporte a Núcleos de Excelência: PRONEX, 653/2009.
CNPq: 475559/2013-4.
Programa de Pesquisa em Biodiversidade (PPBio/RAPELD).
Centro Integrado de Estudos da Biodiversidade Amazônica (INCT—CENBAM).
FAPEAM.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Anthony S. Ferreira conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Robert Jehle authored or reviewed drafts of the paper, approved the final draft.
- Adam J. Stow authored or reviewed drafts of the paper, approved the final draft.
- Albertina P. Lima conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All sampling procedures were discussed and approved by the ethics animal welfare committees of the Instituto Nacional de Pesquisas da Amazônia (CEUA/INPA: 041/2015) in accordance with good scientific practice guidelines and current Brazilian legislation.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

The research project was approved by the appropriate governmental bodies: Ministério do Meio ambiente (MMA), through the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio license 13777) and Sistema de Autorização e Informação em Biodiversidade (SISBIO license 7836-1) for the sampling of A. femoralis.

Data Availability

The following information was supplied regarding data availability:

The raw data are provided in the Supplemental File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5424#supplemental-information.

REFERENCES

Aguiar NO, Gualberto TL, Franklin E. 2006. A medium-spatial scale distribution pattern of Pseudoscorpionida (Arachnida) in a gradient of topography (altitude and inclination), soil factors, and litter in a central Amazonia forest reserve, Brazil. *Brazilian Journal Biology* **66(3)**:791–802 DOI 10.1590/S1519-69842006000500004.

Aleixo A. 2006. Historical diversification of floodplain forest specialist species in the Amazon: a case study with two species of the avian genus *Xiphorhynchus* (Aves: Dendrocolaptidae). *Biological Journal of the Linnean Society* **89(2)**:383–395 DOI 10.1111/j.1095-8312.2006.00703.x.

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. 2014. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* **22(6)**:711–728 DOI 10.1127/0941-2948/2013/0507.

Amézquita A, Castellanos L, Hödl W. 2005. Auditory matching of male *Epipedobates femoralis* (Anura: Dendrobatidae) under field conditions. *Animal Behaviour* **70(6)**:1377–1386 DOI 10.1016/j.anbehav.2005.03.012.

Amézquita A, Hödl W, Lima AP, Castellanos L, Erdtmann L, Araújo MC. 2006. Masking interference and the evolution of the acoustic communication system of the Amazonian poison frog *Epipedobates femoralis*. *Evolution* **60**:1874–1887.

Amézquita A, Lima AP, Jehle R, Castellanos L, Ramos O, Crawford AJ, Gasser H, Hödl W. 2009. Calls, colours, shapes, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. *Biological Journal of the Linnean Society* **98(4)**:826–838 DOI 10.1111/j.1095-8312.2009.01324.x.

Araripe J, Tagliaro CH, Rêgo PS, Sampaio I, Ferrari SF, Schneider H. 2008. Molecular phylogenetics of large-bodied tamarins, *Saguinus* spp. (Primates, Platyrrhini). *Zoologica Scripta* **37(5)**:461–467 DOI 10.1111/j.1463-6409.2008.00343.x.

Araujo MB, Luoto M. 2007. The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography* **16(6)**:743–753 DOI 10.1111/j.1466-8238.2007.00359.x.

Baccaro FB, Rocha IF, Aguila BEG, Schietti J, Emilio T, Pinto JLPV, Lima AP, Magnusson WE. 2013. Changes in ground-dwelling ant functional diversity are correlated with water-table level in an Amazonian terra firme forest. *Biotropica* **45(6)**:755–763 DOI 10.1111/btp.12055.

Barrio-Amorós CL, Santos JC. 2010. Amphibia, Anura, Dendrobatidae, *Allobates femoralis* (Boulenger, 1884): First confirmed country records, Venezuela. *Check List* **6(2)**:208–209 DOI 10.15560/6.2.208.

Bartoń K. 2018. *MuMIn: multi-model inference*. R package version 1.40.4. *Available at https://CRAN.R-project.org/package=MuMIn*.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software* **67(1)**:1–48 DOI 10.18637/jss.v067.i01.

Beck KB, Loretto M, Ringler M, Hödl W, Pašukonis A. 2017. Relying on known or exploring for new? Movement patterns and reproductive resource use in a tadpole-transporting frog. *PeerJ* **5**:e3745 DOI 10.7717/peerj.3745.

Beck H, Thebpanya P, Filiaggi M. 2010. Do Neotropical peccary species (Tayassuidae) function as ecosystem engineers for anurans? *Journal of Tropical Ecology* **26(4)**:407–414 DOI 10.1017/s0266467410000106.

Becker CG, Fonseca CR, Haddad CFB, Batista RF, Prado PI. 2007. Habitat split and the global decline of amphibians. *Science* **318(5857)**:1775–1777 DOI 10.1126/science.1149374.

Betancourth-Cundar M, Lima AP, Hödl W, Amézquita A. 2016. Decoupled evolution between senders and receivers in the neotropical *Allobates femoralis* frog complex. *PLOS ONE* **11(6)**:e0155929 DOI 10.1371/journal.pone.0155929.

Betts RA, Malhi Y, Roberts JT. 2008. The future of the Amazon: new perspectives from climate, ecosystem and social sciences. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363(1498)**:1729–1735 DOI 10.1098/rstb.2008.0011.

Bitar YOC, Tuomisto H, Pinheiro LC, Juen L, Santos-Costa MC. 2017. Species turnover in Amazonian frogs: low predict ability and large differences among forests. *Biotropica* **49(5)**:695–705 DOI 10.1111/btp.12428.

Boulenger GA. 1883. On a collection of frogs from Yurimaguas, Huallaga River, Northern Peru. *Proceedings of the Zoological Society of London* **51(1)**:635–638 DOI 10.1111/j.1469-7998.1883.tb06669.x.

Breheny P, Burchett W. 2017. *visreg: visualization of regression models*. R package Version 2.4-1. *Available at https://CRAN.R-project.org/package=visreg*.

Bueno AS, Bruno RS, Pimentel TP, Sanaiotti TM, Magnusson WE. 2012. The width of riparian habitats for understory birds in an Amazonian forest. *Ecological Applications* **22(2)**:722–734 DOI 10.1890/11-0789.1.

Cadle JE, Greene HW. 1993. Phylogenetic patterns, biogeography, and the ecological structure of Neotropical snake assemblages. In: Ricklefsand RE, Schluter D, eds. *Species Diversity in Ecological Communities*. Chicago: University of Chicago Press, 281–293.

Castilho CV, Magnusson WE, Araújo NRO, Luizão RCC, Luizão FJ, Lima AP, Higuchi N. 2006. Variation in aboveground tree live biomass in a central Amazonian forest: effects of soil and topography. *Forest Ecology and Management* **234(1–3)**:85–96 DOI 10.1016/j.foreco.2006.06.024.

Cintra BBL, Schietti J, Emillio T, Martins D, Moulatlet G, Souza P, Levis C, Quesada CA, Schöngart J. 2013. Soil physical restrictions and hydrology regulate stand age and wood biomass turnover rates of Purus–Madeira interfluvial wetlands in Amazonia. *Biogeosciences* **10(11)**:7759–7774 DOI 10.5194/bg-10-7759-2013.

Cohen MCL, Rossetti DF, Pessenda LCR, Friaes YS, Oliveira PE. 2014. Late Pleistocene glacial forest of Humaitá—Western Amazonia. *Palaeogeography, Palaeoclimatology, Palaeoecology* **415**:37–47 DOI 10.1016/j.palaeo.2013.12.025.

Cracraft J. 1985. Historical biogeography and patterns of differentiation within the South American avifauna: areas of endemism. *Ornithological Monographs* **36**:49–84 DOI 10.2307/40168278.

Crump ML, Scott NJ Jr. 1994. Visual encounter surveys. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS, eds. *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Washington: Smithsonian Institution Press, 84–92.

De Abreu FHT, Schietti J, Anciães M. 2018. Spatial and environmental correlates of intraspecific morphological variation in three species of passerine birds from the Purus–Madeira interfluvium, Central Amazonia. *Evolutionary Ecology* **32(2–3)**:191–214 DOI 10.1007/s10682-018-9929-4.

De Fraga R, Stow AJ, Magnusson W, Lima AP. 2014. The costs of evaluating species densities and composition of snakes to assess development impacts in Amazonia. *PLOS ONE* **9(8)**:e105453 DOI 10.1371/journal.pone.0105453.

Dias-Terceiro RG, Kaefer IL, Fraga R, Araújo MC, Simões PI, Lima AP. 2015. A matter of scale: historical and environmental factors structure anuran assemblages from the Upper Madeira River, Amazonia. *Biotropica* **47(2)**:259–266 DOI 10.1111/btp.12197.

Donagema GK, Campos DVB, Calderano SB, Teixeira WG, Viana JHM. 2011. *Manual de Métodos de Análise de Solo*. Rio de Janeiro: Embrapa solos.

Elith J, Leathwick JR. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics* **40(1)**:677–697 DOI 10.1146/annurev.ecolsys.110308.120159.

Emilio T, Quesada CA, Costa FRC, Magnusson WE, Schietti J, Feldpausch TR, Brienen RJW, Baker TW, Chave J, Álvarez E, Araújo A, Bánki O, Castilho CV, Honorio EN, Killeen TJ, Malhi Y, Mendoza EMO, Monteagudo A, Neill D, Parada JA, Peña-Cruz A, Ramirez-Angulo H, Schwarz M, Silveira M, Steege H, Terborgh JW, Thomas R, Torres-Lezama A, Vilanova E, Phillips OL. 2013. Soil physical conditions limit palm and tree basal area in Amazonian forests. *Plant Ecology & Diversity* **7(1–2)**:215–229 DOI 10.1080/17550874.2013.772257.

Endler JA. 1977. *Geographic Variation, Speciation, and Clines*. Princeton: Princeton University Press.

Ernst R, Rödel MO. 2008. Patterns of community composition in two tropical tree frog assemblages: separating spatial structure and environmental effects in disturbed and undisturbed forests. *Journal of Tropical Ecology* **24(2)**:111–120 DOI 10.1017/S0266467407004737.

Espírito-Santo FDB, Keller M, Braswell B, Nelson BW, Frolking S, Vicente G. 2010. Storm intensity and old-growth forest disturbances in the Amazon region. *Geophysical Research Letters* **37(11)**:1–6 DOI 10.1029/2010GL043146.

Fan Y, Miguez-Macho G. 2010. Potential groundwater contribution to Amazon evapotranspiration. *Hydrology and Earth System Sciences* **14(10)**:2039–2056 DOI 10.5194/hess-14-2039-2010.

Fearnside PM, Graça PMLA, Keizer EWH, Maldonado FD, Barbosa RI, Nogueira EM. 2009. Modelagem de desmatamento e emissões de gases de efeito estufa na região sob influência da rodovia Manaus–Porto Velho (Br-319). *Revista Brasileira de Meteorologia* **24(2)**:208–233 DOI 10.1590/s0102-77862009000200009.

Feldpausch TR, Banin L, Phillips OL, Baker TR, Lewis SL, Quesada CA, Affum Baffoe K, Arets EJMM, Berry NJ, Bird M, Brondizio ES, Camargo P, Chave J, Djagbletey G, Domingues TF, Drescher M, Fearnside PM, França MB, Fyllas NM, Lopez-Gonzalez G, Hladik A, Higuchi N, Hunter MO, Iida Y, Salim KA, Kassim AR, Keller M, Kemp J, King DA, Lovett JC, Marimon BS, Marimon-Junior BH, Lenza E, Marshall AR, Metcalfe DJ, Mitchard ETA, Moran EF, Nelson BW, Nilus R, Nogueira EM, Palace M, Patiño S, Peh KSH, Raventos MT, Reitsma JM, Saiz G, Schrodt F, Sonké B, Taedoumg HE, Tan S, White L, Woll H, Lloyd J. 2011. Height-diameter allometry of tropical forest trees. *Biogeosciences* **8(5)**:1081–1106 DOI 10.5194/bg-8-1081-2011.

Ferrão M, Colatreli O, de Fraga R, Kaefer IL, Moravec J, Lima AP. 2016. High species richness of *Scinax* treefrogs (Hylidae) in a threatened Amazonian landscape revealed by an integrative approach. *PLOS ONE* **11(11)**:e0165679 DOI 10.1371/journal.pone.0165679.

Ferrão M, Fraga R, Moravec J, Kaefer IL, Lima AP. 2018. A new species of Amazonian snouted treefrog (Hylidae: *Scinax*) with description of a novel species-habitat association for an aquatic breeding frog. *PeerJ* **6**:e4321 DOI 10.7717/peerj.4321.

Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ. 2007. Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLOS ONE* **2(10)**:e1109 DOI 10.1371/journal.pone.0001109.

Franklin E, Magnusson WE, Luizão FJ. 2005. Relative effects of biotic and abiotic factors on the composition of soil invertebrates communities in an Amazonian savannah. *Applied Soil Ecology* **29(3)**:259–273 DOI 10.1016/j.apsoil.2004.12.004.

Fraterrigo JM, Wagner S, Warren RJ. 2004. Local-scale biotic interactions embedded in macroscale climate drivers suggest Eltonian noise hypothesis distribution patterns for an invasive grass. *Ecology Letters* **17(11)**:1447–1454 DOI 10.1111/ele.12352.

Gardner TA, Barlow J, Araujo IS, Avila-Pires TC, Bonaldo AB, Costa JE, Esposito MC, Ferreira LV, Hawes J, Hernandez MI, Hoogmoed MS, Leite RN, Lo-Man-Hung NF, Malcolm JR, Martins MB, Mestre LA, Miranda-Santos R, Overal WL, Parry L, Peters SL, Ribeiro-Junior MA, Da Silva MN, Da Silva Motta C, Peres CA. 2008. The cost-effectiveness of biodiversity surveys in tropical forests. *Ecology Letters* **11(2)**:139–150 DOI 10.1111/j.1461-0248.2007.01133.x.

Gascon C. 1995. Tropical larval anuran fitness in the absence of direct effects of predation and competition. *Ecology* **76(7)**:2222–2229 DOI 10.2307/1941695.

Göd M, Franz A, Hödl W. 2007. The influence of internote-interval variation of the advertisement call on the phonotactic behaviour in male *Allobates femoralis* (Dendrobatidae). *Amphibia-Reptilia* **28(2)**:227–234 DOI 10.1163/156853807780202468.

Gu W, Swihart RK. 2003. Absent or undetected? Effects of non-detection of species occurance on wildlife-habitats models. *Biological Conservation* **116(2)**:195–203 DOI 10.1016/s0006-3207(03)00190-3.

Grant T, Frost DR, Caldwell JP, Gagliardo R, Haddad CFB, Kok PJR, Means DB, Noonan BP, Schargel WE, Wheeler W. 2006. Phylogenetic systematics of dart poison frogs and their relatives (Anura: Athesphatanura: Dendrobatidae). *American Museum of Natural History Research Library* **299**:1–262 DOI 10.5531/sd.sp.14.

Grant T, Rada M, Anganoy-Criollo M, Batista A, Dias PH, Jeckel AM, Machado DJ, Rueda-Almonacid JV. 2017. Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology* **12(s1)**:S1–S90 DOI 10.2994/SAJH-D-17-00017.1.

Hadden SA, Westbrooke ME. 1996. Habitat relationships of the herpetofauna of remnant Buloke Woodlands of Wimmera Plains, Victoria. *Wildlife Research* **23(3)**:363–372 DOI 10.1071/wr9960363.

Hanski I. 1999. *Metapopulation Ecology*. Oxford: Oxford University Press.

Hartig F. 2017. *DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models*. R package Version 0.1.5. *Available at http://florianhartig.github.io/DHARMa/*.

Hödl W, Amézquita A, Narins P. 2004. The rôle of call frequency and the auditory papillae in phonotactic behavior in male dart-poison frogs *Epipedobates femoralis* (Dendrobatidae). *Journal of Comparative Physiology A* **190(10)**:823–829 DOI 10.1007/s00359-004-0536-1.

Holyoak M, Leibold MA, Holt RD. 2005. *Metacommunities: Spatial Dynamics and Ecological Communities*. Chicago: University of Chicago Press.

Hoorn C, Wesselingh FP, Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg L, Stadler T, Särkinen T, Antonelli A. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330(6006)**:927–931 DOI 10.1126/science.1194585.

Huey RB. 1987. Phylogeny, history, and the comparative method. In: Feder ME, Bennett AF, Burggren WW, Huey RB, eds. *New Directions in Ecological Physiology*. Cambridge: Cambridge University Press, 76–101.

IBGE. 1997. *Recursos naturais e meio ambiente: uma visão do Brasil*. Second Edition. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística (IBGE), 208.

Jorge RF, Simões PI, Magnusson WE, Lima AP. 2016. Fine-scale habitat heterogeneity explains the local distribution of two Amazonian frog species of concern for conservation. *Biotropica* **48(5)**:697–703 DOI 10.1111/btp.12333.

Kaefer IL, Montanarin A, Da Costa, Rosangela S, Lima AP. 2012. Temporal patterns of reproductive activity and site attachment of the brilliant-thighed frog *Allobates femoralis* from central Amazonia. *Journal of Herpetology* **46(4)**:549–554 DOI 10.1670/10-224.

Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions* **9(2)**:99–110 DOI 10.1046/j.1472-4642.2003.00013.x.

Keller A, Rödel MO, Linsenmair KE, Grafe TU. 2009. The importance of environmental heterogeneity for species diversity and assemblage structure in Bornean stream frogs. *Journal of Animal Ecology* **78(2)**:305–314 DOI 10.1111/j.1365-2656.2008.01457.x.

Krebs CJ. 1972. *Ecology: The Experimental Analysis of Distribution and Abundance*. New York: Harper & Row Press, 694.

Landeiro VL, Waldez F, Menin M. 2014. Spatial and environmental patterns of Amazonian anurans: Differences between assemblages with aquatic and terrestrial reproduction, and implications for conservation management. *Natureza & Conservação* **12(1)**:42–46 DOI 10.4322/natcon.2014.008.

Latrubesse EM, Cozzuol M, Silva-Caminha SAF, Rigsby CA, Absy ML, Jaramillo C. 2010. The late Miocene paleogeography of the Amazon basin and the evolution of the Amazon river system. *Earth-Science Reviews* **99(3–4)**:99–124 DOI 10.1016/j.earscirev.2010.02.005.

Laurance WF, Albernaz AKM, Fearnside PM, Vasconcelos HL, Ferreira LV. 2004. Deforestation in Amazonia. *Science* **304(5674)**:1109b–1111b DOI 10.1126/science.304.5674.1109b.

Lawton JH. 1999. Are there general laws in ecology? *Oikos* **84**:177–192 DOI 10.2307/3546712.

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* **7(7)**:601–613 DOI 10.1111/j.1461-0248.2004.00608.x.

Leite RN, Rogers DS. 2013. Revisiting Amazonian phylogeography: insights into diversification hypotheses and novel perspectives. *Organisms Diversity & Evolution* **13(4)**:639–664 DOI 10.1007/s13127-013-0140-8.

Lescure J, Marty C. 2000. Atlas des amphibiens de Guyane. *Patrimoine Naturel* **45**:1–288.

Levis C, Souza PF, Schietti J, Emilio T, Pinto JLPV, Clement CR, Costa FRC. 2012. Historical human footprint on modern tree species composition in the Purus-Madeira Interfluve, Central Amazonia. *PLOS ONE* **7(11)**:e48559 DOI 10.1371/journal.pone.0048559.

Lima AP, Magnuson WE, Menin M, Erdtmann LK, Rodrigues DJ, Keller C, Hödl W. 2006. *Guia de sapos da Reserva Adolpho Ducke, Amazônia Central*. Manaus: Áttema Design Editorial, 168.

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America* **103(9)**:3165–3170 DOI 10.1073/pnas.0506889103.

Losos JB. 1990. Ecomorphology, performance capability, and scaling of West Indian Anolis lizards: an evolutionary analysis. *Ecological Monographs* **60(3)**:369–388 DOI 10.2307/1943062.

Lougheed SC, Gascon C, Jones DA, Bogart JP, Boag PT. 1999. Ridges and rivers: a test of competing hypothesis of Amazonian diversification using a dart-poison frog (*Epipedobates femoralis*). *Proceedings of the Royal Society B: Biological Sciences* **266(1431)**:1829–1835 DOI 10.1098/rspb.1999.0853.

Luizão RCC, Luizão FJ, Paiva RQ, Monteiro TF, Sousa LS, Kruijt B. 2004. Variation of carbon and nitrogen cycling processes along a topographic gradient in a central Amazonian forest. *Global Change Biology* **10(5)**:592–600 DOI 10.1111/j.1529-8817.2003.00757.x.

MacKenzie DI, Nichols JD, Hines JE, Knutson MG, Franklin AB. 2003. Estimating site occupancy, colonization and local extinction when a species is detected imperfectly. *Ecology* **84(8)**:2200–2207 DOI 10.1890/02-3090.

MacKenzie DI, Nichols JD, Lachman GB, Droege S, Royle JA, Langtimm CA. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* **83(8)**:2248–2255 DOI 10.2307/3072056.

MacKenzie DI, Nichols JD, Royle AR, Pollock KH, Bailey LL, Hines JE. 2006. *Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence.* Burlington: Elsevier/Academic Press, 324.

Magnusson WE, Braga-Neto R, Pezzini F, Baccaro F, Bergallo H, Penha J, Rodrigues D, Lima AP, Albernaz A, Hero JM, Lawson B, Castilho C, Drucker C, Franklin E, Mendonça F, Costa F, Galdino G, Castley G, Zuanon J, Vale J, Santos JLC, Luizão R, Cintra R, Barcosa RI, Lisboa A, Koblitz RV, Cunha CN, Pontes ARM. 2013. *Biodiversidade e monitoramento ambiental integrado: o sistema RAPELD na Amazônia.* Santo Andreñ: Attema.

Magnusson WE, Lima AP, Luizão R, Luizão F, Costa FRC, Castilho CV, Kinupp V. 2005. RAPELD: a modification of the Gentry method for biodiversity surveys in long-term ecological research sites. *Biota Neotropica* **5(2)**:19–24 DOI 10.1590/s1676-06032005000300002.

Martins DL, Schietti J, Feldpausch TR, Luizão FJ, Phillips OL, Andrade A, Castilho CV, Laurance SG, Oliveira A, Amaral IL, Toledo JJ, Lugli LF, Pinto JLPV, Mendoza EMO, Quesada CA. 2014. Soil-induced impacts on forest structure drive coarse woody debris stocks across central Amazonia. *Plant Ecology & Diversity* **8(2)**:229–241 DOI 10.1080/17550874.2013.879942.

Mazerolle MJ, Bailey LL, Kendall WL, Royle JA, Converse SJ, Nichols JD. 2007. Making great leaps forward: accounting for detectability in herpetological field studies. *Journal of Herpetology* **41(4)**:672–689 DOI 10.1670/07-061.1.

McGarigal K, Wan HY, Zeller KA, Timm BC, Cushman SA. 2016. Multi-scale habitat selection modeling: a review and outlook. *Landscape Ecology* **31(6)**:1161–1175 DOI 10.1007/s10980-016-0374-x.

Menin M, Lima AP, Magnusson WE, Waldez F. 2007. Topographic and edaphic effects on the distribution of terrestrially reproducing anurans in Central Amazonia: mesoscale spatial patterns. *Journal of Tropical Ecology* **23(5)**:539–547 DOI 10.1017/S0266467407004269.

Menin M, Waldez F, Lima AP. 2011. Effects of environmental and spatial factors on the distribution of anuran species with aquatic reproduction in central Amazonia. *Herpetological Journal* **21**:255–261.

Montanarin A, Kaefer IL, Lima AP. 2011. Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. *Ethology Ecology & Evolution* **23(2)**:141–150 DOI 10.1080/03949370.2011.554884.

Narins PM, Hödl W, Grabul DS. 2003. Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. *Proceedings of the National Academy of Sciences of the United States of America* **100(2)**:577–580 DOI 10.1073/pnas.0237165100.

Narins PM, Grabul DS, Soma KK, Gaucher P, Hödl W. 2005. Cross-modal integration in a dart-poison frog. *Proceedings of the National Academy of Sciences of the United States of America* **102**:2425–2429 DOI 10.1073/pnas.0406407102.

Nazareno AG, Dick CW, Lohmann LG. 2017. Wide but not impermeable: Testing the riverine barrier hypothesis for an Amazonian plant species. *Molecular Ecology* **26(14)**:3636–3648 DOI 10.1111/mec.14142.

Nogueira ACR, Silveira R, Guimarães JTF. 2013. Neogene–Quaternary sedimentary and paleovegetation history of the eastern Solimões Basin, central Amazon region. *Journal of South American Earth Sciences* **46**:89–99 DOI 10.1016/j.jsames.2013.05.004.

Oliveira U, Vasconcelos MF, Santos AJ. 2017. Biogeography of Amazon birds: rivers limit species composition, but not areas of endemism. *Scientific Reports* **7(1)**:29–92 DOI 10.1038/s41598-017-03098-w.

Ortiz DA, Lima AP, Werneck FP. 2018. Environmental transition zone and rivers shape intraspecific population structure and genetic diversity of an Amazonian rain forest tree frog. *Evolutionary Ecology* **32(4)**:359–378 DOI 10.1007/s10682-018-9939-2.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20(2)**:289–290 DOI 10.1093/bioinformatics/btg412.

Pašukonis A, Beck KB, Fischer MT, Weinlein S, Stückler S, Ringler E. 2017. Induced parental care in a poison frog: a tadpole cross-fostering experiment. *Journal of Experimental Biology* **220(21)**:3949–3954 DOI 10.1242/jeb.165126.

Pašukonis A, Loretto MC, Landler L, Ringler M, Hödl W. 2014. Homing trajectories and initial orientation in a Neotropical territorial frog, *Allobates femoralis* (Dendrobatidae). *Frontiers in Zoology* **11(1)**:29 DOI 10.1186/1742-9994-11-29.

Pašukonis A, Ringler M, Brandl HB, Mangione R, Ringler E, Hödl W. 2013. The homing frog: high homing performance in a territorial dendrobatid frog *Allobates femoralis* (Dendrobatidae). *Ethology* **119(9)**:762–768 DOI 10.1111/eth.12116.

Pašukonis A, Trenkwalder K, Ringler M, Ringler E, Mangione R, Steininger J, Warrington I, Hödl W. 2016. The signifiance of spatial memory for water fiding in a tadpole-transporting frog. *Animal Behaviour* **116**:89–98 DOI 10.1016/j.anbehav.2016.02.023.

QGIS Development Team. 2016. QGIS Geographic Information System. *Open Source Geospatial Foundation Project. Available at http://qgis.osgeo.org*.

Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Cz- imczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmer-ler J, Santos AJB, Hodnett MG, Herrera R, Luizão FJ, Arneth A, Lloyd G, Dezzeo N, Hilke I, Kuhlmann I, Raessler M, Brand WA, Geilmann H, Moraes Filho JO, Carvalho FP, Araujo Filho RN, Chaves JE, Cruz Junior OF, Pimentel TP, Paiva R. 2010. Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* **7(5)**:1515–1541 DOI 10.5194/bg-7-1515-2010.

Quesada CA, Phillips OL, Schwarz M, Czimczik CI, Baker TR, Patiño S, Fyllas NM, Hodnett MG, Herrera R, Almeida S, Alvarez Dávila E, Arneth A, Arroyo L, Chao KJ, Dezzeo N, Erwin T, di Fiore A, Higuchi N, Honorio Coronado E, Jimenez EM, Killeen T, Lezama AT, Lloyd G, López-González G, Luizão FJ, Malhi Y, Monteagudo A, Neill DA, Núñez Vargas P, Paiva R, Peacock J, Peñuela MC, Peña Cruz A, Pitman N, Priante Filho N, Prieto A, Ramírez H, Rudas A, Salomão R, Santos AJB, Schmerler J, Silva N, Silveira M, Vásquez R, Vieira I, Terborgh J, Lloyd J. 2012. Basin-wide variations in Amazon forest structure and function are mediated by both soils and climate. *Biogeosciences* **9(6)**:2203–2246 DOI 10.5194/bg-9-2203-2012.

R Core Team. 2018. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. *Available at* https://www.R-project.org/.

Ribas CC, Aleixo A, Nogueira ACR, Miyaki CY, Cracraft JA. 2012. Palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society B: Biological Sciences* **279(1729)**:681–689 DOI 10.1098/rspb.2011.1120.

Ricklefs RE, Schluter D. 1993. Species diversity in ecological communities: historical and geographic perspectives. Chicago: University of Chicago Press, 424.

Ringler E, Beck KB, Weinlein S, Huber L, Ringler M. 2017a. Adopt, ignore, or kill? Male poison frogs adjust parental decisions according to their territorial status. *Scientific Reports* **7(1)**:43544 DOI 10.1038/srep43544.

Ringler M, Hödl W, Ringler E. 2015. Populations, pools, and peccaries: simulating the impact of ecosystem engineers on rainforest frogs. *Behavioral Ecology* **26(2)**:340–349 DOI 10.1093/beheco/aru243.

Ringler M, Szipl G, Hödl W, Khil L, Kofler B, Lonauer M, Provin C, Ringler E. 2017b. Acoustic ranging in poison frogs—it is not about signal amplitude alone. *Behavioral Ecology and Sociobiology* **71(8)**:114 DOI 10.1007/s00265-017-2340-2.

Ringler E, Pašukonis A, Fitch WT, Huber L, Hödl W, Ringler M. 2015. Flexible compensation of lost uniparental care in a poison frog. *Behavioral Ecology* **26(4)**:1219–1225 DOI 10.1093/beheco/arv069.

Ringler E, Pašukonis A, Ringler M, Huber L. 2016. Sex-specific offspring discrimination reflects respective risks and costs of misdirected care in a poison frog. *Animal Behaviour* **114**:173–179 DOI 10.1016/j.anbehav.2016.02.008.

Ringler E, Pašukonis A, Hödl W, Ringler M. 2013. Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. *Frontiers in Zoology* **10(1)**:67 DOI 10.1186/1742-9994-10-67.

Ringler M, Ursprung E, Hödl W. 2009. Site fidelity and patterns of short- and long-term movement in the brilliant-thighed poison frog *Allobates femoralis* (Aromobatidae). *Behavioral Ecology and Sociobiology* **63(9)**:1281–1293 DOI 10.1007/s00265-009-0793-7.

Roithmair ME. 1992. Territoriality and male mating success in the Dart-poison Frog, *Epipedobates femoralis* (Dendrobatidae, Anura). *Ethology* **92(4)**:331–343 DOI 10.1111/j.1439-0310.1992.tb00970.x.

Roithmair ME. 1994. Field studies on reproductive behaviour in two dart-poison frog species (*Epipedobates femoralis*, *Epipedobates trivittatus*) in Amazonian Peru. *Herpetological Journal* **4**:77–85.

Rojas-Ahumada DP, Landeiro VL, Menin M. 2012. Role of environmental and spatial processes in structuring anuran communities across a tropical rain forest. *Austral Ecology* **37(8)**:865–873 DOI 10.1111/j.1442-9993.2011.02330.x.

Rossetti DF, Toledo PM, Góes AM. 2005. New geological framework for Western Amazônia (Brazil) and implications for biogeography and evolution. *Quarternary Research* **63(1)**:78–89 DOI 10.1016/j.yqres.2004.10.001.

Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC. 2009. Amazonian amphibian diversity is primarily derived from Late Miocene Andean lineages. *PLOS Biology* **7(3)**:e1000056 DOI 10.1371/journal.pbio.1000056.

Schietti J, Martins D, Emilio T, Souza PF, Levis C, Baccaro FB, Pinto JLPV, Moulatlet JM, Stark SC, Sarmento K, Araújo NO, Costa FRC, Schöngart J, Quesada CA, Saleska SR, Tomasella J, Magnusson WE. 2016. Forest structure along a 600 km transect of natural disturbances and seasonality gradients in central-southern Amazonia. *Journal of Ecology* **104(5)**:1335–1346 DOI 10.1111/1365-2745.12596.

Simões PI, Lima AP, Farias IP. 2010. The description of a cryptic species related to the pan Amazonian frog *Allobates femoralis* (Boulenger 1883) (Anura: Aromobatidae). *Zootaxa* **2406**:1–28.

Simões PI, Lima AP, Magnusson WE, Hödl W, Amézquita A. 2008. Acoustic and morphological differentiation in the frog *Allobates femoralis*: relationships with the upper Madeira River and other potential geological barriers. *Biotropica* **40(5)**:607–614 DOI 10.1111/j.1744-7429.2008.00416.x.

Smith DHV, Jones B, Randall L, Prescott DRC. 2014. Difference in detection and occupancy between two anurans: the importance of species-specific monitoring. *Herpetological Conservation and Biology* **9**:267–277.

Smith MA, Green DM. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28(1)**:110–128 DOI 10.1111/j.0906-7590.2005.04042.x.

Sombroek W. 2000. Amazon landforms and soils in relation to biological diversity. *Acta Amazonica* **30(1)**:81 DOI 10.1590/1809-43922000301100.

Sombroek W. 2001. Spatial and temporal patterns of Amazon rainfall. *AMBIO: A Journal of the Human Environment* **30(7)**:388–396 DOI 10.1579/0044-7447-30.7.388.

Sousa TEL. 2007. Distribuição de palmeiras (Arecaceae) ao longo de gradientes ambientais no baixo interflúvio Purus-Madeira, Brasil. Dissertação de mestrado, INPA/UFAM.

Stephens PA, Pettorelli N, Barlow J, Whittingham MJ, Cadotte MW. 2015. Management by proxy? The use of indices in applied ecology. *Journal Applied Ecology* **52(1)**:1–6 DOI 10.1111/1365-2664.12383.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* **306(5702)**:1783–1786 DOI 10.1126/science.1103538.

Tews J, Brose U, Grimm V, Tielbörger K, Wichmann MC, Schwager M, Jeltsch F. 2004. Animal species diversity driven by habitat heterogeneity: the importance of keystone structures. *Journal of Biogeography* **31(1)**:79–92 DOI 10.1046/j.0305-0270.2003.00994.x.

Tuomisto H, Ruokolainen K, Aguilas M, Sarmiento A. 2003. Floristic patterns along a 43 km long transect in Amazonian rain Forest. *Journal of Ecology* **91(5)**:743–756 DOI 10.1046/j.1365-2745.2003.00802.x.

Tyre AJ, Tenhumberg B, Field SA, Niejalke D, Parris K, Possingham HP. 2003. Improving precision and reducing bias in biological surveys: estimating false-negative error rates. *Ecological Applications* **13(6)**:1790–1801 DOI 10.1890/02-5078.

Ursprung E, Ringler M, Jehle R, Hödl W. 2011. Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. *Molecular Ecology* **20(8)**:1759–1771 DOI 10.1111/j.1365-294x.2011.05056.x.

Warren DL. 2012. In defense of 'niche modeling'. *Trends in Ecology & Evolution* **27(9)**:497–500 DOI 10.1016/j.tree.2012.03.010.

Woinarski JCZ, Fisher A, Milne D. 1999. Distribution patterns of vertebrates in relation to an extensive rainfall gradient and variation in soil texture in the tropical savannas of the Northern Territory, Australia. *Journal of Tropical Ecology* **15(4)**:381–398 DOI 10.1017/s0266467499000905.

Zimmerman BL. 1994. Audio strip transects. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LAC, Foster MS, eds. *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Washington, D.C.: Smithsonian Institution Press, 92–96.

Zimmerman BL, Bierregaard R. 1986. Relevance of the equilibrium theory of island biogeography and species-area relations to conservation with a case from Amazonia. *Journal of Biogeography* **13(2)**:133–143 DOI 10.2307/2844988.

Zimmerman BL, Simberloff D. 1996. An historical interpretation of habitat use by frogs in a central Amazonian forest. *Journal of Biogeography* **23(1)**:27–46 DOI 10.1046/j.1365-2699.1996.d01-218.x.

Zurell D, Thuiller W, Pagel J, Cabral JS, Münkemüller T, Gravel D, Dullinger S, Normand S, Schiffers KH, Moore KA, Zimmermann NE. 2016. Benchmarking novel approaches for modeling species range dynamics. *Global Change Biology* **22(8)**:2651–2664 DOI 10.1111/gcb.13251.

Zuur A, Ieno EN, Walker N, Savellev AA, Smith GM. 2009. *Mixed Effects Models and Extensions in Ecology with R.* New York: Springer.

CAPÍTULO 2

Ferreira, A.S.; Lima, A.P.; Jehle, R.; Ferrão, M.; Stow, A. landscape genomics across a large Amazonian interfluve supports the gradient diversification hypothesis for the frog *Allobates femoralis*. Manuscrito em preparação para a ***Molecular Ecology***.

Landscape genomics across a large Amazonian interfluve supports the gradient diversification hypothesis for the frog *Allobates femoralis*

ANTHONY S. FERREIRA[1*], ALBERTINA P. LIMA[2], ROBERT JEHLE[3], MIQUÉIAS FERRÃO[1] and ADAM STOW[4]

[1] Programa de Pós-Graduacão em Ecologia, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil

[2] Coordenacão de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil

[3] School of Environment and Life Sciences, University of Salford, M5 4WT, Salford, UK

[4] Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia

[*] Corresponding author: anthony.ferreira@inpa.gov.br

Abstract

Landscape genomics offers a powerful approach to understanding species' dispersal patterns. However, ecological processes of diversification acting across geographically continuous populations have been rarely documented in Amazonia, due to lack of thorough taxonomic, geographic and molecular (genome-wide) sampling over extensive areas. We address this challenge by applying multilevel approaches to model landscape drivers of genetic structure in the brilliant-thighed poison frog (*Allobates femoralis*). More specifically, we focus on a 880 km long transect along the Purus-Madeira interfluve (PMI) and analyze > 10 000 SNPs from 66 individuals to test the hypothesis that, due to environmental gradients and the geographic extent of the region, connectivity between sampling points will be governed by isolation by geographic distance (IBD) as well as environmental resistance (IBR) modeled through a suite of ecological traits. We show that strong IBD as well as IBR were evident in the spatial patterns of genetic variability, supporting the gradient hypothesis for diversification. We however also identified four genetic clusters which partly paralleled phenotypic variation (femoral spot colour), and which could be explained by historical processes in the northeastern part of the PMI, and the presence of different forest types in the southwest. This study sheds light on the processes that can generated diversity across continuous Amazonian landscapes.

Keywords: Purus-Madeira interfluve, RADseq, single nucleotide polymorphism, landscape genetics, evolutionary genetics, circuit theory.

Introduction

The processes generating the high biodiversity in Amazonia result from long and complex interactions between geological, climatic, ecological and evolutionary processes (Rossetti, Mann de Toledo & Góes 2005; Hoorn et al. 2010; Smith et al. 2014). The evolutionary models proposed to explain distribution patterns of Amazonian biota largely depend on barriers imposed by major Amazonian rivers (the riverine barrier hypothesis; Wallace 1852; Ribas et al. 2012; Nazareno, Dick & Lohmann 2017; Godinho & Da Silva 2018), historical geological and hydrological dynamics (Antonelli et al. 2010), ancient ridges (Bush 1994), saline lakes resulting from marine incursions (Rasanen, Linna, Santos & Negri 1995; Lovejoy, Bermingham & Martin 1998), and climatic cycles that reduced forest areas in the Amazon to small forest refuges, isolating populations and promoting allopatric speciation (Haffer 1969; Vanzolini & Williams 1981). The strengths of these hypotheses, however, are still the subject of debate (Colinvaux, De Oliveira & Bush 2000; Oliveira, Vasconcelos & Santos 2017; Santorelli, Magnusson & Deus 2018).

A further framework for diversification focusing on populations in sympatry or parapatry is the gradient hypothesis (Endler 1977; see reviews in Moritz, Patton, Schneider & Smith 2000; Leite & Rogers 2013). The gradient hypothesis predicts that biotic or abiotic environmental differences may be sufficient to lead to divergence among populations, resulting in parapatric divergence along a continuous geographical or environmental cline, in a process that later led to the term ecological speciation (Schluter 2009). Under this scenario, allele frequencies will vary among localities following isolation-by-distance patterns with higher gene flow among geographically closer localities, in addition to ecological adaptation of populations in adjacent but different environments generating genetic and phenotypic differentiation (Wright 1943;

Endler 1977; Nosil 2012; Calsbeek, Smith & Bardeleben 2007; Price 2008). Transition zones can result from primary contact, both when parapatric populations differentiate in situ, and from secondary contact, when initially isolated populations reestablish contact after differentiation (Thorpe 1984), although defining the nature of contact zones is still a major challenge (Endler 1977; Thorpe 1984; Grahame, Wilding & Butlin 2006). In continuous populations, contact zones generally reflect the degree of environmental variation experienced by different populations of the same species at different sites (De Abreu, Schietti & Anciães 2018). Therefore, it is often difficult to evaluate the mechanisms interacting between geographical, environmental variables and ecologically-based selection on the differentiation patterns of populations (Nosil 2012; Stein, Gerstner & Kreft 2014).

The Amazonian basin is generally viewed to be governed by the riverine barrier hypothesis, with intervening land areas (interfluves) between the largest Amazonian rivers termed "areas of endemism" (AoE; Cracraft 1985; Ribas et al. 2012; Borges & Silva 2012; Oliveira, Vasconcelos & Santos 2017). However, the observation that the distribution of taxa is heterogeneous within AoEs, apparently associated with limits concordant with transition zones, smaller tributaries and with other characteristics of the landscape, has added complexity to our understanding of their dynamics (Simões, Lima, Magnusson, Hödl & Amézquita 2008; Naka et al. 2012; Fernandes et al. 2013; Ferreira, Jehle, Stow & Lima 2018; Ortiz, Lima & Werneck 2018).

To assess how environmental parameters influence the extent of genetic variation within and among populations, it is helpful to use taxa that retain ancestral differentiation at small spatial scales (Manel & Holderegger 2013; Richardson 2012; Wang 2012; Ortiz, Lima & Werneck 2018; Barratt et al. 2018). Amphibians are ideal candidates due to their strong association with local environmental conditions, physiological restrictions, high philopatry and low dispersal abilities (Lougheed, Austin, Bogart, Boag & Chek 2006; Zeisset & Beebee 2008). Therefore, studies investigating spatial genetic structure in tropical anurans within AoE would provide novel insights into the factors that drive population differentiation processes associated with variation on environmental variables of rainforests. In this context, western Amazonian lowlands are especially challenging, with a recent and dynamic geologic history associated to the transition from a wetland-dominated landscape to system drained by rivers that are historically highly dynamic, with large and recent spatial changes in their courses (Rossetti, Mann de Toledo & Góes 2005; Hoorn et al. 2010; Ruokolainen, Moulatlet, Zuquim, Hoorn & Tuomisto 2018).

One of the main problems regarding standardized and fine-scale studies of intraspecific diversity within Amazonia AoE is the difficulty to access the remote areas distant from large rivers. The Program for Planned Biodiversity and Ecosystem Research (PPBio) installed research sites along the Purus-Madeira interfluve (PMI), within the Inambari AoE, providing an opportunity to study intraspecific diversity. Combined, all research sites represent the largest geographic gradient sampled within a single Amazonian interfluve (Magnusson et al. 2013). The PMI is crossed by the BR-319 highway, which has been abandoned since 1988. But currently the Brazilian government is starting new efforts to reconstructing and paving the BR-319 highway, which will strongly impact the deforestation process and biodiversity loss in this region (Fearnside & Graça 2006; Fearnside et al. 2009), where habitat loss models predict the loss of a third of the Purus-Madeira interfluve, which is equivalent to approximately 5.4 million hectares by 2050 (Maldonado, Keizer, Graça, Fearnside & Vitel 2012). The Amazonas state government's State Secretariat for the Environment and Sustainable Development (SDS) and the federal government's Ministry of Environment (MMA) have plans for creating a series of protected areas within the PMI (Fearnside et al. 2009). Studies have shown an environmental gradient along the PMI, where physical soil and hydrologic characteristics combined with weather conditions drive the forest structure and its dynamics (Cintra et al. 2013; Emilio et al. 2013; Martins et al. 2014; Schietti et al. 2016). This system partially explains the distribution, abundance and change of species composition (Ferrão, Fraga, Moravec, Kaefer & Lima 2018; Ferreira, Jehle, Stow & Lima 2018; Ortiz, Lima & Werneck 2018), providing an ideal situation to investigate ecological and historical factors associated to the current diversity patterns.

Here, we apply a genetic-based multilevel modelling approach to assess for parapatric divergence across geographic and ecological gradients in the brilliant-thighed poison frog (*Allobates femoralis*), a terrestrial frog widely distributed to Amazon basin. We adopt a landscape resistance approach based on circuit theory that uses Single Nucleotide Polymorphism (SNPs) data and georeferenced occurrences. Our study spans over an approximately 880 km long transect within the PMI, and represents an appropriate management scale for effective conservation strategies of species. Therefore, we used the intraspecific population structure of *A. femoralis* as a model for testing geographic variations effects on isolation-by-distance (IBD) and current landscape features effects on isolation-by-resistance (IBR). In doing so, using our analytical framework, this study provides an important first step towards understanding fine-scale diversification patterns and processes in this highly-threatened AoE.

Materials and methods

Study species

The brilliant-thighed poison frog, *Allobates femoralis* (Aromobatidae, Grant et al.

2017), is a small (~ 33 mm) and ground-dwelling diurnal frog which feeds on a wide range of invertebrate taxa (Caldwell 1996; Simões, Lima, Magnusson, Hödl & Amézquita 2008). This species is widely distributed throughout primary, non-flooded forest areas of the Amazon basin and Guiana Shield (Amézquita et al. 2009; Ferreira, Jehle, Stow & Lima 2018), suggesting that flooding areas of major Amazonian rivers may represent barriers for gene flow. Phylogeographic and taxonomic studies suggest that *A. femoralis* comprises a suite of cryptic species distributed across the Amazonian basin (Grant et al. 2017; Fouquet et al. 2007b; Santos et al. 2009; Simões, Lima & Farias 2010), and habitat loss is a major factor causing population declines and extinction risk (Simões et al. 2014). *Allobates femoralis* move via leaf litter or on fallen tree trunks (Roithmair 1992; Montanarin, Kaefer & Lima 2011), and the configuration of forest habitat is an important determinants of the *A. femoralis* distributions (Ferreira, Jehle, Stow & Lima 2018). Males exhibit territorial behaviour and females lay eggs on dead leaves in male territories during the rainy season (Ringler, Ursprung & Hödl 2009; Montanarin, Kaefer & Lima 2011). The availability and location of sites for tadpole deposition influences year-to-year displacement of individuals that survive more than one breeding season (Ringler, Ursprung & Hödl 2009; Ringler, Beck, Weinlein, Huber & Ringler 2017). Dispersal is male-biased and the ephemeral occurrence of suitable bodies of water also sometimes forces male *A. femoralis* to deposit tadpoles more than 180 m away from their territories (Ringler, Pašukonis, Hödl & Ringler 2013), to which they reliably return (Pašukonis, Loretto, Landler, Ringler & Hödl 2014). In our study area there are three morphotypes of *A. femoralis* in relation to the coloration of the femoral spot (yellow, red and orange), being distributed in separate localities and finding in one contact zone.

Study area and sampling

In November and March 2010-2015, we sampled *A. femoralis* in 16 locations along an 880 km transect which is crossed by a federal highway (BR-319) and delimited by the Purus and Madeira rivers (Fig. 1; Table S1). The PMI is located in the south of the Amazon River covering approximately 15.4 million hectares, and presents gradients of vegetation cover type, soil and climatic seasonality (Cintra et al. 2013; Schietti et al. 2016; Ferreira, Jehle, Stow & Lima 2018). The PMI is characterized by a complex hydrography with hundred small rivers that flood large areas in northeastern region and patches of forest in the southwest by overflow during the rainy season, and with most streams disappearing in the dry season (Fan & Miguez-Macho 2010; Schietti et al. 2016). This interfluve has a recent sedimentary origin from Late Pleistocene-Early Holocene, and ranges between 30 m and 100 ma.s.l. (Sombroek 2000; Rossetti, Toledo & Góes 2005). The mean annual precipitation varies from 2200 to 2800 mm, and is highest in the middle portion of the PMI (Alvares, Stape, Sentelhas, de Moraes Gonçalves & Sparovek 2013; Fick & Hijmans 2017). Northeastern and central regions of the PMI are characterized by lowland dense rainforest with a mean tree basal area of 56.45 m^2 ha^{-1} (Schietti et al. 2016; Ferreira, Jehle, Stow & Lima 2018), and soil is mainly Plinthosols with predominance of silt (Cintra et al. 2013; Martins et al. 2014). Southwestern parts (Madeira River, Porto Velho) are characterized by lowland open rainforest with a mean tree basal area of 19.31 m^2 ha^{-1} (Ferreira, Jehle, Stow & Lima 2018) and soil in this region is Podzolic with predominant texture of clay (Cintra et al. 2013). Also, considerable areas of savanna are present between open and dense rainforest (IBGE 1997). Of the 16 sampling sites included in this study, 14 were previously used by Ferreira et al. (2018) to describe associations between soil and forest structure on the occurrence and relative abundance of *A. femoralis*.

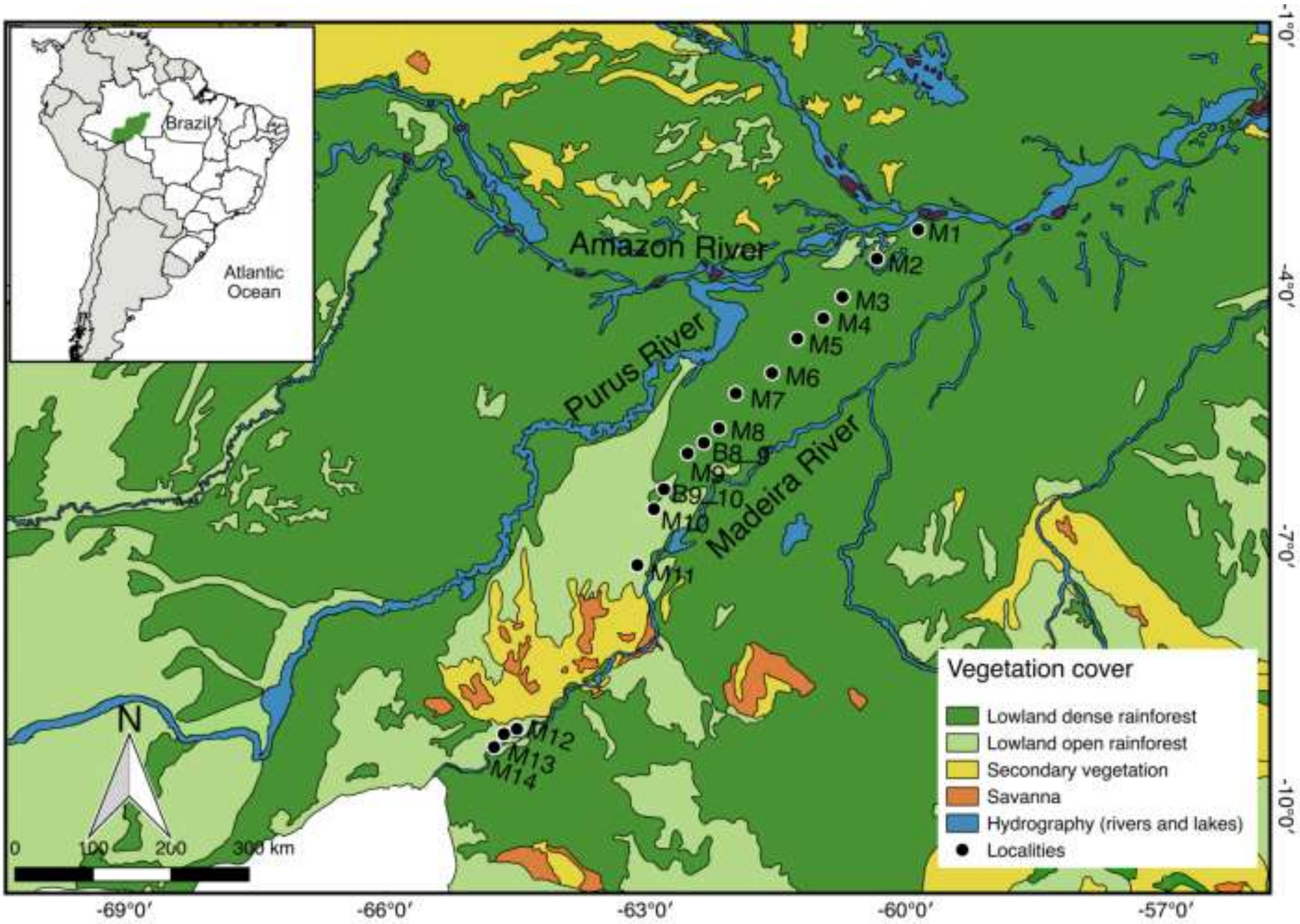


Figure 1. Map of sites at which samples of *Allobates femoralis* were collected in the central-southern Amazonia, Brazil. For results from population structure analyses see Figure 3 and sample sizes see Table 3.

We collected a total of 66 *A. femoralis* individuals from 13 localities in the PMI, 11 of which were PPBio research sites (modules, M), which follow the RAPELD configuration (see Magnusson et al. 2013 for more details), and from two localities in areas between modules in dense rainforest (BM8_9 and BM9_10) in the northeast region. We did not find *A. femoralis* in multiple surveys in three modules in the northeast of the PMI (M3-M5) due to locally unsuitable habitat (see Ferreira, Jehle, Stow & Lima 2018 for more details). *Allobates femoralis* was searched by acoustic and visual sampling during the daily periods of peak vocalization for the species (7:00-

10:00 a.m. and 14:00-18:00 p.m.). We captured frogs by hand and maintained them in sealed plastic bags until arrival in the laboratory, where they were sacrificed and fixed after tissue (leg muscle) was removed for genetic analyses. Specimens were deposited in the herpetological section of the zoological collection of the Instituto Nacional de Pesquisas da Amazônia (INPA-H, Manaus, Brazil). We collected *A. femoralis* under permits from IBAMA/SISBIO (Ministry of Environment, Government of Brazil) process numbers 13.777 and 7836-1.

Genotyping and filtering

Tissue from all 66 *A. femoralis* was processed for DNA extraction, sequencing, genotyping, and SNP discovering at Diversity Arrays Technology Pty. Ltd. (Canberra, Australia). The DArTSeq^{TM} genotyping-by-sequencing protocol used is based on a combination of Diversity Arrays (DArT) markers (Jaccoud, Peng, Feinstein & Kilian 2001; Kilian et al. 2002; Luikart, England, Tallmon, Jordan & Taberlet 2003) and sequencing with Illumina platforms (Sansaloni et al. 2011; Petroli et al. 2012) to identify single nucleotide polymorphisms (SNPs) across the genome of a target species. We used the genome of *Nanorana parkeri* (Sun et al. 2015) as a reference. Detailed descriptions of DArT marker technologies for SNP discovery and genotyping are provided in Jaccoud, Peng, Feinstein & Kilian (2001), Kilian et al. (2012) and Thompson, Stow & Raftos (2017).

A data set of 147.595 SNPs genotypes was filtered for missing data at both the individual and locus level using the R-package RADIATOR v. 0.010 (Gosselin 2017). Only individuals and loci with ≥ 95% available data were retained. SNPs were also screened for allele coverage, with any SNPs displaying a call rate < 60%, and local and global minimum allele frequencies (MAF) < 1% removed from the dataset.

Additionally, we excluded monomorphic loci and in cases where multiple polymorphisms were found within the same sequence length, only one SNP per locus was retained, and all other duplicates were removed to avoid statistical bias from physical linkage (Lemay & Russello 2015; Zheng et al. 2012). Two samples from M14 failed to pass for quality control and were excluded, retaining 64 individuals from 13 populations genotyped for 10.275 filtered SNPs (see Table 1 for all filtering step). During the filtering, the RADIATOR package converted the dataset in several formats (i.e. genotype coding system) required by several packages in further analyses. All other required file types were created using two file conversion programs PGDSpider v. 2.1.1.3 (Lischer & Excoffier 2012) and PLINK v. 1.9 (Chang et al. 2015), and the packages RADIATOR, *LEA* (Frichot & François 2015) and *adegenet* (Jombart 2008; Jombart & Ahmed 2011). The output files were used for the following analyses.

Table 1. Number of putative SNPs retained following each filtering step.

From reads to SNP	SNP count
Stacks catalog	147 595
Reproducibility	
≥ 95% of markes	147 350
Call rate	
≥ 60% of markers	48 986
Population filters	
monomorphic markers	20 220
markers commom in all populations	14 994
Individual threshold (%)	
≥ 60% of markers	14 967
MAF filters	
Global MAF = 0.01	
Local MAF = 0.01	14 967

SNPs	
SNPs per locus = 1	10 373
Heterozygosity	10 275

Our initial analysis involving all localities showed that the M1 and M2 modules were highly genetically differentiated from all other sampling sites along the PMI (e.g. F_{ST} ranged from 0.72-0.83; Supplementary Figure S1). These two modules also formed a separate cluster in our genetic structure and phylogenomic relationships analyses (Supplementary Figure S2 and S3). Because of this large genetic divergence, we did not consider the individuals of these two modules in the landscape genetics analyses.

Detection of putatively neutral SNPs

We searched for loci with a level of genetic divergence that exceeding neutral expectations using two different approaches. First, we detected putatively neutral SNPs using F_{ST} outlier analysis (OA) with BAYESCAN v. 2.1 (Foll & Gaggiotti 2008), a Bayesian method based on a logistic regression model that separates locus-specific effects of selection (adaptive genetic variation) from population-specific effects of demography (neutral genetic variation). BAYESCAN is powerful in detecting outliers in scenarios with low-admixtured samples (Luu, Bazin & Blum 2017), and we used a sample size of 200 000 and a prior model (prior odds parametrization) of 100, a thinning interval of 10-20 pilot runs of length 10 000, and a burn-in of 50 000 steps. Second, we used environmental association analysis (EAA) with Latent Factors Mixed Models (LFMM), which tests for association between loci and environmental variables using the R-package *LEA* v. 2.1.0 (Frichot & François 2015). LFMM uses a hierarchical Bayesian mixed model based on the residual of a PCA to take population genetic structure into account (Benestan et al. 2016). We selected four environmental variables that are potentially related to signatures of selection in frogs: vegetation cover type, silt content, and temperature seasonality (BIO4 as in e.g. www.worldclim.org/bioclim, showing the annual range in temperature, and the Walsh index which reflects the intensity and duration of the dry season). We summarized the variables by using the first axis of a Principal Component Analysis (PCA). To avoid biases in PCA to due scales differences in variables, we scaled the PCA by dividing the (centered) columns by their standard deviations. LFMM was run using 10 000 iterations, a burn-in of 5000 steps, 5 repetitions, and three genetic clusters (using Bayesian clustering programs, we found that K = 3 ancestral populations could better explain the data). We set both BAYESCAN and *LEA* with false discovery rate (FDR) of 0.05. This study is focused on spatially explicit neutral genetic processes thus the following analyses were performed only with putatively neutral SNPs data set (i.e. Manel, Schwartz, Luikart & Taberlet 2003).

Accessing Intraspecific Genetic Structure

We estimated intraspecific genetic structure between localities using the algorithm sNMF in the R-package LEA v. 2.1.0 (Frichot, Mathieu, Trouillon, Bouchard & François 2014), using a likelihood algorithm implemented in the programs ADMIXTURE v. 1.3.0 (Alexander, Novembre & Lange 2009), and using discriminant analysis of principal components (DAPC) in the R-package *adegenet* v. 2.1.1 (Jombart, Devillard & Balloux 2010). sNMF is a method based on sparse Non-negative Matrix Factorization algorithms (NMF) and least-squares optimization (Frichot, Mathieu, Trouillon, Bouchard & François 2014). We tested values of genetic groups (*K*) ranging from 1 to 11 (upper limit equal to *n* localities) with 20 independent runs per test, alpha set at 100, a tolerance error of 0.00001, entropy set as true (where the cross-entropy criterion is calculated), a random seed of 50 and 10 000 interactions in the algorithm. The best-supported *K* and the best run were selected by estimation of the lowest error value of ancestry through the cross-entropy criterion. The program ADMIXTURE simultaneously estimates the probability of the observed genotypes using ancestry proportions and population allele frequencies (Alexander, Novembre & Lange 2009). Significance of the methods was defined at $p < 0.05$, otherwise individuals were considered pure. We ran ADMIXTURE using cross-validation of a random seed as 43, the point estimation method as block relaxation algorithm, QuasiNewton as convergence acceleration algorithm and a delta of < 0.0001 to terminate point estimation. The number of *K* with the best fit was determined by the lowest cross-validation error value. DAPC is a multivariate method that performs discriminant functions to describe the relationships between clusters, and membership probabilities of each individual for different groups, optimizing variance between groups while minimizing variance within groups (Jombart, Devillard & Balloux 2010). We used cross-validation to define the number of PCs retained in the analysis. Cross-validation provides an objective optimization procedure for identifying the "golidlocks point" in the trade-off between retaining too few and too many PCs in the model. We used the number of PCs associated with the lowest Root Mean Squared Error - RMSE as the optimum number of PCA in the DAPC analysis. Eight PCs and two DAs were retained for the analyses, and explained 41% of the total variance.

Measuring Genetic Differentiation

We tested for significant deviation from Hardy-Weinberg Equilibrium (HWE) for each locality and locus to evaluate the null hypothesis of random mating. Each locality was considered as a population (folloeinng Oliveira et al. 2017). Observed (H_O) and expected (H_E) heterozygosity and inbreeding coefficients (F_{IS}) were calculated using the R-package *diveRsity* v. 1.9.90 (Keenan, McGinnity, Cross, Crozier & Prodöhl 2013), based on 999 bootstraps with 95% confidence interval and 1000 replicates for the Monte Carlo test. Limited gene flow and the presence of genetic structure will result in a spatial Wahlund effect (Wahlund 1928). This is because pooling genetic data where genetic structure is present will result in a heterozygosity deficit compared to HWE expectation.

To assess genetic differentiation between localities, we calculated pairwise genetic distances based on genotypic relatedness. Genotypes are shuffled at each generation, and genotypic structure derived from genotypic similarity between individuals can detect short-term processes such as the spatial distribution of close relatives (Stow, Sunnucks, Briscoe & Gardner 2001). We used the R-package *adegenet* v. 1.3.1 (Jombart & Ahmed 2011) to estimate pairwise relatedness with five different indices. We further used distance matrices based on Nei's distance to test the effect of IBD and IBR on genetic differentiation and resistance surface, and as recommended by Rousset (1997) we linearized F_{ST} using the formula $F_{ST}/(1-F_{ST})$. We also tested differences between populations and groups of populations (clusters) with analysis of molecular variance (AMOVA) using 1000 permutations, as described by Excoffier, Smouse & Quattro (1992) using the R-package *poppr* v. 2.7.1 (Kamvar, Tabima & Grünwald 2014).

Phylogenomic relationships

We explored phylogenomic relationships using SNAPP v. 1.4.1 (Bryant, Bouckaert, Felsenstein, Rosenberg & Roychoudhury 2012) implemented in BEAST v. 2.5

(Bouckaert et al. 2014). To reduce computational requirements and run times, we selected 2–3 representative individuals per population without signatures of between-population admixture to avoid bias. Mutation rates (*u* and *v*) were estimated from the data in SNAPP, with the birth rate (λ) of the Yule prior based on the number of samples used. The trial run for each dataset used a chain length of 1.000.000 generations, sampling every 1.000 trees. We inspected final log files, and created maximum clade credibility trees (median node heights) by combining three independent runs in TreeAnnotator v. 2.5 after discarding 25% as burn-in. We used the SNAPP analysis to verify the relationship between the individuals (clusters), and specifically between individuals from M1 and M2 modules of the rest of the PMI (see Supplementary Figure S3).

Constructing the Environmental Resistance Surfaces

The study area is characterized by a mosaic of different vegetation types influenced by differences in soil gradients and climatic seasonality. We selected four continuous landscape variables to be the most important resistors of *A. femoralis* gene flow: vegetation-cover type (as discrete variable), silt content, temperature seasonality (BIO4) and the Walsh index (a proxy for climate conditions that represent intensity and duration of the dry season) as continuous variables. These variables likely affect the life history of *A. femoralis* across all seasons, and reflect large-scale environments important for *A. femoralis* occurrence and abundance (Ferreira, Jehle, Stow & Lima 2018). We obtained the environmental variables in raster files from the public repository Ambdata (Amaral, Costa, Arasato, Ximenes & Rennó 2013; www.dpi.inpe.br/Ambdata). Ambdata provides environmental data for the entire Amazon basin, which we reduced to our study area coordinates using the R-package *raster* v. 2.6.7 (Hijmans 2017) with a cell resolution of 30 arcsecond (1 km^2). We then extracted the values of each sampling location and quantified correlations between variables. To avoid model overparameterization, we removed strongly correlated variables (r > 0.85) based on their presumed biological relevant for *A. femoralis*. We then standardized all raster file to values between 1 and 100, a valid approach to standardized both the range and distribution of resistance values to facilitate comparisons among surfaces (Row, Knick, Oyler-McCance, Lougheed & Fedy 2017). All environmental raster files are available at Supplementary Figure S4. We modelled resistance across the landscape following Dudaniec et al. (2013) and Dudaniec et al. (2016), evaluating each resistance surface model separately. We assumed that resistance was a function of environmental variables as follows:

$$ri = 1 + \alpha\left(\frac{Fi - 1}{100 - 1}\right)^{\gamma}$$

where ri is the resistance of raster cell i; Fi is the environmental variables value of cell i, in our case ($1 \leq Fi \geq 100$); $\alpha > 0$ is a parameter that determines the maximum possible resistance value, with $1 + \alpha$ being the maximum resistance value possible; the exponent γ is a parameter that determines the shape of the relationship between environmental variable values (Fi) and resistance (ri), being linear when $\gamma = 1$ and nonlinear when $\gamma \neq 1$ (Shirk, Wallin, Cushman, Rice & Warheit 2010; Dudaniec et al. 2013; 2016). The equation expresses resistance as a function of the effect of landscape features on resistance, and explicitly assumes that the effects of vegetation-cover type and temperature seasonality on resistance are negative and positive, respectively (Fig. 2).

We assumed a range of values for the parameters α and γ. These values were 0, 5, 10, 100, 1000 for α and 0.01, 0.1, 0.5, 1, 5, 10, 100 for γ (see Fig. 2). Raster models were created using the R-packages *sp* v. 1.2.7 (Bivand, Pebesma & Gomez-Rubio 2013), *rgdal* v. 1.2.18 (Bivand, Keitt & Rowlingson 2018), *raster* v. 2.6.7 (Hijmans 2017), *DT* v. 0.4 (Xie 2018) and *dbplyr* v. 1.2.1 (Wickham & Ruiz 2018). For each possible combination of these values (i.e. 35 models), we calculated the resistance value of each raster cell for the four landscape features, and used circuit theory (Hanks & Hooten 2013; McRae, Dickson, Keitt & Shah 2008) as implemented in CIRCUITSCAPE v. 4.0.5 (McRae 2006) to calculate pairwise resistance distance between our sampling locations for each landscape resistance surface. This approach assesses all current and total resistance between any two points and may better represent gene flow that occurs over multiple generations (McRae & Beier 2007; MacRae & Shah 2009).

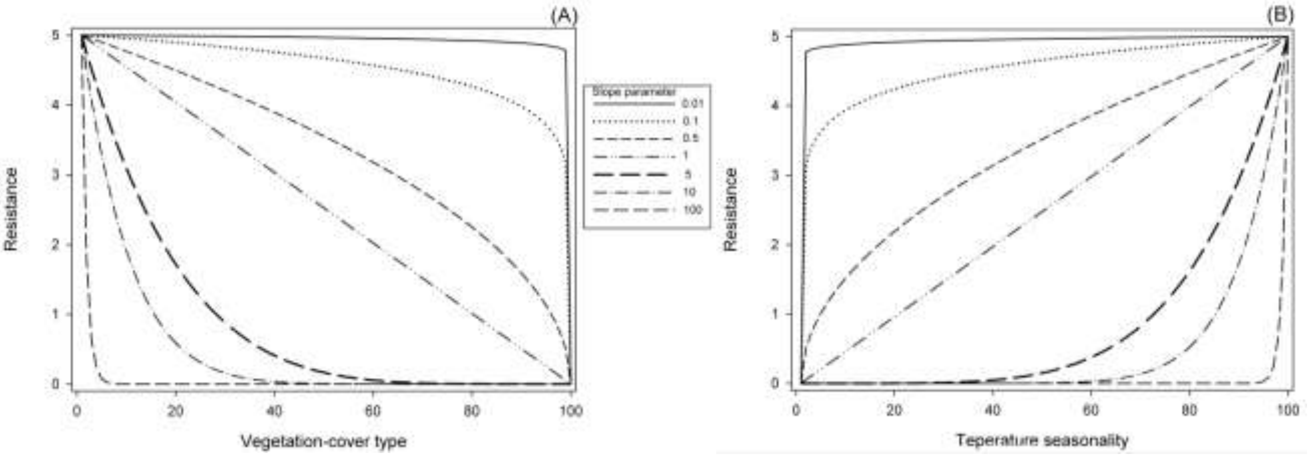


Figure 2. Shapes of the isolation-by-resistance (IBR) relationships tested for the effect of temperature seasonality and vegetation-cover type on genetic distance $F_{ST}/(1-F_{ST})$ using seven values of γ (0.01, 0.1, 0.5, 1, 5, 10, 100). The different slopes are not shown (α values) and is set at 5 here for simplicity. The curves show decreasing landscape resistances from right to left for vegetation-cover type (A) and left to right for temperature seasonality (B).

We then fitted a Maximum-Likelihood Population-Effects (MLPE) mixed-effects models for each combination of α and γ to evaluate the contribution of landscape features in explaining genetic differentiation in *A. femoralis*. This model used pairwise metrics for genetic differentiation and landscape resistance (or cost), where each pairwise data point is considered an observation. The lack of independence is incorporated as a population-level factor that distinguishes between data points that share a common deme, and those that do not (Clarke, Rothery & Raybould 2002; Row, Knick, Oyler-McCance, Lougheed & Fedy 2017). A comparison of the fit of models in a biologically relevant model set can readily accommodate ecological complexity (Burnham & Anderson 2002). Pairwise genetic distance $F_{ST}/(1-F_{ST})$ was used as the dependent variable, resistance and connectivity distance as the independent variable and population as the random variable. We fitted the mixed-effects models using parameterization to account for the non-independence of values within pairwise distance matrices without restricting maximum-likelihood (Clarke, Rothery & Raybould 2002; Van Strien, Keller & Holderegger 2012). We constructed 35 different models of all combinations of the predictor variables assuming that, in every model, the predictors for variation in the intercept were the same as for those in the slope; setting REML=FALSE in the function *mlpe_rga*, allowed the calculation of valid AIC scores (see below). Next, we performed a model selection approach to characterize parameter uncertainty using Akaike Information Criteria (AIC) to estimate the relative contributions of each parameter combination of the four landscape features. Then, we compared models by calculating the difference between the AIC of each model and the minimum AIC value found for the set of models compared (Burnham & Anderson 2002; Diniz-Filho, Rangel & Bini 2008). Note that models where α is equal to 0 (seven models for each landscape features) are identical regardless of γ values, and were thus considered to be identical, reducing the analysis to 29 unique models. These analyses were performed using R-package *ResistanceGA* v. 4.0-4 (Peterman 2018), the general function *mlpe_rga* for flexibly fitting MLPE models using the standard *lme4* v. 1.1-17 formula interface (Clarke, Rothery & Raybould 2002; Bates, Maechler, Bolker & Walker 2015), *magrittr* v. 1.5 (Bache & Wickham 2014) and *dplyr* v. 0.7.4 (Wickham, Francois, Henry & Müller 2017).

Effect of isolation by distance vs. isolation by resistance on genetic differentiation

We hypothesized that five potential drivers are largely responsible for genetic differentiation in *A. femoralis* across the Purus-Madeira interfluve: (i) geographical distance, (ii) resistance through differences in temperature seasonality, (iii) resistance through differences in silt content, (iv) resistance through differences in vegetation-cover type, and (v) resistance through differences in intensity and duration of the dry season (Walsh index). The first driver (geographical distance) represents the predicted pattern of isolation by distance (IBD), whereas the others are based on assumptions regarding the permeability of landscape features to dispersal and represent the probable isolation by resistance (IBR) pattern. These hypotheses are not mutually exclusive and, in combination, may better explain the genetic pattern we observe.

We assumed that geographical distance and temperature seasonality are inhibitors of gene flow, and thus resistance increased as the geographical distance and seasonality of temperature increased. Vegetation-cover type, silt content and Walsh index were considered promotors of gene flow (i.e. higher values would have lower resistance). In addition, large rivers represent an important barrier for anurans (Ron 2000; Kaefer, Tsuji-Nishikido, Mota, Farias & Lima 2013; Dias-Terceiro et al. 2015; Simões 2016; Godinho & Da Silva 2018; Ortiz, Lima & Werneck 2018), including *A. femoralis* (see Simões, Lima, Magnusson, Hödl & Amézquita 2008; 2014). We therefore reasoned that, because the Purus-Madeira interfluve is delimited by these rivers and *A. femoralis* strongly avoids flooded habitats, gene flow is likely restricted by these areas. Consequently, we assigned these rivers a prohibitively high resistance in each resistance distance raster. All rasters are available in Figure S4.

The effect of isolation by distance (association between geographic and $F_{ST}/(1-F_{ST})$) was tested among all sampling localities through a Mantel test (Mantel 1967) using the function *mantel.randtest* implemented in the *ade4* v. 1.7-11 R-package (Dray & Dufour 2017), under 10.000 permutations. Geographic distance was modeled with Cartesian coordinates and Nei's distance. The shortest distance between sampling sites was 17.77 km between M12 and M13 and the longest was 601.44 km between M6 and M14 as measured with a Global Positioning System (Supporting information Table S2). We also carried out an independent test for spatial autocorrelation between geographic distance and genetic differentiation along classes of distances using a Mantel correlogram (Oden & Sokal 1986), computed using the function *mantel.correlog* with 10.000 permutations, number of classes as selected by the Strurges equation, using Pearson correlation and correction of *P*-values through FDR in the vegan v. 2.5-1 R-package (Oksanen et al. 2018). We set the null hypothesis where no genetic differentiation is associated with classes of geographic distance at $p > 0.05$.

The effect of isolation by resistance (association between genetic and cost distance) decoupled from geographic distance (IBD) was calculated using distance-based redundancy analysis (dbRDA) in the *vegan* v. 2.5.1 R-package (Oksanen et al. 2018). dbRDA is a direct extension of multiple regression to model multivariate response data (Legendre & Gallagher 2001; Benestan et al. 2016). We performed an dbRDA to quantify the correlation between the best MLPE model for each landscape feature and $F_{ST}/(1-F_{ST})$, assuming models with genetic differentiation as dependent variable and cost distances as independent variables, conditioned on IBD (geographic distance in km). These models were useful to investigate the separate influences of landscape features on genetic variation among samples when IBD is not taken into account. dbRDA is also more accurate for continuously distributed species, and its type II error rates are lower than partial Mantel tests (Kierepka & Latch 2015). We obtained statistical significance from each dbRDA model using analyses of variance (ANOVAs; 1000 permutations).

Results

Data filtration and detection of putatively neutral SNPs

The individuals from the northern modules M1 and M2 were found to constitute evolutionary units unique from the rest of the PMI (see Supplementary Figures S1, S2 and S3), and therefore were excluded from the landscapes genetic analyses.

Using 54 individuals of *A. femoralis* from 11 localities, we obtained a total of 10.275 filtered and informative SNPs. *BAYSCAN* detected 174 SNPs (2,28%) and LFMM identified 639 SNPs (8,37%) putatively under divergent selection at the 5% significance level.

Population Structure

ADMIXTURE, sNMF and DAPC analyses produced congruent results for the number of inferred ancestral populations (*K* = 3; Fig. 3). The three analyses also identified the same grouping pattern: a cluster in the first three modules (M6 to M8), a cluster in the five subsequent sites (from BM8_M9 to M11), and another cluster confined to the eastern bank of upper Madeira river (M12 to M14). The largest cluster B was comprised of 24 individuals and had the largest geographic distribution across dense and open forest ecotypes, however with a considerable admixture with individuals of cluster A (cluster A = 0.90 ± 0.17; cluster B = 0.88 ± 0.12; cluster C = 0.99 ± 0.00). Cluster A was situated in dense forest area and comprised 14 individuals (M6-M8). Cluster C was comprised of 16 individuals from open forest area separated from the open forest further northeast (M11) by transition area with savanna patches (see Fig. 1). Individuals from the three clusters did not overlapped in the DAPC plots (Fig. 4).

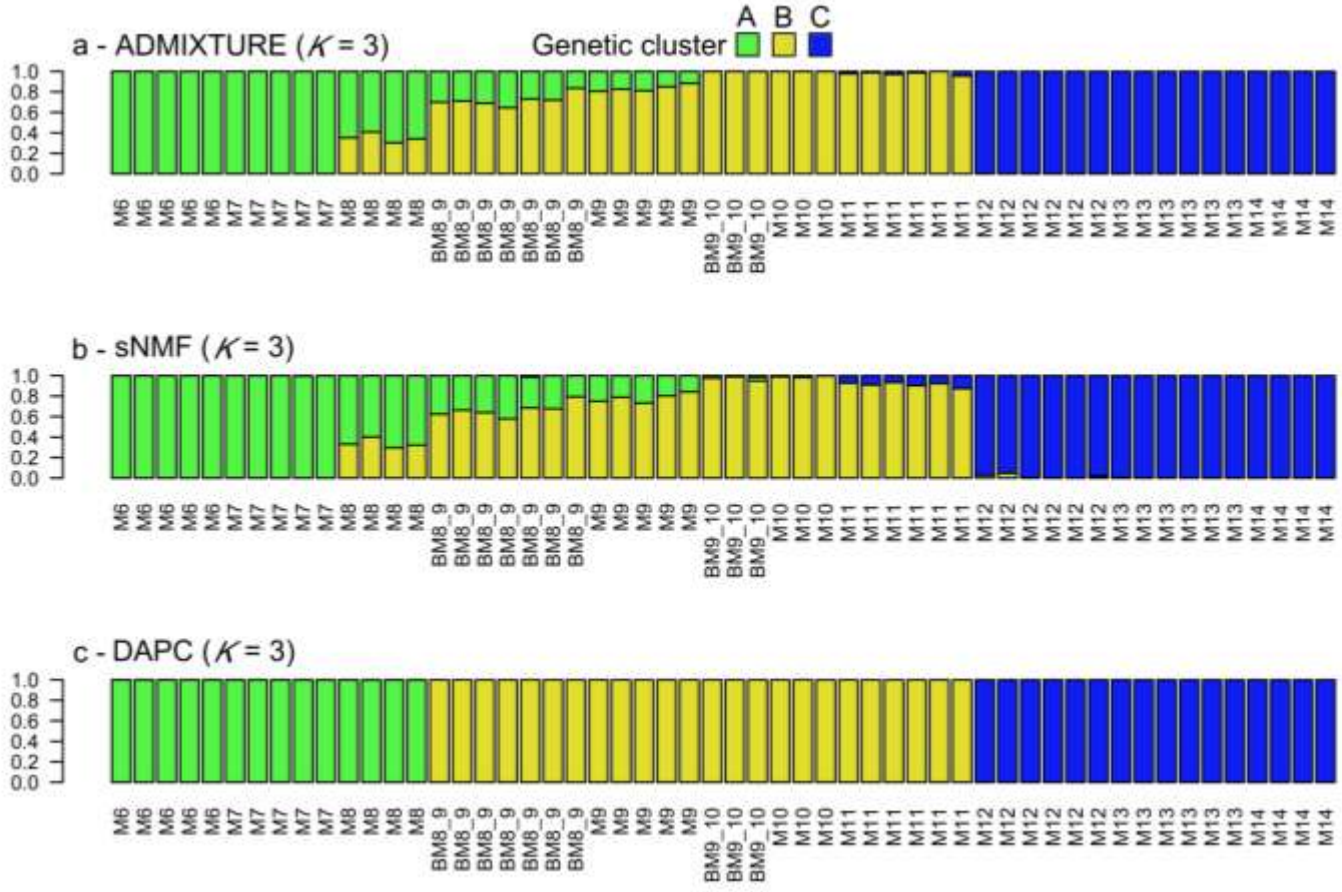


Figure 3. Barplots of population structure analyses conducted for *Allobates femoralis* along Purus-Madeira interfluve in central-southern Amazonia, resulting from SNPs. A) ADMIXURE, B) sNMF and C) DAPC. Sampling locations and the most probable number of genetic clusters (k) is provided. Each genetic cluster is assigned to a different color, and each bar represent an individual with the proportion of their admixed genome ($p < 0.05$), except to DAPC.

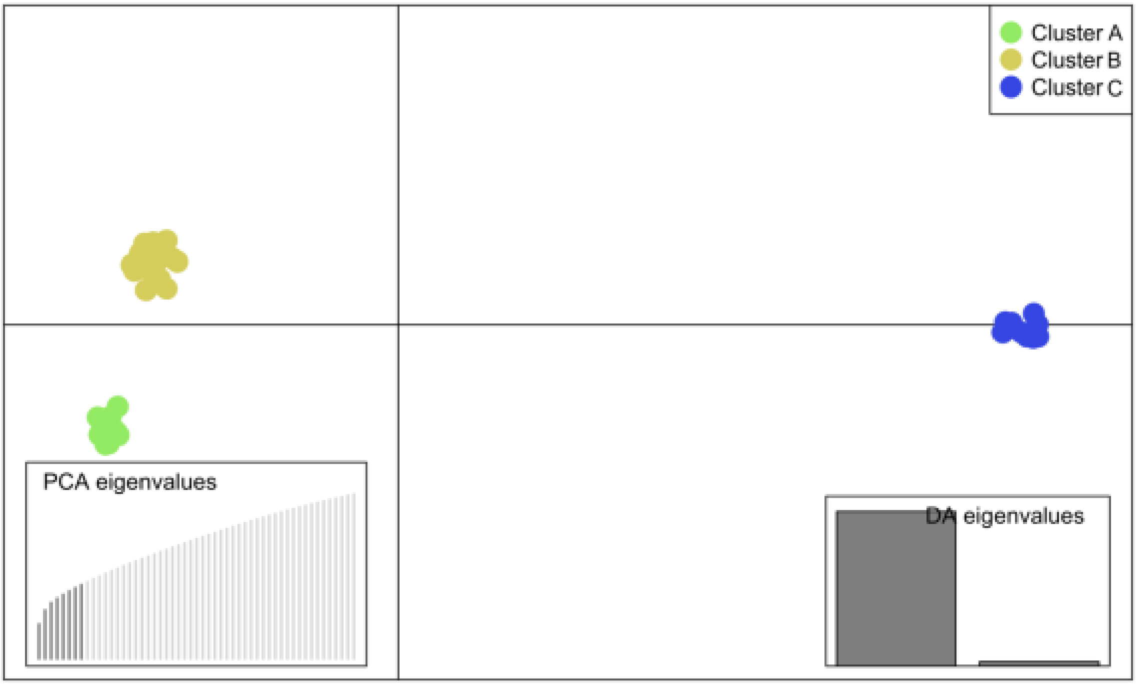


Figure 4. Results of the DAPC analysis among geographic populations of *Allobates femoralis* using 10 275 SNPs. Custer A (green circles), samples from M6-M8; cluster B (yellow circles), samples from B8_9-M11 and Cluster C (blue circles), samples from M12-M14.

Genetic Differentiation

Levels of genetic variation are given in Table 2. No significantly deviations from Hardy-Weinberg equilibrium (HWE) were observed within populations. However, when pooling data across populations we found significant deviations from HWE for 1.614 (21.15%) putatively neutral SNPs, and for 1.117 SNPs (14.63%) when adjusted for false discovery rates (FDR). Observed heterozygosity ranged from 0.08 (M14) to 0.12 (M8), and expected heterozygosity ranged from 0.08 (M14) to 0.13 (BM8_9 and M9). Inbreeding coefficients ranged between -0.077 (M14) and 0.167 (BM8_9).

Table 2. Genetic differentiation indexes calculated for *Allobates femoralis* sampling sites with number of collected individuals along Purus-Madeira interfluve in central-southern Amazonia.

	Parameter						
Site	N_{TOTAL}	H_O	H_E	F_{IS}	F_{IS} low	F_{IS} high	HWE
M6	5	0.09	0.09	0.0434	-0.0341	0.0827	1
M7	5	0.10	0.10	0.0126	-0.0756	0.0719	1
M8	4	0.12	0.12	0.0389	-0.1102	0.1448	1
BM8_9	7	0.11	0.13	0.1672	0.0524	0.239	1
M9	5	0.11	0.13	0.1202	-0.0517	0.2236	1
BM9_10	3	0.11	0.11	0.0023	-0.053	0.0985	1
M10	3	0.11	0.11	-0.0151	-0.0719	0.077	1
M11	6	0.10	0.11	0.1244	-0.0025	0.1777	1
M12	6	0.09	0.10	0.0386	-0.0846	0.1041	1
M13	6	0.09	0.09	0.009	-0.128	0.0742	1
M14	4	0.08	0.08	-0.077	-0.2999	0.0515	1

Parameters are as follows: number of sampled individuals (N_{TOTAL}), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}) and their low and high values are presented for each locality and Hardy-Weinberg equilibrium (HWE). Sampling localities abbreviations: M (modules), BM (between modules).

Pairwise genetic distances (F_{ST}) ranged from 0.020 (M13 vs. M14) to 0.207 (M6 vs. M14, Table 3), and generally increased with geographic distance (highest between M6-M8 in the northeast and M12-M14 inn the southeast, see Table 3 and Table S2). AMOVAs for populations and genetic clusters revealed that most of the genetic variation was expressed among individuals (59.13% and 53.80%, resopectively), followed by the variation among the populations (34.97%) and clusters (33.91%) as well as among individuals within populations (5.90%) and genetic clusters (12.29%).

Statistical significance recovered by permutation tests on both AMOVAs are below 0.001. Detailed information on sample size per localities are shown in Table 2.

Table 3. Pairwise genetic distances (F_{ST}) based on Nei's distance estimates between sampling sites.

	M6	M7	M8	BM8_9	M9	BM9_10	M10	M11	M12	M13	M14
M6	-	0.037	0.058	0.080	0.094	0.121	0.127	0.137	0.198	0.201	0.207
M7		-	0.048	0.071	0.082	0.108	0.114	0.125	0.185	0.189	0.194
M8			-	0.043	0.050	0.076	0.082	0.092	0.154	0.157	0.162
BM8_9				-	0.032	0.055	0.063	0.073	0.135	0.138	0.143
M9					-	0.051	0.058	0.070	0.133	0.137	0.141
BM9_10						-	0.051	0.061	0.130	0.133	0.139
M10							-	0.053	0.133	0.137	0.141
M11								-	0.116	0.122	0.125
M12									-	0.021	0.029
M13										-	0.020
M14											-

Isolation by geographic distance

There were significant associations between genetic and geographic distances, supporting IBD among sampling sites in *A. fe*moralis (Fig. 5, Mantel test: $p < 0.0001$, $r^2 = 0.96$). The Mantel correlogram calculated for seven classes of geographic distance (km) over genetic distances showed spatial autocorrelation in four cases (Fig. 6). Genetic distance was positively correlated with geographic distances within 60 km ($r = 0.67$, $p < 0.001$) and 143 km ($r = 0.24$, $p = 0.02$), while a negatively correlation was observed for scales of 476 km ($r = -0.61$, $p = 0.03$) and 560 km ($r = -0.61$, $p < 0.001$).

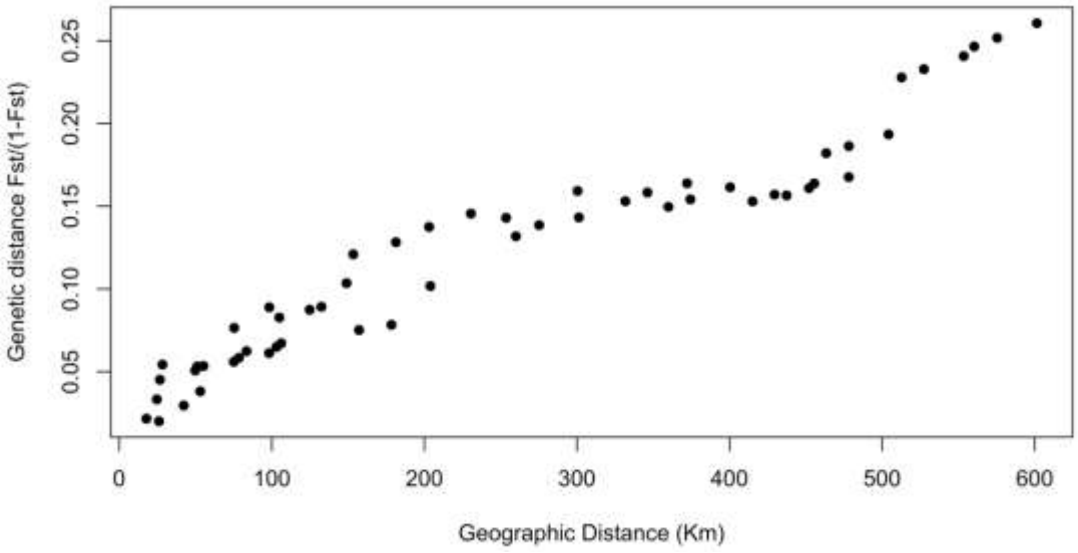


Figure 5. Patterns of gene flow based on correlation between genetic distance F_{ST}/(1-F_{ST}) and geographic distance, indicating isolation by geographic distance in *A. femoralis* across Purus-Madeira interfluve.

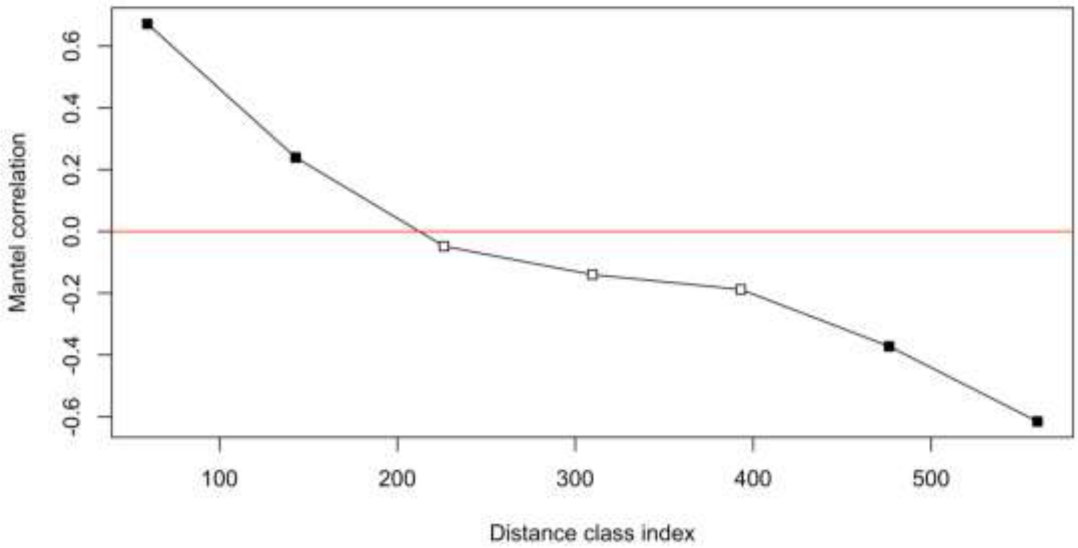


Figure 6. Spatial autocorrelation of Mantel calculated for seven classes of geographic distance (km) over genetic distances *F*st/(1-*F*st) in *Allobates femoralis* across Purus-Madeira interfluve. Black squares indicate significant distance classes ($p \leq 0.05$).

Isolation by environmental resistance

The best model (lowest AICc) for the effect of vegetation-cover type on genetic distance in *A. femoralis* was $\alpha = 5$, $\gamma = 10$, and explained 98% of the genetic variation (Table 4). Walsh index explained 96% of the genetic variation at $\alpha = 100$ and $\gamma = 5$ (Table 4). Temperature seasonality and silt content explained 95% of the genetic variation in both cases, with the maximum-likelihood parameters of $\alpha = 10$ and 1000, and $\gamma = 5$ and 1, respectively (Table 4). This parameter combination indicates that IBR is at the maximum value when all landscape features have values of 100 (see Fig. 2). All the ΔAIC values were the same for the four landscape features (0.00), supporting the maximum-likelihood models. In the dbRDA models, the Walsh index best explained the observed genetic variation of *A. femoralis* and captured 8.4% of the observed variation ($F_{1,52} = 41.72$; $p = 0.001$), followed by vegetation-cover type (6.4%; $F_{1,52} = 26.85$, $p = 0.001$), temperature seasonality (5.3%; $F_{1,52} = 20.54$, $p = 0.001$) and silt content (3.5%; $F_{1,52} = 11.79$, $p = 0.001$; Table 4). The figures from the dbRDA models are available in Figure S5.

Table 4. Summary of model selection, maximum likelihood population effects mixed effects models (MLPE) and distance-based redundancy analyses (dbRDA) showing the effects of isolation by resistance (IBR) on gene flow in *Allobates femoralis* along the Purus-Madeira Interfluve.

			MLPE				dbRDA			
Variables	α	γ	AICc	ΔAIC	r^2	SE	*t*-value	r^2	F	*p*
Vegetation-cover type	5	10	36.66	0.00	0.98	0.0800	21.223	0.064	26.85	**0.001**
Walsh index	100	5	51.81	0.00	0.96	0.0707	17.025	0.084	41.72	**0.001**
Temperature seasonality	10	5	52.73	0.00	0.95	0.0903	16.053	0.053	20.54	**0.001**
Silt content	1000	1	50.69	0.00	0.95	0.0887	16.557	0.035	11.79	**0.001**

AIC, Akaike Information Criteria. The parameter combination of the best models with the maximum-likelihood for each landscape features is shown (‘α and γ’). The AICc and ΔAIC of this parameter combination is shown, as well as the model correlation coefficient (‘r^2’ = proportion of variance explained by the model) and standard error (‘SE’) and finally, the size of the difference relative to the variation of the data (‘*t*-value’) is shown. The F and *p* values in dbRDA were obtained by ANOVA. Bolded *p* values show significant effects of IBR on genetic distance in *Allobates femoralis*. Coefficients from MLPE and dbRDA were obtained using genetic distance as response variable and environmental cost as predictor variables. We used log(F_{ST}/1-F_{ST}) and geographic distance in km both in the MLPE and in the dbRDA.

Discussion

The localities M1 and M2 had very high genetic distances relative to individuals from other localities along the PMI, and taxonomic considerations of our findings will be addressed in future research. In addition to this region undergoing several profound historical changes in drainage systems (see Hoorn et al. 2010; Latrubesse et al. 2010; Nogueira, Silveira & Guimarães 2013), these two localities are situated on very recent holocenic terraces that have been dated to the last 6 to 2 mya (Rossetti, Mann de Toledo & Góes 2005), characterized by a mosaic of seasonally flooded and upland forests (Brasil 1978). Besides the recent geologic history, the forests in this northern portion of the interfluve are comprised of smaller and younger trees, and have high rates of biomass turnover when compared to forests that grow in well drained soils within the interfluve (Cintra et al. 2013; Schietti et al. 2016).

Differentiation and genetic structure within the Purus-Madeira interfluve

Our findings corroborate the gradient hypothesis as a mechanism for Neotropical diversification (Wright 1943; Endler 1977; Nosil 2012). *Allobates femoralis* populations within the PMI showed strong spatial genetic structure, with higher gene flow among geographically closer localities combined with ecological adaptation to dense and open forest ecotypes (Fig. 1). The pattern found for *A. femoralis* in this study is reflected in other vertebrates within the PMI. An arboreal frog (*Osteocephalus taurinus*) also has a sharp phylogeographic break in its populations (Ortiz, Lima & Werneck 2018), and an abrupt cline in color for the bird populations (*Gymnopithys salvini*; De Abreu, Schietti & Anciães 2018). In the past this region experienced an extremely cold and dry climate during glacial periods in the late Pleistocene (Cohen et al. 2014), which may explain this break of gene flow in different vertebrates. We believe that clusters C and part of B of *A. femoralis,* as well as *O. taurinos* and *G. salvini* could have been historically exposed to more climatically unstable and ephemeral conditions, followed by isolation of cluster C in the open forest from cluster B occupying drier forest with patches of savanna. The southwestern PMI is drier as well as colder, with more concentrated rains over short periods (IBGE 1997; Alvares, Stape, Sentelhas, de Moraes Gonçalves & Sparovek 2013).

Individuals cluster B who have the color of the femoral spot yellow expand into the dense forest until they come into contact with individuals cluster A who have the color of the femoral spot red. Cluster A is dominant within the dense forest. In the contact zone between different morphotypes in relation to femoral spot color (between M8-M9), we found hybrids with orange colors. We believe that this phenotypic variation may be responsible for the structuring of these two populations. Although the geographic variation of the color of the femoral spot of *A. femoralis* has been previously documented (Amézquita et al. 2009), still do not have a quantitative assessment of such variation and how it relates to the phenotypic divergence among populations. Our captive reproduction experiments (mate-recognition traits) indicated that the *A. femoralis* females have strong sexual selection in relation to the color of the femoral spot of males (AP Lima and AS Ferreira, unpublished data), and future studies need to test how the levels of connectivity can help explain the phenotypic variation in coloration of this species. Rodríguez et al. (2015) reported that forest-specialist anurans tend to have stronger genetic structure than open-area specialists, however, *A. femoralis* is generalist and use breeding habitats inside and outside the forest (Gascon 1993).

Suitable areas may maximize individual fitness and shelter large population sizes (Gugger, Ikegami & Sork 2013; Nagaraju et al. 2013), and hence it is expected that individuals will immigrate from neighboring, less suitable areas. Therefore, differences in the macro habitat (forest structure and climate) could have produced distinct ecological responses (e.g., in physiological preferences and dispersal capabilities) in continuous *A. femoralis* populations distributed along the PMI environmental gradient, thus promoting their genetic differentiation. In addition, historical processes, for example, ancient geomorphological barriers have been proposed as drivers of geographic differentiation involving secondary contact for Amazonian frogs (Gascon et al. 1998; Lougheed, Gascon, Jones, Bogart & Boag 1999; Symula et al. 2003; Simões et al 2008). However, we could not determine the type of contact (primary or secondary) for *A. femoralis* populations within PMI, an issue which must be addressed in future research.

Isolation by resistance and by distance

Our study demonstrates that the genetic variation in *A. femoralis* is affected both by resistance imposed by landscape features and geographic distance. Landscape features can potentially influence the spatial connectivity and rates of gene flow among populations. The four landscape features used by us had significant effects on genetic differentiation among *A. femoralis* populations, although they only explained around

5,9% of its variance (Table 4). A previous study carried out in this interfluve showed that *A. femoralis* is more abundant in open forests with clay-rich soils (Ferreira, Jehle, Stow & Lima 2018). The main factors related to the variation in vegetation structure are climatic conditions, biotic interactions, soil conditions and its occurrence in areas subject to seasonal flooding (Sombroek 2000; Thuiller 2007; Quesada et al. 2010; Olivares, Svenning, Van Bodegom & Balslev 2015; Figueiredo et al. 2017). Although within the PMI does not have physical barriers, this area is not spatially uniform due to the climatic and environmental changes that occur throughout its entire length. The correlation between genetic and current landscape features found in *A. femoralis* reflect the climatic and environmental discontinuity of this interfluve. The foraging mode of garden treeboa snake (*Corallus hortulanus*) was associated with genetic divergence with significant effects of IBR on gene flow within PMI (Fraga, Lima, Magnusson, Ferrão & Stow 2017), for this species, nonflooded rainforests are habitats providing genetic connectivity. Amphibians are potentially affected by landscape features (Zeisset & Beebee 2008; Dudaniec, Spear, Richardson & Storfer 2012; Peterman, Connette, Semlitsch & Eggert 2014). Previous studies reporting their associations between landscape feature without physical barriers with processes of population connectivity (gene flow), population structure and speciation (e.g., Graham, Ron, Santos, Schneider & Moritz 2004; Richardson 2012; Wang 2012; Richter-Boix, Quintela, Kierczak, Franch & Laurila 2013; Buskirk 2014).

Our results show that gene flow in *A. femoralis* is heavily influenced by IBD, given that genetic distance increases with geographic distance (Table 3). IBD is especially common among ectotherms, suggesting a metabolic basis underlying gene flow (Jenkins et al. 2010, Oliveira et al. 2017). Ectotherms like frogs must modulate activity and location based on external temperature. Thus, they are necessarily constrained to disperse within strict temperature limits and temporal windows of opportunity (Janzen 1967; Ghalambor, Huey, Martin, Tewksbury & Wang 2006; Ortiz, Lima & Werneck 2018). Although our results support more IBD, our landscape features can be considered measure of habitat quality for *A. femoralis*, and add unique contributions to the observed genetic pattern. Some evidence suggests that variation in habitat quality drives differential dispersal and, therefore, population differentiation (Garant, Kruuk, Wilkin, McCleery & Sheldon 2005). We acknowledge that *A. femoralis* individuals can disperse along the entire interfluve because it is found in different regions within the PMI, but they seem to be filtered by ecological and environmental filters as the proportion of these individuals was low in dense forest and abundant in open forest. (Ferreira, Jehle, Stow & Lima 2018).

Our interpretation is supported by previous studies with frog species that reported adaptive divergence of populations to different habitat types across continuum landscapes thus suggesting incipient ecological specialization (Richter-Boix, Quintela, Kierczak, Franch & Laurila 2013; Buskirk 2014; Rodríguez et al. 2015; Ortiz, Lima & Werneck 2018); and by studies that have suggested that steep genetic clines and contact zones are maintained by reinforcement due to morphological, acoustic and genetic character displacement as prezygotic mechanism (Hoskin, Higgie, McDonald & Moritz 2005) and hybrid unviability as postzygotic mechanisms (Simões, Lima & Farias 2012; Smith, Hale, Kearney, Austin & Melville 2013). The evolution of these mechanisms between areas are thought to reduce the likelihood of heterospecific encounters (Nosil 2012), thus maintaining the geographic differentiation of parapatric populations (Endler 1977). Therefore, we propose that differences in the macro habitat (forest structure, soil and climate) could have produced distinct ecological responses (i.e., in physiological preferences and dispersal capabilities) in continuous *A. femoralis* populations distributed along the PMI environmental gradient, thus promoting their genetic differentiation. This may be especially important considering that *A. femoralis* is a diurnal frog with territorial behavior and females present low mobility even across years (Ringler, Ursprung & Hödl 2009; Montanarin, Kaefer & Lima 2011).

Conclusion

This study constitutes a first approach to understanding drivers of genetic variation of a widespread Amazonian forest-floor frog at an interfluvial landscape level, based on a large sampling effort. Our results show that landscape features and geographical distances were enough to explain divergence among populations of *A. femoralis* within one interfluve in the Amazon where, for this species, the presence of barriers to gene flow was limited when compared to multiple interfluvial areas or obvious physical barriers. This study also illustrates how patterns of genetic variation can be studied in a spatially continuous environment with high heterogeneity to investigate broader current biogeographic processes through the coupling of landscape genetics with the use of SNPs, and model-based approaches.

In this perspective, our results showed that *A. femoralis* form geographically structured clusters along the continuous landscapes within PMI, without physical barriers (i.e. rivers, emerging arches and mountains), reflecting complex structuring scenarios related with both the environmental variation and geographic distance, but maybe also influenced by deep historical events in the Amazon Basin diversification (Antonelli et al. 2010; Hoorn et al. 2010), and sexual selection through of different phenotypic traits. Long-term isolation is not essential to generate significant genetic differentiation (Knowles & Alvarado-Serrano 2010), but this seems to be the case for the *A. femoralis* complex, where our approach yielded stronger support for the occurrence and abundance models (Ferreira, Jehle, Stow & Lima 2018) that showed that despite the potential for dispersal of *A. femoralis,* environmental variation act as an environmental filter. These characteristics make *A. femoralis* an important evolutionary system to track signs of ecologically based diversification in Amazonia. This approach is particularly relevant for poorly studied regions, such as the Amazon basin where threats are escalating.

Acknowledgments

This study was funded by the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) under grants conceded to Albertina PL (CNPq: Programa Ciência sem Fronteiras process 401327/2012- 4; FAPEAM/CNPq/PRONEX process 586/10, Edital 003/2009 – number 137) and Anthony SF received a PhD scholarship by CNPq (number 161883/2014-1) and by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) through Programa de Doutorado-Sanduíche no Exterior (PDSE) (Finance Code 001, number 88881.133683/2016-01). Specimens were collected under permit numbers 13777-1 (Albertina PL) issued by Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio of the Ministry of Environment, Government of Brazil. Fieldwork and infrastructure were funded by the Instituto Nacional de Pesquisas da Amazônia (INPA), Programa de Pesquisa em Biodiversidade (PPBio/RAPELD), Centro Integrado de Estudos da Biodiversidade Amazônica (INCT - CENBAM) and Santo Antônio Energia S.A. We thank Chris Barratt and Rachael Dudaniec for providing R scripts, and Sonu Yadav and Alex Carey for helping with R scripts. We are grateful to all members of the Conservation Genetics Lab of Macquarie University for valuable discussion during early stages of this study. We are very grateful to the field assistants for their help in collecting *Allobates femoralis* over the years, and to W. Magnusson for providing comments on the study design.

Author contributions

ASF, APL, and MF conceived the study. APL acquired funding for this study. APL, ASF conducted field work. APL and AS supervised the study. ASF conducted bioinformatic analyses and wrote the first draft of this manuscript. ASF, APL, RJ, MF and AS reviewed, edited and approved the final draft of this manuscript.

Conflict of interest

The authors declare there are no competing interests.

Data accessibility

Raw SNP data (rad file), putatively neutral SNPs (genepop file), raster files of environmental variables, genetic and geographical distance matrices, and R scripts are available at the Dryad Digital Repository (datadryad.org) at the DOI:

References

Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, **19**, 1655-1664. DOI: 10.1101/gr.094052.109

Alvares CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G (2013) Köppen’s climate classification map for Brazil. *Meteorologische Zeitschrift*, **22**, 711-728. DOI: 10.1127/0941-2948/2013/0507

Amaral S, Costa CB, Arasato LS, Ximenes AC, Rennó CD (2013) AMBDATA: variáveis ambientais para modelos de distribuição de espécies (MDEs). *Anais do XVI Simpósio Brasileiro de Sensoriamento Remoto* (SBSR), **16**, 6930-6937.

Amézquita A, Lima AP, Jehle R, Castellanos L, Ramos O, Crawford AJ, Gasser H, Hödl W (2009) Calls, colours, shapes, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. *Biological Journal of the Linnean Society*, **98**, 826-838. DOI: 10.1111/j.1095-8312.2009.01324.x

Antonelli A, Quijada-Mascareñas A, Crawford AJ, Bates JM, Velazco PM, Wüster W (2010) Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. Amazonia, landscape and species evolution: A look into the past. (ed. by C. Hoorn and F.P. Wesselingh). pp. 386-404. Blackwell Scientific Publications, Oxford.

Bache SM, Wickham H (2014) magrittr: A Forward-Pipe Operator for R. R package version 1.5. https://CRAN.R-project.org/package=magrittr

Barratt CD, Bwong BA, Jehle R, Liedtke HC, Nagel P, Onstein RE, Portik DM, Streicher J, Loader S (2018) Vanishing refuge? Testing the forest refuge hypothesis in coastal East Africa using genome-wide sequence data for seven amphibians. *Molecular Ecology*. ? DOI: 10.1111/mec.14862

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1-48. DOI: 10.18637/jss.v067.i01.

Benestan L, Quinn BK, Maaroufi H, Laporte M, Clark FK, Greenwood SJ, Rochette R, Bernatchez L (2016) Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (*Homarus americanus*). *Molecular Ecology*, **25**, 5073-5092. DOI: 10.1111/mec.13811

Bivand R, Keitt T, Rowlingson B (2018) rgdal: bindings for the 'geospatial' data abstraction library. R package version 1.2-18. https://CRAN.Rproject.org/package=rgdal

Bivand RS, Pebesma E, Gomez-Rubio V (2013) Applied spatial data analysis with R, Second edition. Springer, NY. http://www.asdar-book.org/

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, **10**, e1003537.

Borges S, Silva J Da (2012) A new area of endemism for Amazonian birds in the Rio Negro Basin. *The Wilson Journal of Ornithology*, **124**, 15-23.

Brasil (1978) RADAMBRASIL Folha SB-20 Purus; geologia, geomorfologia, pedologia, vegetação e uso potencial da terra. Rio de Janeiro. Departamento Nacional de Produção Mineral.

Bryant D, Bouckaert R, Felsenstein J, Rosenberg NA, Roychoudhury A (2012) Inferring species trees directly from biallelic genetic markers: Bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution*, **29**, 1917-1932.

Burnham K, Anderson D (2002) Model selection and multi-model inference: A Practical Information Theoretic Approach, 2nd edn. Springer-Verlag, New York, NY.

Bush MB (1994). Amazonian speciation: a necessarily complex model. *Journal of Biogeography*, **21**, 5-17. DOI: 10.2307/2845600

Buskirk JV (2014) Incipient habitat race formation in an amphibian. *Journal of Evolutionary Biology*, **27**, 585-592. DOI: 10.1111/jeb.12327

Caldwell JP (1996) The evolution of myrmecophagy and its correlates in poison frogs (Family Dendrobatidae). *Journal of Zoology*, **240**, 75-101. DOI: 10.1111/j.1469-7998.1996.tb05487.x

Calsbeek R, Smith TB, Bardeleben C (2007) Intraspecific variation in *Anolis sagrei* mirrors the adaptive radiation of Greater Antillean anoles. *Biology Journal of Linnean Society*, **90**, 189-199.

Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*, **25**, 4-7. DOI: 10.1186/s13742-015-0047-8

Cintra BBL, Schietti J, Emillio T, Martins D, Moulatlet G, Souza P, Levis C, Quesada CA, Schöngart, J (2013) Soil physical restrictions and hydrology regulate stand age and wood biomass turnover rates of Purus–Madeira interfluvial wetlands in Amazonia. *Biogeosciences*, **10**, 7759-7774. DOI: 10.5194/bg-10-7759-2013

Clarke RT, Rothery P, Raybould AF (2002) Confidence limits for regression relationships between distance matrices: Estimating gene flow with distance. *Journal of Agricultural, Biological, and Environmental Statistics*, **7**, 361-372. DOI: 10.1198/108571102320

Cohen MCL, Rossetti DF, Pessenda LCR, Friaes YS, Oliveira PE (2014) Late Pleistocene glacial forest of Humaitá–western Amazonia. *Palaeogeography, Palaeoclimatology, Palaeoecolgy*, **415**, 37-47. DOI: 10.1016/j.palaeo.2013.12.025

Colinvaux PA, De Oliveira PJ, Bush MB (2000). Amazonian and neotropical plant communities on glacial time-scales: The failure of the aridity and refuge hypotheses. *Quaternary Science Reviews*, **19**, 141-169.

Cracraft J (1985) Historical biogeography and patterns of differentiation within the South American avifauna: areas of Endemism. *Ornithological Monographs*, **36**, 49-84.

De Abreu FHT, Schietti J, Anciães M (2018) Spatial and environmental correlates of intraspecific morphological variation in three species of passerine birds from the Purus–

Madeira interfluvium, Central Amazonia. *Evolutionary Ecology*, **32**, 191-214. DOI: 10.1007/s10682-018-9929-4

Dias-Terceiro RG, Kaefer IL, Fraga R, Araújo MC, Simões PI, Lima AP (2015) A matter of scale: historical and environmental factors structure anuran assemblages from the Upper Madeira River, Amazonia. *Biotropica*, **47**, 259-266 DOI: 10.1111/btp.12197.

Diniz-Filho JAF, Rangel TFLVB, Bini LM (2008) Model selection and information theory in geographical ecology. *Global Ecology Biogeography*, **17**, 479-488. DOI: 10.1111/j.1466-8238.2008.00395.x

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22**, 1-20. DOI: 10.18637/jss.v022.i04

Dudaniec RY, Rhodes JR, Wilmer JW, Lyons M, Lee KE, Mcalpine CA, Carrick FN (2013) Using multilevel models to identify drivers of landscape-genetic structure among management areas. *Molecular Ecology*, **22**, 3752-3765. DOI: 10.1111/mec.12359

Dudaniec RY, Spear SF, Richardson JS, Storfer A (2012) Current and historical drivers of landscape genetic structure differ in core and peripheral salamander populations. *PLoS One*, **7**, e36769. DOI: 10.1371/journal.pone.0036769

Dudaniec RY, Wilmer JW, Hanson JO, Warren M, Bell S, Rhodes JR (2016) Dealing with uncertainty in landscape genetic resistance models: a case of three co-occurring marsupials. *Molecular Ecology*, **25**, 470-486. DOI: 10.1111/mec.13482

Emilio T, Quesada CA, Costa FRC, Magnusson WE, Schietti J, Feldpausch TR, Brienen RJW, Baker TW, Chave J, Álvarez E, Araújo A, Bánki O, Castilho CV, Honorio EN, Killeen TJ, Malhi Y, Mendoza EMO, Monteagudo A, Neill D, Parada JA, Peña-Cruz A, Ramirez-Angulo H, Schwarz M, Silveira M, Steege H, Terborgh JW, Thomas R, Torres-Lezama A, Vilanova E, Phillips OL (2013) Soil physical conditions limit palm and tree basal area in Amazonian forests. *Plant Ecology & Diversity*, **7**, 215-229. DOI 10.1080/17550874.2013.772257.

Endler JA (1977) Geographic variation, speciation, and clines. Princeton University Press, Princeton, pp 262.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479-491.

Fan Y, Miguez-Macho G (2010) Potential groundwater contribution to Amazon evapotranspiration. *Hydrology and Earth System Sciences*, **14**, 2039–2056. DOI: 10.5194/hess-14-2039-2010

Fearnside PM, Graça PML de A (2006) BR-319: Brazil’s Manaus-Porto Velho highway and the potential impact of linking the arc of deforestation to central Amazonia. *Environmental management*, **38**, 705-16. DOI: 10.1007/s00267-005-0295-y

Fearnside PM, Graça PML de A, Keizer EWH, Maldonado FS, Barbosa RI, Nogueira

EM (2009) Modelagem de desmatamento e emissões de gases de efeito estufa na região sob influência da rodovia Manaus-Porto Velho (BR-319). *Revista Brasileira de Meteorologia*, **24**, 208-233.

Fernandes AM (2013) Fine-scale endemism of Amazonian birds in a threatened landscape. *Biodiversity and Conservation*, **22**, 2683-2694. DOI 10.1007/s10531-013-0546-9

Ferrão M, Fraga R, Moravec J, Kaefer IL, Lima AP (2018) A new species of Amazonian snouted treefrog (Hylidae: Scinax) with description of a novel species-habitat association for an aquatic breeding frog. PeerJ, **6**, e4321 DOI 10.7717/peerj.4321.

Ferreira AS, Jehle R, Stow AJ, Lima AP (2018) Soil and forest structure predicts large-scale patterns of occurrence and local abundance of a widespread Amazonian frog. *Peerj*, **6**, e5424. DOI: 10.7717/peerj.5424

Fick SE, Hijmans RJ (2017) Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, **37**, 4302-4315. DOI: 10.1002/joc.5086

Figueiredo FOG, Zuquim G, Tuomisto H, Moulatlet GM, Balslev H, Costa FRC (2017) Beyond climate control on species range: The importance of soil data to predict distribution of Amazonian plant species. *Journal of Biogeography*, **45**, 190-200. DOI: 10.1111/jbi.13104

Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*, **180**, 977-993. DOI: 10.1534/genetics.108.092221

Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLOS ONE*, **2**, e1109 DOI 10.1371/journal.pone.0001109.

Fraga R, Lima AP, Magnusson WE, Ferrão M, Stow AJ (2017) Contrasting patterns of gene flow for Amazonian snakes that actively forage and those that wait in ambush. *Journal of Heredity*, **108**, 524-534. DOI:10.1093/jhered/esx051

Frichot E, François O (2015) LEA: an R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, **6**, 925-929. DOI: 10.1111/2041-210X.12382

Frichot E, Mathieu F, Trouillon T, Bouchard G, François O (2014) Fast and efficient estimation of individual ancestry coefficients. *Genetics*, **196**, 973-983. DOI: 10.1534/genetics.113.160572

Garant D, Kruuk LE, Wilkin TA, McCleery RH, Sheldon BC (2005) Evolution driven by differential dispersal within a wild bird population. *Nature*, **433**, 60-65. DOI: 10.1038/nature03051

Gascon C (1993) Breeding-habitat use by five Amazonian frogs at forest edge. *Biodiversity and Conservation*, **2**, 438-444.

Gascon C, Lougheed SC, Bogart JP (1998) Patterns of genetic population differentiation in four species of Amazonian frogs: a test of the riverine barrier hypothesis. *Biotropica*, **30**, 104–119

Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G (2006) Are mountain passes higher in the tropics? Janzen’s hypothesis revisited. *Integrative and Comparative Biology*, **46**, 5-17. DOI: 10.1093/icb/icj003

Godinho MBC, Da Silva FR (2018) The influence of riverine barriers, climate, and topography on the biogeographic regionalization of Amazonian anurans. *Scientific Reports*, **8**, 3427. DOI:10.1038/s41598-018-21879-9

Gosselin T (2017) radiator: RADseq Data Exploration, Manipulation and Visualization using R. R package version 0.0.5 https://github.com/thierrygosselin/radiator. DOI: 10.5281/zenodo.154432

Graham CH, Ron SR, Santos JC, Schneider CJ, Moritz C (2004) Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution*, **58**, 1781-1793. DOI: 10.1111/j.0014-3820.2004.tb00461.x

Grahame JW, Wilding CS, Butlin RK (2006) Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. *Evolution*, **60**, 268-278.

Grant T, Rada M, Anganoy-Criollo M, Batista A, Dias PH, Jeckel AM, Machado DJ, Rueda-Almonacid JV (2017) Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology*, **12**, 1-90. DOI: 10.2994/SAJH-D-17-00017.1

Gugger PF, Ikegami M, Sork VL (2013) Influence of late Quaternary climate change on present patterns of genetic variation in valley oak, Quercus lobata Née. *Molecular Ecology,* **22**, 3598–3612

Haffer J (1969) Speciation in Amazonian forest birds. *Science*, **165**, 131-137. DOI: 10.1126/science.165.3889.131

Hanks EM, Hooten MB (2013) Circuit theory and model-based inference for landscape connectivity. *Journal of American Statistical Association*, **108**, 22-33.

Hijmans RJ (2017) raster: Geographic Data Analysis and Modeling. R package version 2.6-7. https://CRAN.R-project.org/package=raster

Hoorn C, Wesselingh FP, Steege H. ter, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler J, Särkinen T, Antonelli A (2010) Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science*, **330**, 927-931. DOI: 10.1126/science.1194585

Hoskin CJ, Higgie M, McDonald KR, Moritz C (2005) Reinforcement drives rapid allopatric speciation. *Nature*, **437**, 1353-1356. DOI: 10.1038/nature04004

IBGE (1997) Recursos naturais e meio ambiente: uma visão do Brasil. Second Edition. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística (IBGE), pp. 208.

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, 29, e25.

Janzen DH (1967) Why mountain passes are higher in the tropics. *The American Naturalist*, **101**, 233-249.

Jenkins DG, Carey M, Czerniewska J, Fletcher J, Hether T, Jones A, Knight S, Knox J, Long T, Mannino M, McGuire M, Riffle A, Segelsky S, Shappell L, Sterner A, Strickler T, Tursi, R (2010) A meta-analysis of isolation by distance: relic or reference standard for landscape genetics? *Ecography*, **33**, 315-320. DOI: 10.1111/j.1600-0587.2010.06285.x

Jombart T, Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*, **27**, 3070-3071. DOI: 10.1093/bioinformatics/btr521

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94. DOI: 10.1186/1471-2156-11-94

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics,* **24**, 1403-1405. DOI: 10.1093/bioinformatics/btn129

Kaefer IL, Tsuji-Nishikido BM, Mota EP, Farias IP, Lima AP (2013) The early stages of speciation in Amazonian forest frogs: phenotypic conservatism despite strong genetic structure. *Evolutionary Biology*, **40**, 228-245. DOI: 10.1007/s11692-012-9205-4

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, **2**, e281. DOI: 10.7717/peerj.281

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) diveRsity: An R package for the estimation of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, **4**, 782-788. DOI: 10.1111/2041-210X.12067

Kierepka E, Latch E (2015) Fine-scale landscape genetics of the American badger (Taxidea taxus): Disentangling landscape effects and sampling artifacts in a poorly understood species. *Heredity*, **116**, 1-11. DOI: 10.1038/hdy.2015.67

Kilian A, Wenzl, P, Huttner, E, Carling J, Xia L, Blois H, Caig V, Heller-Uszynska K, Jaccoud D, Hopper C, Aschenbrenner-Kilian M, Evers M, Peng K, Cayla C, Hok P, Uszynski G (2012) Diversity Arrays Technology: A generic genome profiling technology on open platforms. In: Pompanon F, Bonin A. (eds). Data production and analysis in population genomics. Methods in molecular biology (Methods and Protocols), vol 888. Humana Press, Totowa, NJ.

Knowles LL & Alvarado-Serrano DF (2010) Exploring the population genetic consequences of the colonization process with spatio-temporally explicit models: insights from coupled ecological, demographic and genetic models in montane grasshoppers. *Molecular Ecology*, **19**, 3727-3745. DOI: 10.1111/j.1365-294X.2010.04702.x

Latrubesse EM, Cozzuol M, Silva-Caminha SAF, Rigsby CA, Absy ML, Jaramillo C (2010) The Late Miocene paleogeography of the Amazon Basin and the Evolution of the Amazon River system. *Earth Science Reviews*, **99**, 99-124. DOI: 10.1016/j.earscirev.2010.02.005

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia,* **129**, 271-280. DOI: 10.1007/s004420100716

Leite RN, Rogers DS (2013) Revisiting Amazonian phylogeography: insights into diversification hypotheses and novel perspectives. *Organisms Diversity & Evolution*, **13**, 639-664. DOI:10.1007/s13127-013-0140-8

Lemay MA, Russello MA (2015) Genetic evidence for ecological divergence in kokanee salmon. *Molecular Ecology*, **24**, 798-811. DOI: 10.1111/mec.13066

Lischer HEL, Excoffier L (2012) PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, **28**, 298-299. DOI: 10.1093/bioinformatics/btr642

Lougheed SC, Gascon C, Jones DA, Bogart JP, Boag PT (1999) Ridges and rivers: a test of competing hypothesis of Amazonian diversification using a dart-poison frog (*Epipedobates femoralis*). *Proceedings of the Royal Society B: Biological Sciences*, **266**, 1829-1835 DOI: 10.1098/rspb.1999.0853.

Lougheed SC, Austin JD, Bogart JP, Boag PT, Chek AA (2006) Multi-character perspectives on the evolution of intraspecific differentiation in a neotropical hylid frog. *BMC Evolutionary Biology*, **6**, 23. DOI: 10.1186/1471-2148-6-23

Lovejoy NR, Bermingham E, Martin AP (1998) Marine incursion into South America. *Nature*, **396**, 421-422. DOI: 10.1038/24757

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**, 981-994. DOI: 10.1038/nrg1226

Luu K, Bazin E, Blum MGB (2017) pcadapt: an R package for performing genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, **17**, 67-77. DOI: 10.1111/1755-0998.12592

Magnusson WE, Braga-Neto R, Pezzini F, Baccaro F, Bergallo H, Penha J, Rodrigues D, Lima AP, Albernaz A, Hero JM, Lawson B, Castilho C, Drucker C, Franklin E, Mendonça F, Costa F, Galdino G, Castley G, Zuanon J, Vale J, Santos JLC, Luizão R, Cintra R, Barcosa RI, Lisboa A, Koblitz RV, Cunha CN, Pontes ARM (2013) Biodiversidade e monitoramento ambiental integrado: o sistema RAPELD na Amazônia. Santo André: Publisher Attema.

Maldonado FD, Keizer EWH, Graça PMLA, Fearnside PM, Vitel CS (2012) Previsão temporal da distribuição espacial do desmatamento no interflúvio Purus-Madeira até o ano 2050. Editors. Rio Purus: Água, Território e Sociedade na Amazônia Sul-Ocidental. Ecuador: LibriMundi Press.

Manel S, Holderegger R (2013) Ten years of landscape genetics. *Trends in Ecology & Evolution*, **28**, 614-621.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**, 189-197. DOI: 10.1016/S0169-5347(03)00008-9

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209-220.

Martins DL, Schietti J, Feldpausch TR, Luizão FJ, Phillips OL, Andrade A, Castilho CV, Laurance SG, Oliveira A, Amaral IL, Toledo JJ, Lugli LF, Pinto JLPV, Mendoza EMO, Quesada CA (2014) Soil-induced impacts on forest structure drive coarse woody debris stocks across central Amazonia. Plant *Ecology & Diversity*, **7**, 1-13. DOI: 10.1080/17550874.2013.879942

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 19885-19890. DOI: 10.1073/pnas.0706568104

McRae BH, Dickson BG, Keitt TH, Shah VB (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology*, **89**, 2712-2724. DOI: 10.1890/07-1861.1

McRae BH, Shah VB (2009) Circuitscape user’s guide. Santa Barbara: The University of California. Available from: http://www.circuitscape.org.

McRae BH (2006) Isolation by resistance. *Evolution*, **60**, 1551-1561. DOI: 10.1111/j.0014-3820.2006.tb00500.x

Montanarin A, Kaefer IL, Lima AP (2011) Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. *Ethology Ecology & Evolution*, **23**, 141-150. DOI: 10.1080/03949370.2011.554884

Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of rainforest faunas: an integrated molecular approach. *Annual Reviews of Ecology and Systematics,* **31**, 533-563. DOI: 10.1146/annurev.ecolsys.31.1.533

Nagaraju SK, Gudasalamani R, Barve N, Ghazoul J, Narayanagowda GK, Ramanan US (2013) Do ecological niche model predictions reflect the adaptive landscape of species?: a test using Myristica malabarica Lam., an endemic tree in the Western Ghats, India. *PLoS ONE*, **8**, e82066

Naka LN, Bechtoldt CL, Henriques LMP, Brumfield RT, Heard AESB, McPeek EMA (2012) The role of physical barriers in the location of avian suture zones in the Guiana Shield, northern Amazonia. *The American Naturalist*, **179**, 115-132.

Nazareno AG, Dick CW, Lohmann LG (2017) Wide but not impermeable: testing the riverine barrier hypothesis for an Amazonian plant species. *Molecular Ecology*, **26**, 3636-3648. DOI: 10.1111/mec.14142

Nogueira ACR, Silveira R, Guimarães JTF (2013) Neogene–Quaternary sedimentary and paleovegetation history of the eastern Solimões Basin, central Amazon region. *Journal of South American Earth Sciences*, **46**, 89-99. DOI: 10.1016/j.jsames.2013.05.004

Nosil P (2012) Ecological speciation. Oxford University Press, Oxford, pp 304.

Oden NL, Sokal RR (1986) Directional autocorrelation: an extension of spatial correlograms to two dimensions. Systematic Zoology, **35**, 608-617.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Har RB, Simpson GL, Solymos P, Stevens HMH, Szoecs E, Wagner H (2018) vegan: Community Ecology Package. R package version 2.5-1. https://CRAN.R-project.org/package=vegan

Olivares I, Svenning JC, van Bodegom PM, Balslev H (2015) Effects of warming and drought on the vegetation and plant diversity in the Amazon Basin. *The Botanical Review*, **81**, 42-69. DOI 10.1007/s12229-014-9149-8

Oliveira U, Vasconcelos MF, Santos AJ (2017) Biogeography of Amazon birds: rivers limit species composition, but not areas of endemism. *Scientific Reports*, **7**, 2992. DOI:10.1038/s41598-017-03098-w

Oliveira EF, Martinez PA, São-Pedro VA, Gehara M, Burbrink FT, Mesquita DO, Garda AA, Colli GR, Costa GC (2017) Climatic suitability, isolation by distance and river resistance explain genetic variation in a Brazilian whiptail lizard. *Heredity*, **120**, 251-265. DOI: 10.1038/s41437-017-0017-2

Ortiz DA, Lima AP, Werneck FP (2018) Environmental transition zone and rivers shape intraspecific population structure and genetic diversity of an Amazonian rain forest tree frog. *Evolutionary Ecology*, **32**, 359-378. DOI: 10.1007/s10682-018-9939-2

Pašukonis A, Loretto MC, Landler L, Ringler M, Hödl W (2014) Homing trajectories and initial orientation in a Neotropical territorial frog, *Allobates femoralis* (Dendrobatidae). *Frontiers in Zoology,* **11**, 29. DOI: 10.1186/1742-9994-11-29

Peterman WE, Connette GM, Semlitsch RD, Eggert LS (2014) Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. *Molecular Ecology,* **23**, 2402-2413. DOI: 10.1111/mec.12747

Peterman WE (2018) ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms. *Methods in Ecology and Evolution,* **9**, 1638-1647. DOI: 10.1111/2041-210X.12984.

Petroli CD, Sansaloni CP, Carling J, Steane DA, Vaillancourt RE, Myburg AA, Silva Jr OB da, Pappas Jr GJ, Kilian A, Grattapaglia D (2012) Genomic characterization of DArT markers based on high-density linkage analysis and physical mapping to the Eucalyptus genome. *PLoS One,* **7**, e44684. DOI: 10.1371/journal.pone.0044684

Price T (2008) Speciation in Birds. Roberts and Company Publishers, Greenwood Village.

Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmerler J, Santos AJB, Hodnett MG, Herrera R, Luizão FJ, Arneth A, Lloyd G, Dezzeo N, Hilke I, Kuhlmann I, Raessler M, Brand WA, Geilmann H, Moraes Filho JO, Carvalho FP, Araujo Filho RN, Chaves JE, Cruz Junior OF, Pimentel TP, Paiva R (2010) Variations in chemical and physical properties of

Amazon forest soils in relation to their genesis. *Biogeosciences*, **7**, 1515-1541. DOI: 10.5194/bg-7-1515-2010.

Rasanen ME, Linna AM, Santos JCR, Negri FR (1995) Late Miocene tidal deposits in the Amazonian foreland basin. *Science*, **269**, 386-390. DOI: 10.1126/science.269.5222.386

Ribas CC, Aleixo A, Nogueira ACR, Miyaki CY, Cracraft J (2012) A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society of London Se*ries B, Biological Sciences, **279**, 681-689. DOI 10.1098/rspb.2011.1120.

Richardson JL (2012) Divergent landscape effects on population connectivity in two co-occurring amphibian species. *Molecular Ecology*, **21**, 4437-4451. DOI: 10.1111/j.1365-294X.2012.05708.x

Richter-Boix A, Quintela M, Kierczak M, Franch M, Laurila A (2013) Fine-grained adaptive divergence in an amphibian: genetic basis of phenotypic divergence and the role of nonrandom gene flow in restricting effective migration among wetlands. *Molecular Ecology*, **22**, 1322-1340. DOI: 10.1111/mec.12181

Ringler E, Beck KB, Weinlein S, Huber L, Ringler M (2017) Adopt, ignore, or kill? Male poison frogs adjust parental decisions according to their territorial status. *Scientific Reports*, **7**, 1-6. DOI:10.1038/srep43544

Ringler E, Pašukonis A, Hödl W, Ringler M (2013) Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. *Frontiers in Zoology*, **10**, 67. DOI: 10.1186/1742-9994-10-67

Ringler M, Ursprung E, Hödl W (2009) Site fidelity and patterns of short-and long-term movement in the brilliant-thighed poison frog *Allobates femoralis* (Aromobatidae). *Behavioral Ecology and Sociobiology*, **63**,1281-1293. DOI: 10.1007/s00265-009-0793-7.

Rodríguez A, Börner M, Pabijan M, Gehara M, Haddad CFB, Vences M (2015) Genetic divergence in tropical anurans: deeper phylogeographic structure in forest specialists and in topographically complex regions. *Evolutionary Ecology*, **29**, 765-785. DOI: 10.1007/s10682-015-9774-7

Roithmair ME (1992) Territoriality and male mating success in the Dart-Poison Frog, *Epipedobates femoralis* (Dendrobatidae, Anura). *Ethology*, **92**, 331-343. DOI: 10.1111/j.1439-0310.1992.tb00970.x

Ron SR (2000) Biogeographic area relationships of lowland Neotropical rainforest based on raw distributions of vertebrate groups. *Biological Journal of the Linnean Society*, **71**, 379-402. DOI: 10.1111/j.1095-8312.2000.tb01265.x

Rossetti DF, Mann de Toledo P, Góes AM (2005) New geological framework for Western Amazônia (Brazil) and implications for biogeography and evolution. *Quarternary Research*, **63**, 78-89. DOI: 10.1016/j.yqres.2004.10.001

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219-1228.

Row JR, Knick ST, Oyler-McCance SJ, Lougheed SC, Fedy BC (2017) Developing approaches for linear mixed in landscape genetics through landscape-directed dispersal simulations. *Ecology and Evolution*, **7**, 3751-3761. DOI: 10.1002/ece3.2825

Ruokolainen K, Moulatlet GM, Zuquim G, Hoorn C, Tuomisto H (2018) River Network Rearrangements in Amazonia Shake Biogeography and Civil Security. *Preprints* doi:10.20944/preprints201809.0168.v1

Sansaloni C, Petroli C, Jaccoud D, Carling J, Detering F, Grattapaglia D, Kilian A (2011) Diversity Arrays Technology (DArT) and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus. *BMC Proceedings*, **5**, 54-55. DOI: 10.1186/1753-6561-5-S7-P54

Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC (2009) Amazonian amphibian diversity is primarily derived from Late Miocene Andean lineages. *PLOS Biology*, **7**, e1000056 DOI 10.1371/journal.pbio.1000056.

Santorelli S, Magnusson WE, Deus CP (2018) Most species are not limited by an Amazonian river postulated to be a border between endemism areas. *Scientific Reports*, **8**, 2294. DOI: 10.1038/s41598-018-20596-7

Schietti J, Martins D, Emilio T, Souza PF, Levis C, Baccaro FB, Pinto JLPV, Moulatlet JM, Stark SC, Sarmento K, Araújo NO, Costa FRC, Schöngart J, Quesada CA, Saleska SR, Tomasella J, Magnusson WE (2016) Forest structure along a 600 km transect of natural disturbances and seasonality gradients in central-southern Amazonia. *Journal of Ecology*, **104**, 1335-1346. DOI: 10.1111/1365-2745.12596

Schluter D (2009) Evidence for ecological speciation and its alternative. *Science*, **323**, 737-741.

Shirk AJ, Wallin DO, Cushman SA, Rice CG, Warheit KI (2010) Inferring landscape effects on gene flow: a new model selection framework. *Molecular Ecology*, **19**, 3603-3619. DOI: 10.1111/j.1365-294X.2010.04745.x

Simões PI, Lima AP, Farias IP (2012) Restricted natural hybridization between two species of litter frogs on a threatened landscape in southwestern Brazilian Amazonia. *Conservation Genetics*, **13**, 1145-1159. DOI: 10.1007/s10592-012-0362-x

Simões PI, Lima AP, Magnusson WE, Hödl W, Amézquita A (2008) Acoustic and morphological differentiation in the frog *Allobates femoralis*: relationships with the upper Madeira River and other potential geological barriers. *Biotropica* **40**, 607-614. DOI: 10.1111/j.1744-7429.2008.00416.x

Simões PI, Stow A, Hödl W, Amézquita A, Farias IP, Lima AP (2014) The value of including intraspecific measures of biodiversity in environmental impact surveys is highlighted by the Amazonian brilliant-thighed frog (*Allobates Femoralis*). *Tropical Conservation Science*, **7**, 811-828. DOI: 10.1177/194008291400700416

Simões PI (2016) A new species of nurse-frog (Aromobatidae, Allobates) from the Madeira river basin with a small geographic range. *Zootaxa*, **4083**, 501-525. DOI: 10.11646/zootaxa.4083.4.3

Smith BT, McCormack JE, Cuervo AM, Hickerson MJ, Aleixo A, Cadena CD, Pérez-Emán J, Burney CW, Xie X, Harvey MG, Faircloth BC, Glenn TC, Derryberry EP, Prejean J, Fields S, Brumfield RT (2014) The drivers of tropical speciation. *Nature*, **515**, 406-409. DOI:10.1038/nature13687

Smith KL, Hale JM, Kearney MR, Austin JJ, Melville J (2013) Molecular patterns of introgression in a classic hybrid zone between the Australian tree frogs, *Litoria ewingii* and *L. paraewingi*: evidence of a tension zone. *Molecular Ecology*, **22**, 1869-1883. DOI: 10.1111/mec.12176

Sombroek W (2000) Amazon landforms and soils in relation to biological diversity. *Acta Amazonica*, **30**, 81-100. DOI: 10.1590/1809-43922000301100

Stein A, Gerstner K, Kreft H (2014) Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters*, **17**, 866-880.

Stow AJ, Sunnucks P, Briscoe DA, Gardner MG (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology*, **10**, 867-878. DOI: 10.1046/j.1365-294X.2001.01253.x

Sun Y-B, Xiong Z-J, Xiang X-Y, Liu S-P, Zhou W-W, Tu X-L, Zhong L, Wang L, Wu D-D, Zhang B-L, Zhu C-L, Yang M-M, Chen H-M, Li F, Zhou L, Feng S-L, Huang C, Zhang G-J, Irwin D, Hillis DM, Murphy RW, Yang H-M, Che J, Wang J, Zhang Y-P (2015) Whole-genome sequence of the Tibetan frog *Nanorana parkeri* and the comparative evolution of tetrapod genomes. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, E1257-E1262. DOI: 10.1073/pnas.1501764112

Symula R, Schulte R, Summers K (2003) Molecular systematics and phylogeography of Amazonian poison frogs of the genus Dendrobates. *Molecular Phylogenet Evolution*, **26**, 452–475

Thompson JA, Stow AJ, Raftos DA (2017) Lack of genetic introgression between wild and selectively bred Sydney rock oysters Saccostrea glomerata. *Marine Ecology Progress Series*, **570**, 127-139. DOI: 10.3354/meps12109

Thorpe RS (1984) Primary and secondary transition zones in speciation and population differentiation: a phylogenetic analysis of range expansion. *Evolution*, **38**, 233-243.

Thuiller W (2007) Climate change and the ecologist. *Nature*, **448**, 550-552. DOI: 10.1038/448550a

Van Strien MJ, Keller D, Holderegger R (2012) A new analytical approach to landscape genetic modelling: least-cost transect analysis and linear mixed models. *Molecular Ecology*, **21**, 4010-4023. DOI: 10.1111/j.1365-294X.2012.05687.x

Vanzolini PE, Williams EF (1981) The vanishing refuge: a mechanism for ecogeographic speciation. *Papéis Avulsos de Zoologia*, **34**, 251-255.

Wahlund S (1928) Zusammensetzung von Population und Korrelationserschei- nungen vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas*, **11**, 65-106. English translation in Kenneth M. Weiss and Paul A. Ballonoff, eds., *Demographic Genetics*. Stroudsburg, Pa.: Dowden, Hutchinson and Ross, pp. 224-263.

Wallace AR (1852) On the monkeys of the Amazon. *Proceedings of the Zoological Society of London*, **20**, 107-110. DOI: 10.1080/037454809494374

Wang IJ (2012) Environmental and topographic variables shape genetic structure and effective population sizes in the endangered Yosemite toad. *Diversity and Distributions*, **18**, 1033-1041. DOI: 10.1111/j.1472-4642.2012.00897.x

Wickham H, Francois R, Henry L, Müller K (2017) dplyr: A Grammar of Data Manipulation. R package version 0.7.4. https://CRAN.R-project.org/package=dplyr

Wickham H, Ruiz E (2018) dbplyr: A 'dplyr' back end for databases. R package version 1.2.1. https://CRAN.R-project.org/package=dbplyr

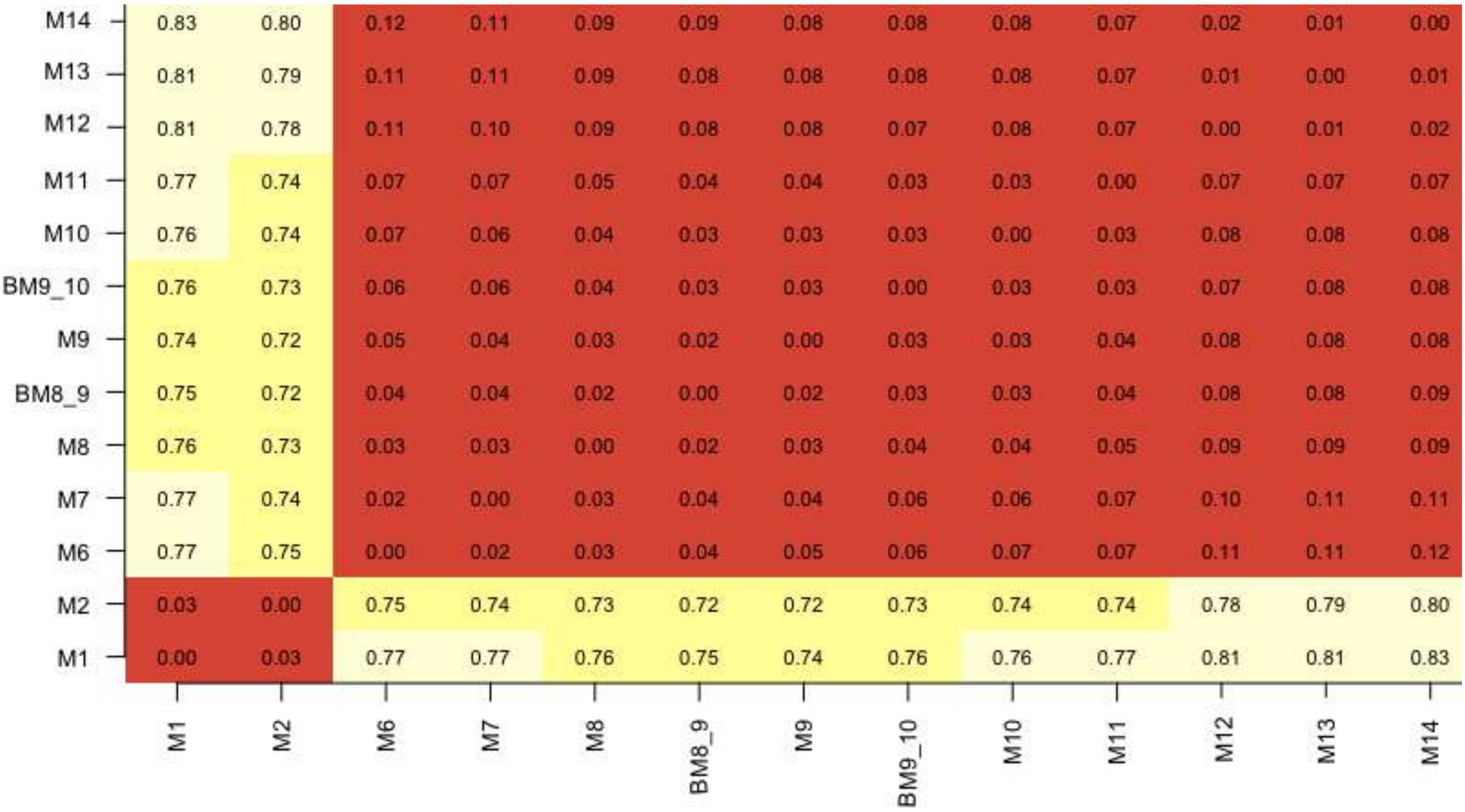
Wright S (1943) Isolation by distance. *Genetics*, **28**, 114-138.

Xie Y (2018) DT: A wrapper of the javascript library 'datatables'. R package version 0.4. https://CRAN.R-project.org/package=DT

Zeisset I, Beebee TJC (2008) Amphibian phylogeography: a model for understanding historical aspects of species distributions. *Heredity*, **101**, 109-119. DOI: 10.1038/hdy.2008.30

Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS (2012) A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, **28**, 3326-3328. DOI: 10.1093/bioinformatics/bts606

Supporting information



	M1	M2	M6	M7	M8	BM8_9	M9	BM9_10	M10	M11	M12	M13	M14
M14	0.83	0.80	0.12	0.11	0.09	0.09	0.08	0.08	0.08	0.07	0.02	0.01	0.00
M13	0.81	0.79	0.11	0.11	0.09	0.08	0.08	0.08	0.08	0.07	0.01	0.00	0.01
M12	0.81	0.78	0.11	0.10	0.09	0.08	0.08	0.07	0.08	0.07	0.00	0.01	0.02
M11	0.77	0.74	0.07	0.07	0.05	0.04	0.04	0.03	0.03	0.00	0.07	0.07	0.07
M10	0.76	0.74	0.07	0.06	0.04	0.03	0.03	0.03	0.00	0.03	0.08	0.08	0.08
BM9_10	0.76	0.73	0.06	0.06	0.04	0.03	0.03	0.00	0.03	0.03	0.07	0.08	0.08
M9	0.74	0.72	0.05	0.04	0.03	0.02	0.00	0.03	0.03	0.04	0.08	0.08	0.08
BM8_9	0.75	0.72	0.04	0.04	0.02	0.00	0.02	0.03	0.03	0.04	0.08	0.08	0.09
M8	0.76	0.73	0.03	0.03	0.00	0.02	0.03	0.04	0.04	0.05	0.09	0.09	0.09
M7	0.77	0.74	0.02	0.00	0.03	0.04	0.04	0.06	0.06	0.07	0.10	0.11	0.11
M6	0.77	0.75	0.00	0.02	0.03	0.04	0.05	0.06	0.07	0.07	0.11	0.11	0.12
M2	0.03	0.00	0.75	0.74	0.73	0.72	0.72	0.73	0.74	0.74	0.78	0.79	0.80
M1	0.00	0.03	0.77	0.77	0.76	0.75	0.74	0.76	0.76	0.77	0.81	0.81	0.83

Firure S1. Pairwise genetic distances (F_{ST}) based on Nei's distance estimates between sampling sites.

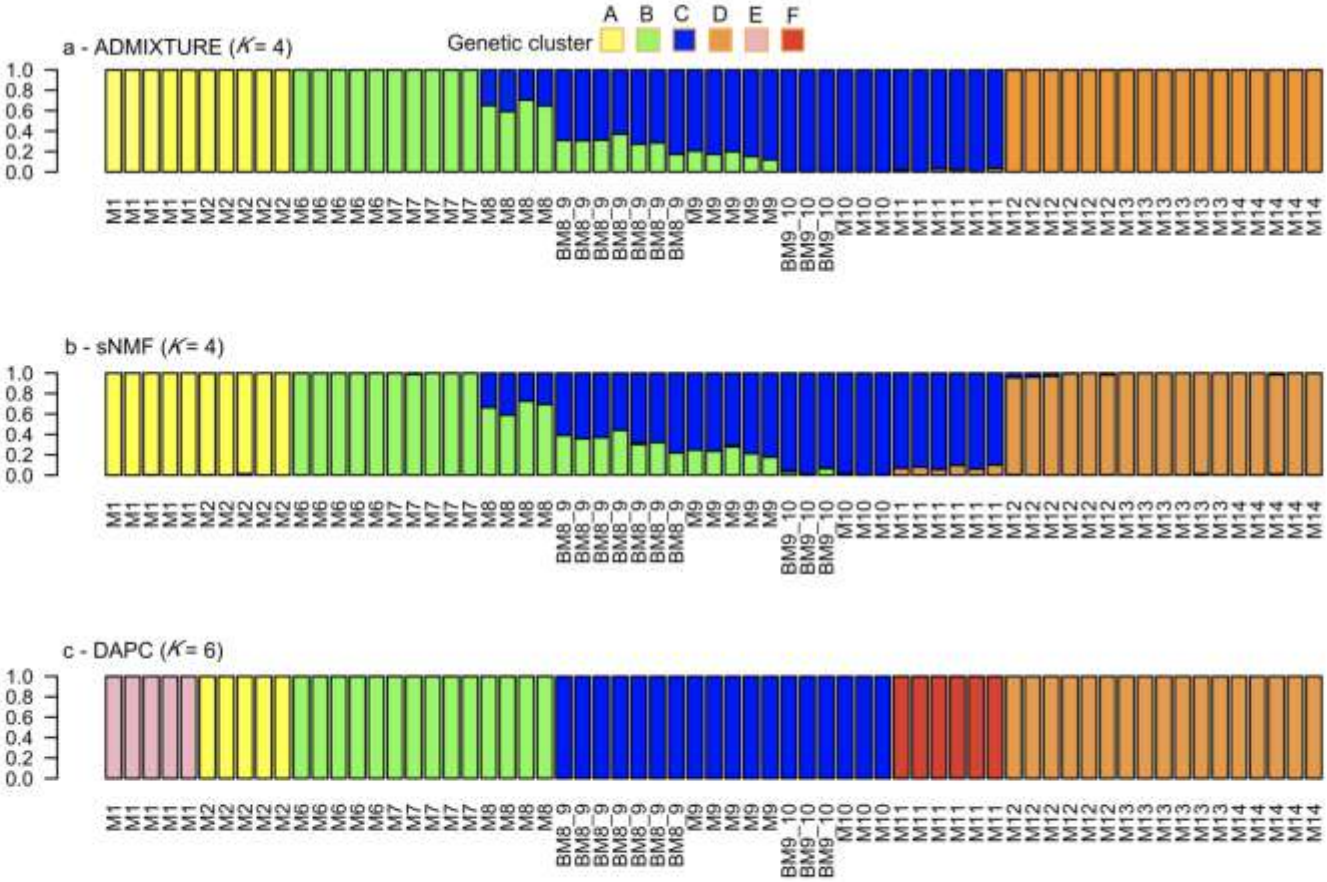


Figure S2. Barplots of population structure analyses conducted for *Allobates femoralis* in central-southern Amazonia, resulting from SNPs. A) ADMIXURE, B) sNMF and C) DAPC. Sampling locations and the most probable number of genetic clusters (k) is provided. Each genetic cluster is assigned to a different color, and each bar represent an individual with the proportion of their admixed genome ($P < 0.05$), except to DAPC.

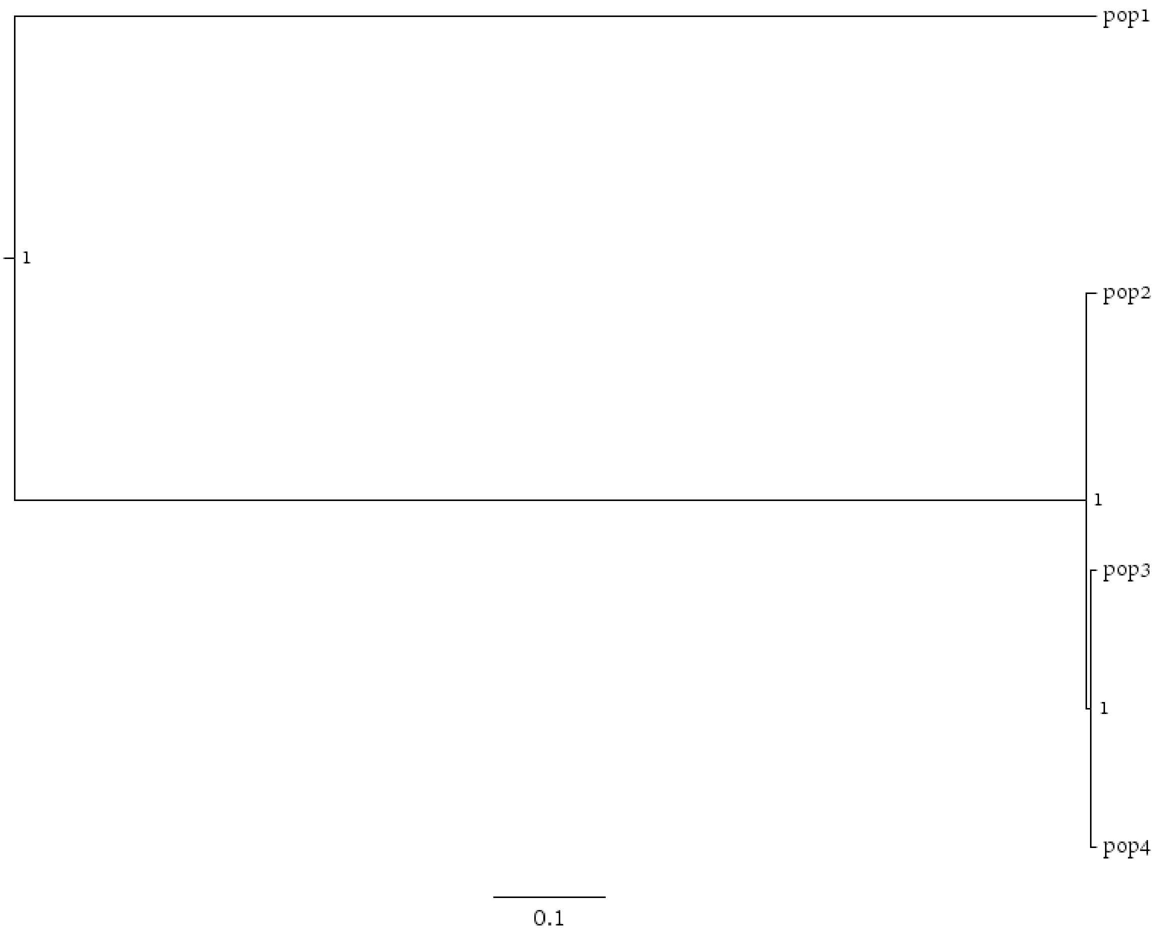


Figure S3. Population tree based on SNAPP analysis from SNPs. Posterior probabilities obtained at each node (between populations) are indicated on the tree. Pop1 correspond to the individuals from localities M1 and M2; Pop2 correspond to individuals from localities M6 to M8; Pop3 correspond to individuals from localities B8_9 to M11 and Pop4 individuals from localities M12 to M14.

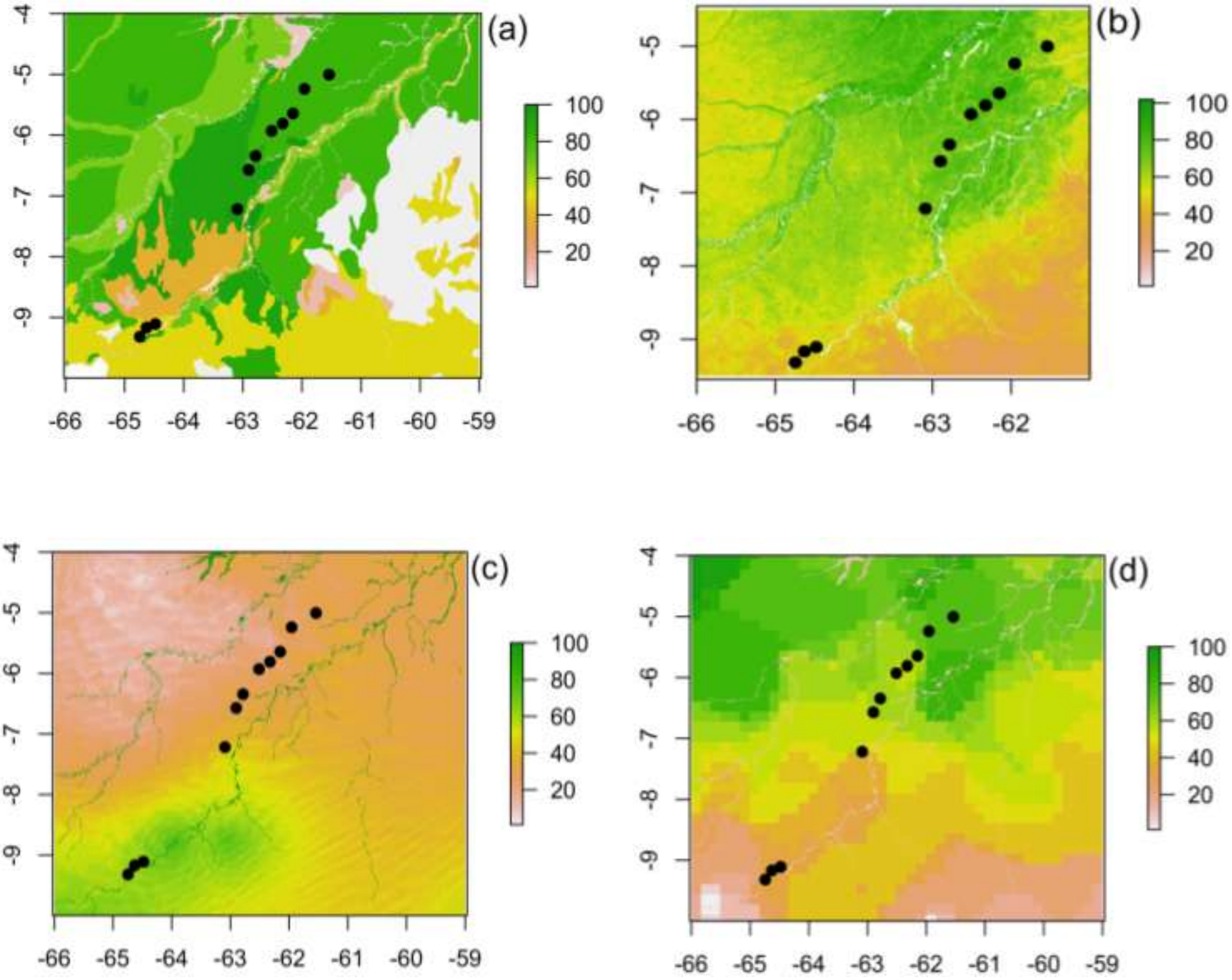


Figure S4. Rasters used in Circuiscape to generate the matrices of pairwise distance to test genetic differentiation in *Allobates femoralis*. Vegetation-cover type (A), silt content (B), temperature seasonality - Bio4 (C) and Walsh index (D).

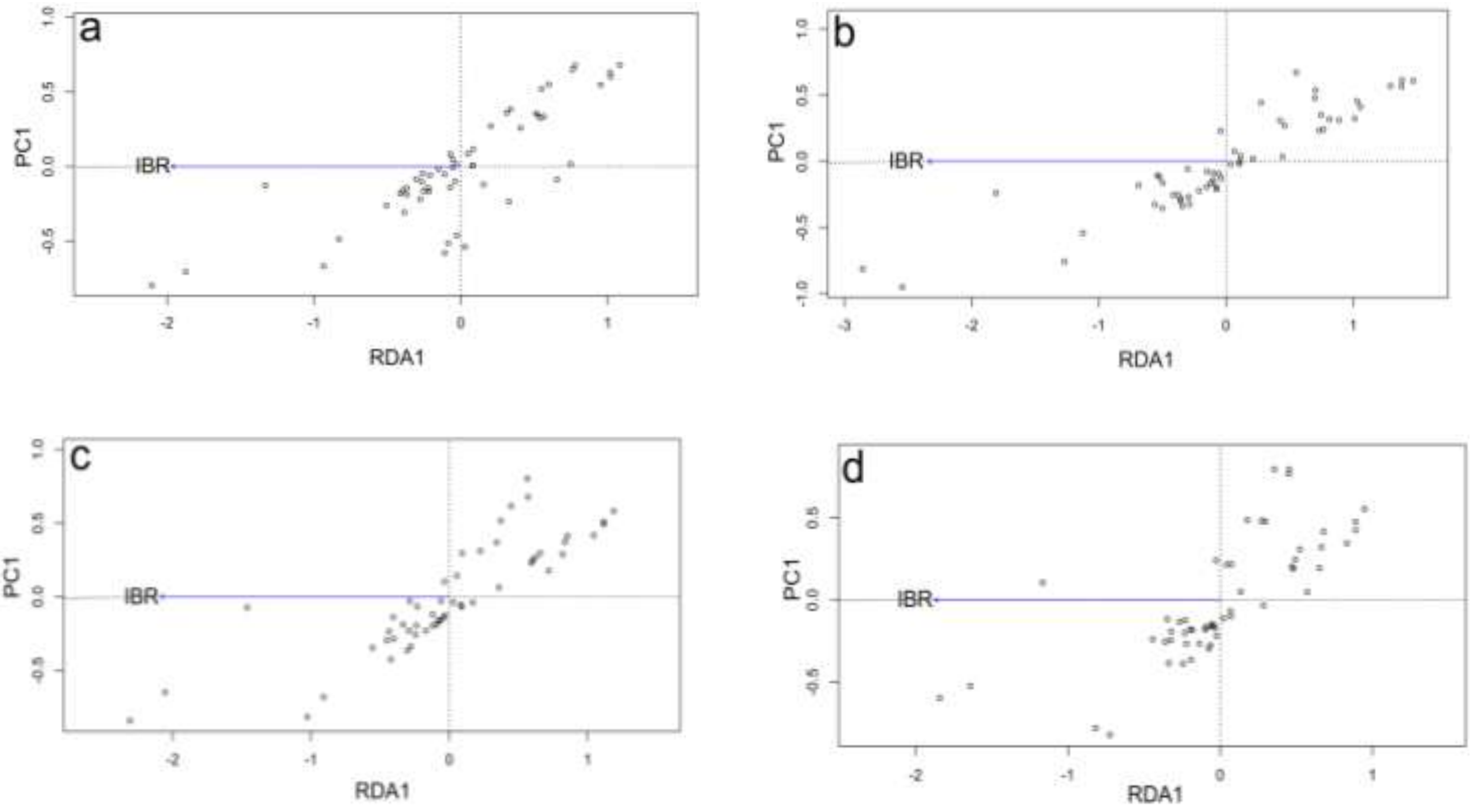


Figure S5. Patterns of gene flow in *Allobates femoralis* based on isolation by cost distance. The biplot shows individuals as open circles and the explanatory variables as vectors (blue arrows). Vegetation-cover type (A), silt content (B), temperature seasonality - Bio4 (C) and Walsh index (D).

Table S1. Geographic position of brilliant-thighed frog (*Allobates femoralis*) sampling locations within the Purus–Madeira interfluve.

Locality	local name	Code	Municipality	State	Latitude	Longitude
PPBio module 1, km 34 of BR-319	Purupuru	M1	Careiro da Várzea	Amazonas	-3,353377	-59,85562996
PPBio module 2, km 100 of BR-319	Manaquiri	M2	Careiro	Amazonas	-3,68750874	-60,33169667
PPBio module 3, km 168 of BR-319	Taboca	M3	Careiro	Amazonas	-4,12758792	-60,72872504
PPBio module 4, km 220 of BR-319	Taquara	M4	Borba	Amazonas	-4,37630304	-60,94880347
PPBio module 5, km 260 of BR-319	Igapó-açu	M5	Beruri	Amazonas	-4,6096445	-61,2500883
PPBio module 6, km 300 of BR-319	Orquestra	M6	Manicoré	Amazonas	-5,0041793	-61,5411712
PPBio module 7, km 350 of BR-319	Rio Novo	M7	Beruri	Amazonas	-5,23999991	-61,95724483
PPBio module 8, km 400 of BR-319	Capanã	M8	Manicoré	Amazonas	-5,6436243	-62,151335
Site between module 8 and 9 of BR-319	Capanã	BM8_9	Manicoré	Amazonas	-5,80877778	-62,32552778
PPBio module 9, km 450 of BR-319	Jari	M9	Tapauá	Amazonas	-5,92982409	-62,5117239
Site between module 9 and 10 of BR-319	Jari	BM9_10	Tapauá	Amazonas	-6,34458333	-62,78458333
PPBio module 10, km 540 of BR-319	Aracá	M10	Humaitá	Amazonas	-6,57142945	-62,90062384
PPBio module 11, km 620 of BR-319	Puruzinho	M11	Humaitá	Amazonas	-7,2185608	-63,0919793
PPBio module 12, left bank of the upper Madeira River	Búfalo	M12	Jirau	Porto Velho	-9,1076	-64,479081
PPBio module 13, left bank of the upper Madeira River	Ilha da Pedra	M13	Jirau	Porto Velho	-9,167074	-64,629109
PPBio module 14, left bank of the upper Madeira River	Jirau esquerdo	M14	Abumã	Porto Velho	-9,317073	-64,743354

Table S2. Distance between *Allobates femoralis* sampling locations within the Purus-Madeira interfluve.

	M6	M7	M8	BM8_9	M9	BM9_10	M10	M11	M12	M13	M14
M6	-	53.08	98.19	124.81	148.94	203.06	230.45	300.38	560.32	575.38	601.44
M7		-	49.81	75.33	98.34	153.35	181.32	253.53	512.75	527.40	553.44
M8			-	26.65	51.07	104.90	132.46	203.87	463.37	478.16	504.21
BM8_9				-	24.63	78.36	106.11	178.37	437.42	452.09	478.13
M9					-	55.17	83.39	157.16	415.05	429.48	455.50
BM9_10						-	28.33	103.05	359.91	374.31	400.33
M10							-	75.08	331.67	346.14	372.17
M11								-	259.96	275.19	301.25
M12									-	17.77	42.23
M13										-	26.06
M14											-

SÍNTESE

Ocupando uma área estimada em mais de seis milhões de Km^2, a Amazônia concentra uma das mais ricas biodiversidades terrestres do planeta (Gentry 1988; Mittermeier *et al.* 2003; Betts *et al.* 2008). No entanto, apesar do reconhecimento da floresta amazônica como um dos últimos biomas terrestres tropicais a apresentar grandes extensões contínuas de floresta e da sua importância na regulação climática, ela vem sendo ameaçada pelo avanço do desmatamento, da fronteira agrícola e pela instalação de grandes hidroelétricas (Fearnside 2006; Nobre 2014). De maneira geral, essas pressões afetam sua complexa rede de interações ecológicas e sua capacidade de continuar gerando processos vitais responsáveis por manter o funcionamento dos serviços ecossistêmicos (Lewis *et al.* 2011; Nobre 2014). Portanto, é urgente e necessário entender quais os fatores que controlam a distribuição, abundância, variabilidade genética e fluxo gênico de sua biodiversidade.

No primeiro capítulo desta tese, nós identificamos e caracterizamos importantes parâmetros ambientais associados com a distribuição e abundância relativa de *Allobates femoralis* usando amostragens padronizadas em módulos de pesquisas que seguem o padrão RAPELD em um transecto de 880 km de paisagem contínua ao longo do interflúvio Purus-Madeira. Nós mostramos que *Allobates femoralis* apresenta alta probabilidade de detecção. Usamos modelos lineares generalizados de efeito misto (para o nível das parcelas) e regressões lineares simples (para o nível de módulos), e levando em conta a detectabilidade nos modelos, nós mostramos que *A. femoralis* é mais abundante nas florestas abertas do que nas florestas densas e é positivamente associado com solos ricos em argila. Nós demostramos que variáveis ambientais relativamente fáceis de amostrar podem explicar a distribuição e abundância relativa de uma espécie amplamente distribuída em diferentes escalas espaciais. Desta forma, nossos resultados sugerem que a distribuição e abundância relativa de *A. femoralis* é moldada por gradientes ecológicos graduais.

No segundo capítulo, nós aplicamos uma abordagem de modelagem multinível baseada na teoria dos circuitos utilizando Polimorfismos de Nucleotídeos Únicos (SNPs) para testar a hipótese de gradientes ambientais em *A. femoraslis*. Nós mostramos que tanto os efeitos do isolamento por distância (IBD) quanto os efeitos do isolamento por resistência (IBR) de paisagens atuais e contínuas são evidentes nos padrões espaciais de variabilidade genética e no fluxo gênico em *A. femoralis* ao longo das paisagens heterogêneas ao longo do IPM, suportando assim a hipótese dos gradientes para diversificação.

Os resultados obtidos nessa tese são inéditos e importantes para diversas áreas, tais como, ecologia, evolução e biologia da conservação e podem ajudar ecologistas e conservacionistas que trabalham em grandes e inacessíveis áreas, tais como, a bacia Amazônica. Especialmente devido os avanços do desmatamento na Amazônia e recursos cada vez mais limitados para aplicar em conservação, ter dados disponíveis e métodos rápidos e com bom custo benefício para predizer impactos antrópicos são fundamentais. Ao usar uma estrutura analítica atual e sofisticada, esta tese também fornece um importante primeiro passo para compreender os padrões e processos que podem gerar diversidade em paisagens contínuas da Amazônia.

REFERÊNCIAS BIBLIOGRÁFICAS

Aleixo, A. 2004. Historical diversification of a terra-firme forest bird superspecies: a phylogeographic perspective on the role of different hypotheses of Amazonian diversification. *Evolution*, **58**, 1303-1317.

Avise, J.C. 2000. Phylogeography: the history and formation of species. Cambridge: Harvard University Press. **650p**.

Baker, T.R., Pennington, R.T., Magallon, S., Gloor, E., Laurance, W.F., Alexiades, M., Alvarez, E., Araujo, A., Arets, E.J.M.M., Aymard, G., de Oliveira, A.A., Amaral, I., Arroyo, L., Bonal, D., Brienen, R.J.W., Chave, J., Dexter, K.G., Di Fiore, A., Eler, E., Feldpausch, T.R., Ferreira, L., Lopez-Gonzalez, G., van der Heijden, G., Higuchi, N., Honorio, E., Huamantupa, I., Killeen, T.J., Laurance, S., Leaño, C., Lewis, S.L., Malhi, Y., Marimon, B.S., Marimon Junior, B.H., Monteagudo Mendoza, A., Neill, D., Peñuela- Mora, M.C., Pitman, N., Prieto, A., Quesada, C.A., Ramírez, F., Ramírez Angulo, H., Rudas, A., Ruschel, A.R., Salomão, R.P., de Andrade, A.S., Silva, J.N.M., Silveira, M., Simon, M.F., Spironello, W., Steege, H. ter, Terborgh, J., Toledo, M., Torres-Lezama, A., Vasquez, R., Vieira, I.C.G., Vilanova, E., Vos, V.A. & Phillips, O.L. 2014. Fast demographic traits promote high diversification rates of Amazonian trees. *Ecology Letters*, **17**, 527–536.

Benestan, L., Quinn, B.K., Maaroufi, H., Laporte, M., Clark, F.K., Greenwood, S.J., Rochette, R., Bernatchez, L. 2016. Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (*Homarus americanus*). *Molecular Ecology*, **25**, 5073-5092.

Boubli, J.P., Ribas, C., Lynch Alfaro, J.W., Alfaro, M.E., Silva, M.N.F., Pinho, G.M. & Farias, I.P. 2015. Spatial and temporal patterns of diversification on the Amazon: A test of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in Brazil. *Molecular Phylogenetics and Evolution*, **82**, 400-412.

Borges S. 2007. Análise biogeográfica da avifauna da região oeste do baixo Rio Negro, amazônia brasileira. *Revista Brasileira de Zoologia*, **24**, 919-940.

Borges, S., Silva, J. Da. 2012. A new area of endemism for Amazonian birds in the Rio Negro Basin. *The Wilson Journal of Ornithology*, **124**,15-23.

Bush, MB. 1994. Amazonian speciation: a necessarily complex model. *Journal of Biogeography*, **21**, 5-17.

Cintra, B.B.L., Schietti, J., Emillio, T., Martins, D., Moulatlet, G., Souza, P., Levis, C., Quesada, C.A. & Schöngart, J. 2013. Soil physical restrictions and hydrology regulate stand age and wood biomass turnover rates of Purus–Madeira interfluvial wetlands in Amazonia. *Biogeosciences*, **10**, 7759-7774.

Cohn-Haft, M. 2000. A case study Amazonian biogeography: vocal and DNA-sequence variation in *Hemitriccus flycatchers*. Tese de doutorado, Louisiana State University, Baton Rouge, EUA, **136p**.

Collinvaux, P.A., De Oliveira, P.J. & Bush, M.B. 2000. Amazonian and neotropical plant communities on glacial time-scales: The failure of the aridity and refuge hypotheses. *Quaternary Science Reviews*, **19**, 141-169.

Cracraft, J. 1985. Historical biogeography and patterns of differentiation within the south american avifauna: areas of endemism. *Ornithological Monographs*, **36**, 49-84.

Dexter, K.G., Lavin, M., Torke, B., Twyford, A.D., Kursar, T.A., Coley, P.D., Drake, C., Hollands, R. & Pennington, R.T. 2017. Dispersal assembly of rain forest tree communities across the Amazon basin. *Proceedings of the National Academy of Sciences*, I.

Dias-Terceiro, R.G., Kaefer, I.L., Fraga, R., Araújo, M.C., Simões, P.I. & Lima, A.P. 2015. A Matter of Scale: Historical and Environmental Factors Structure Anuran Assemblages from the Upper Madeira River, Amazonia. *Biotropica*, **47**, 259-266.

Diniz-Filho, J.A.F., Telles, M.P.C., Bonatto, S.L., Eizirik, E., Freitas, T.R.O., Marco, P., Santos, F.R., Sole-Cava, A. & Soares, T.N. 2008. Mapping the evolutionary twilight zone: molecular markers, populations and geography. *Journal of Biogeography*, **35**, 753-763.

Drucker, D.P., Costa, F.R.C. & Magnusson, W.E. 2008. How wide is the riparian zone of small streams in tropical forests? A test with terrestrial herbs. *Journal of Tropical Ecology*, **24**, 65-74.

Dudaniec, R.Y., Rhodes, J.R., Wilmer, J.W., Lyons, M., Lee, K.E., Mcalpine, C.A. & Carrick, F.N. 2013. Using multilevel models to identify drivers of landscape-genetic structure among management areas. *Molecular Ecology*, **22**, 3752-3765.

Dudaniec, R.Y., Wilmer, J.W., Hanson, J.O., Warren, M., Bell, S. & Rhodes, J.R. 2016. Dealing with uncertainty in landscape genetic resistance models: a case of three co-occurring marsupials. *Molecular Ecology*, **25**, 470-486.

Duellman, W.E. & Trueb, L. 1986. Biology of Amphibians. The Johns Hopkins University Press, Baltimore, USA. **670pp**.

Duellman, W.E. 1988. Patterns of species diversity in anuran amphibians in the American Tropics. *Annals of the Missouri Bot. Garden*, **75**, 79-104.

Duellman, W.E. 1995. Temporal fluctuations in abundance of anuran amphibians in a seasonal amazonian rainforest. *Journal of Herpetology*, **29**, 13-21.

Duellman, W.E. 1999. Distribution patterns of amphibians in South America. In: W.E. Duellman (ed.). *Patterns of distribution of amphibians: a global perspective*. The Johns Hopkins University Press, Baltimore, USA. **255-328p**.

Endler, J. 1977. Geographic variation, speciation, and clines. Princeton University Press. Princeton, New York, **480-496p**.

Endler, J.A. 1982. Pleistocene refuge: fact or fancy? Biological diversification in the tropics. Columbia University Press. New York, **641-657p**.

Fearnside, P.M. 2006. Dams in the Amazon: Belo Monte and Brazil's hydroelectric development of the Xingu River Basin. *Environmental Management*, **38**, 16-27.

Fernandes, A.M., Cohn-Haft, M., Hrbek, T. & Farias, I.P. 2014. Rivers acting as barriers for bird dispersal in the amazon. *Revista Brasileira de Ornitologia*, **22**, 363-373.

Fine, P.V.A., Daly, D.C., Muñoz, G. V., Mesones, I. & Cameron, K.M. 2005. The contribution of edaphic heterogeneity to the evolution and diversity of burseraceae trees in the Western Amazon. *Evolution*, **59**, 1464-1478.

Fittkau, E.J., Junk, W., Klinge, H. & Sioli, H. 1975. Substrate and vegetation in the Amazon region. *Berichte der Internationalen Symposien der Internationalen Vereinigung für Vegetationskunde Herausgegeben von Reinhold Tüxen*. (ed J. Cramer), 7390pp. J. Cramer, Vaduz.

Fraga, R., Lima, A.P., Magnusson, W.E., Ferrão, M. & Stow, A.J. 2017. Contrasting patterns of gene flow for Amazonian snakes that actively forage and those that wait in ambush. *Journal of Heredity*, **108**, 524-534.

Funk, W.C., Caldwell, J.P., Peden, C.E., Padial, J.M., De la Riva, I. & Cannatella, D.C. 2007. Tests of biogeographic hypotheses for diversification in the Amazonian forest frog, *Physalaemus petersi*. *Molecular Phylogenetics and Evolution*, **44**, 825-837.

Gascon, C., Lougheed, S.C. & Bogart, J.P. 1996. Genetic and morphological variation in *Vanzolinius discodactylus*: a test of the river hypothesis of speciation. *Biotropica*, **28**, 376-387.

Gascon, C., Lougheed, S.C. & Bogart, J.P. 1998. Patterns of genetic population differentiation in four species of Amazonian frogs: a test of the riverine barrier hypothesis. *Biotropica*, **30**, 104-119.

Gascon, C., Malcolm, J.R., Patton, J.L., Silva, M.N.F., Bogarti, J.P., Lougheed, S.P., Peres, C.A., Neckel, S. & Boag, P.T. 2000. Riverine barriers and the geographic distribution of Amazonian species. *PNAS*, **97**, 13672-13677.

Gaston, K.J. & Blackburn, T.M. 2003. Macroecology and conservation biology. In T. M. Blackburn, & K. J. Gaston (Eds.), *Macroecology: Concepts and consequences*. Oxford: Blackwell Science.

Gentry, A.H. 1988. Tree species richness of upper Amazonian forests. Proceedings of the *National Academy of Science USA*, **85**, 156-159.

Haddad, C.F.B. & Prado, C.P.A. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic forest of Brazil. *BioScience*, **55**, 207-217.

Haffer, J. 1969. Speciation in Amazonian Forest Birds. *Science*, **165**, 131-137.

Haffer, J. 1974. Avian speciation in Tropical South America: With a systematic survey of the toucans (Ramphastidae) and jacamars (Galbulidae). *Publications of the Nuttall Ornithological Club*, **14**, 1-390.

Hayes, F.E. & Sewlal, J.A.N. 2004. The Amazon River as a dispersal barrier to passerine birds: effects of river width, habitat and taxonomy. *Journal of Biogeography*, **31**, 1809-1818.

Hoorn, C., Wesselingh, F.P., Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, J., Särkinen, T. & Antonelli, A. 2010. Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science,* **330**, 927-931.

Hoorn, C. & Wesselingh, F.P. 2010. Amazonia, landscape and species evolution. Blackwell Publishing, Oxford. **390p.**

Hoorn, C. 1994. An environmental reconstruction of the palaeo-Amazon River system (Middle–Late Miocene, NW Amazonia). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **112**, 187-238.

Hughes, C.E., Pennington, R.T. & Antonelli, A. 2013. Neotropical Plant Evolution: Assembling the Big Picture. *Botanical Journal of the Linnean Society*, **171**, 1-18.

Jorge, R.F., Simões, P.I., Magnusson, W.E. & Lima, A.P. 2016. Fine-scale habitat heterogeneity explains the local distribution of two Amazonian frog species of concern for conservation. *Biotropica*, **48**, 694-703.

Lack, D. 1969. The numbers of bird species on islands. *Bird Study*, v.16, **193-209p.**

Legendre, P. & Fortin, J.M. 1989. Spatial pattern and ecological analysis. *Vegetatio*, v.80, **107-138p**.

Legendre, P. 1993. Spatial Autocorrelation: Trouble or new paradigm? *Ecology*, v.74, **1659-1673p**.

Leite, R.N. & Rogers, D.S. 2013. Revisiting Amazonian phylogeography: insights into diversification hypotheses and novel perspectives. *Organisms Diversity & Evolution*, **13**, 639-664.

Lemmon, E.M. & Lemmon, A.R. 2013. High-throughput genomic data in systematics and phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 99-121.

Lewis, S.L., Brando, P.M., Phillips, O.L., van der Heijden, G.M.F. & Nepstad, D. 2011. The 2010 Amazon drought. *Science (New York, N.Y.),* **331**, 554.

Lovejoy, N.R., Bermingham, E. & Martin, A.P. 1998. Marine incursion into South America. *Nature*, **396**, 421-422.

Lowe, W.H. & Allendorf, F.W. 2010. What can genetics tell us about population connectivity? *Molecular Ecology*, **19**, 3038-3051.

MacArthur, R.H. & Wilson, E.O. 1967. The theory of island biogeography. Princeton University Press, Princeton.

Manthey, J.D. & Moyle, R.G. 2015. Isolation by environment in white-breasted nuthatches (*Sitta carolinensis*) of the Madrean Archipelago sky islands: a landscape genomics approach. *Molecular Ecology*, **24**, 3628-3638.

Marrote, R.R., Gonzales, A. & Millien, V. 2014. Landscape resistance and habitat combine to provide an optimal model of genetic structure and connectivity at the range margin of a small mammal. *Molecular Ecology*, **23**, 3983-3998.

Martins, D.L., Schietti, J., Feldpausch, T.R., Luizão, F.J., Phillips, O.L., Andrade, A., Castilho, C.V., Laurance, S.G., Oliveira, A., Amaral, I.L., Toledo, J.J., Lugli, L.F., Pinto, J.L.P.V., Mendoza, E.M.O. & Quesada, C.A. 2014. Soil-induced impacts on forest structure drive coarse woody debris stocks across central Amazonia. *Plant Ecology & Diversity,* **7**, 1-13.

McKinney, G.J., Larson, W.A., Seeb, L.W. & Seeb, J.E. 2016. RADseq provides unprecedented insights into molecular ecology and evolutionary genetics: comment on Breaking RAD by Lowry et al. (2016). *Molecular Ecology Resources*, **17**, 356-361.

Menin, M., Waldez, F. & Lima, A.P. 2011. Effects of environmental and spatial factors on the distribution of anuran species with aquatic reproduction in central Amazonia. *Herpetological Journal*, **21**, 255-261.

Menin, M., Lima, A.P., Magnusson, W.E. & Waldez, F. 2007. Topographic and edaphic effects on the distribution of terrestrially reproducing anurans in Central Amazonia: mesoscale spatial patterns. *Journal of Tropical Ecology*, **23**, 539-547.

Mittermeier, R.A., Mittermeier, C.G., Brooks, T.M., Pilgrim, J.D., Konstant, W.R. da, Fonseca, G.A.B. & Kormos, C. 2003. Wilderness and biodiversity conservation. *PNAS*, **100**, 10309-10313.

Moritz, C., Patton, J.L., Schneider, C.J. & Smith, T.B. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics*, **31**, 533-563.

Morrone, J.J. 2009. Evolutionary biogeography: an integrative approach with case studies. New York: Columbia University Press, **345-351p**.

Nielsen, R. 2005. Molecular signatures of natural selection. *Annual Review of Genetics*, **39**, 197-218.

Nobre, A.D. 2014. *O Futuro Climático Da Amazônia.* São José dos Campos.

Nores, M. 1999. An alternative hypothesis of the origin of Amazonian bird diversity. *Journal of Biogeography*, **26**, 275-485.

Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford, **304pp**.

Oden, N.L. & Sokal, R.R. 1986. Directional autocorrelation: an extension of spatial correlograms to two dimensions. *Systematic Zoology*, **35**, 608-617.

Oliveira, U., Vasconcelos, M.F. & Santos, A.J. 2017. Biogeography of Amazon birds: rivers limit species composition, but not areas of endemism. *Scientific Reports*, **7**, 2992.

Opdam, P. & Wascher, D. 2004. Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation*, **117**, 285-297.

Ortiz, D.A., Lima, A.P. & Werneck, F.P. 2018. Environmental transition zone and rivers shape Intraspecific population structure and genetic diversity of an Amazonian rain forest tree frog. *Evolutionary Ecology*, **32**, 359-378.

Peloso, P.L., Sturaro, M.J., Forlani, M.C., Gaucher, P., Motta, A.P. & Wheeler, W.C. 2014. Phylogeny, taxonomic revision, and character evolution of the genera Chiasmocleis and Syncope (Anura: Microhylidae) in the Amazonia, with descriptions of three new species. *Bulletin of American Museum of Natural History*, **386**, 1-112.

Peterman, W.E., Connette, G.M., Semlitsch, R.D. & Eggert, L.S. 2014. Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. *Molecular Ecology*, **23**, 2402-2413.

Pianka, E.R. 1966. Convexity, desert lizard, and special heterogeneity. *Ecology*, v.47, **1055-1059p.**

Pinto, M.P., Bini, L.M. & Diniz-Filho, J.A.F. 2003. Análise quantitativa da influência de um novo paradigma ecológico: autocorrelação espacial. Maringá, v.25, n. 1, **137-143p**.

Pough, F.H., Andrews, R.M., Cadle, J.E., Crump, M.L., Savitzky, A.H. & Wells, K.D. 2003. Herpetology. 3rd Edition. Prentice-Hall, New Jersey, USA. **736p.**

Quesada, C.A., Phillips, O.L., Schwarz, M., Czimczik, C.I., Baker, T.R., Patiño, S., Fyllas, N.M., Hodnett, M.G., Herrera, R., Almeida, S., Alvarez Dávila , E., Arneth, A., Arroyo, L., Chao, K.J., Dezzeo, N., Erwin, T., Di Fiore, A., Higuchi, N., Honorio Coronado, E., Jimenez, E.M., Killeen, T., Lezama, A.T., Lloyd, G., López-González, G., Luizão, F.J., Malhi, Y., Monteagudo, A., Neill, D.A., Núñez Vargas, P., Paiva, R., Peacock, J., Peñuela, M.C., Peña Cruz, A., Pitman, N., Priante Filho, N., Prieto, A., Ramírez, H., Rudas, A., Salomão, R., Santos, A.J.B., Schmerler, J., Silva, N., Silveira, M., Vásquez, R., Vieira, I., Terborgh, J. & Lloyd, J. 2012. Basin-wide variations in Amazon forest structure and function are mediated by both soils and climate. *Biogeosciences*, **9**, 2203-2246.

Radespiel, U, & Bruford, M.W. 2014. Fragmentation genetics of rainforest animals: insights from recent studies. *Conservation Genetics*, **15**, 245-260.

Rasanen, M.E., Linna, A.M., Santos, J.C.R. & Negri, F.R. 1995. Late Miocene tidal deposits in the Amazonian foreland basin. *Science,* **269**, 386-390.

Ribas, C.C., Aleixo, A., Gubili, C., d'Horta, F.M., Brumfield, R.T. & Cracraft, J. 2018. Biogeography and diversification of Rhegmatorhina (Aves: Thamnophilidae): Implications for the evolution of Amazonian landscapes during the Quaternary. *Journal of Biogeography*, **45**, 917-928.

Ribas, C.C., Aleixo, A., Nogueira, A.C.R., Miyaki, C.Y. & Cracraft, J. 2012. A paleobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society of London Series B*, **279**, 681-689.

Ribeiro, J.W., Lima, A.P. & Magnusson, W.E. 2012. The effect of riparian zones on species diversity of frogs in Amazonian forest. *Copeia*, **2012**, 375-381.

Ricklefs, R.E. 2003. A economia da natureza. 5ª Edição. Guanabara Koogan. **542p**.

Ricklefs, R.E. 2004. A comprehensive framework for global patterns in biodiversity. *Ecology Letters*, **7**, 1-15.

Rojas-Ahumada, D.P., Landeiro, V.L. & Menin, M. 2012. Role of environmental and spatial processes in structuring anuran communities across a tropical rain forest. *Austral Ecology*, **37**, 865-873.

Rossetti, D.F., Toledo, P.M., Góes, A.M. 2005. New geological framework for Western Amazônia (Brazil) and implications for biogeography and evolution. *Quarternary Research*, **63**, 78-89.

Row, J.R., Knick, S.T., Oyler-McCance, S.J., Lougheed, S.C. & Fedy, B.C. 2017. Developing approaches for linear mixed in landscape genetics through landscape-directed dispersal simulations. *Ecology and Evolution*, **7**, 3751-3761.

Ruiz-Lopez, M.J., Barelli, C., Rovero, F., Hodges, K., Roos, C., Peterman, W.E., Ting, N. 2016. A novel landscape genetic approach demonstrates the effects of human disturbance on the Udzungwa red colobus monkey (*Procolobus gordonorum*). *Heredity (Edinb)*, **116**, 167-176.

Rull, V. 2011. Neotropical biodiversity: timing and potential drivers. *Trends in ecology & evolution*, **26**, 508-513.

Santorelli, S., Magnusson, W.E. & Deus, C.P. 2018. Most species are not limited by an Amazonian river postulated to be a border between endemism areas. *Scientific Reports*, **8**, 2294.

Savolainen, O., Lascoux, M. & Merilä, J. 2013. Ecological genomics of local adaptation. *Nature Reviews Genetics*, **14**, 807-820.

Schietti, J., Martins, D., Emilio, T., Souza, P.F., Levis, C., Baccaro, F.B., Pinto, J.L.P.V., Moulatlet, J.M., Stark, S.C., Sarmento, K., Araújo, N.O., Costa, F.R.C., Schöngart, J., Quesada, C.A., Saleska, S.R., Tomasella, J. & Magnusson, W.E. 2016. Forest structure along a 600 km transect of natural disturbances and seasonality gradients in central-southern Amazonia. *Journal of Ecology*, **104**, 1335-1346.

Smith, B.T., McCormack, J.E., Cuervo, A.M., Hickerson, M.J., Aleixo, A., Cadena, C.D., Pérez-Emán, J., Burney, C.W., Xie, X., Harvey, M.G., Faircloth, B.C., Glenn, T.C., Derryberry, E.P., Prejean, J., Fields, S. & Brumfield, R.T. 2014. The drivers of tropical speciation. *Nature*, **515**, 406-409.

Sombroek, W. 2000. Amazon landforms and soils in relation to biological diversity. *Acta Amazonica*, **30**, 81-100.

Stow, A.J., Sunnucks, P., Briscoe, D.A. & Gardner, M.G. 2001. The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology*, **10**, 867-878.

Symula, R., Schulte, R. & Summers, K. 2003. Molecular systematics and phylogeography of Amazonian poison frogs of the genus Dendrobates. *Molecular Phylogenetics and Evolution*, **26**, 452-475.

Tews, J., Brose, U., Grimm, V., Tielbörger K., Wichmann M.C., Schwager M. & Jeltsch, F. 2004. Animal species diversity driven by habitat heterogeneity: the importance of keystone structures. *Journal of Biogeography*, **31**, 79-92.

Tuomisto, H. 2007. Interpreting the biogeography of South America. *Journal of Biogeography*, *34*, 1294-1295.

Wallace, A.R. 1852. On the monkeys of the Amazon. *Proceedings of the Zoological Society of London*, **20**, 107-110.

Wang, I.J. & Bradburd, G.S. 2014. Isolation by environment. *Molecular Ecology*, **23**, 5649-5662.

Wiens, J.J. & Donoghue, M.J. 2004. Historical biogeography, ecology and species richness. *Trends in ecology & evolution*, **19**, 639-44.

Wiens, J.A. 1989. Spatial scaling in ecology. *Functional Ecology*, **3**, 385-397.

Willette, D.A., Allendorf, F.W., Barber, P.H., Barshis, D.J., Carpenter, K.E., Crandall, E.D., Cresko, W.A., Fernandez-Silva, I., Matz, M.V., Meyer, E., Santos, M.D., Seeb, L.W. & Seeb, J.E. 2014. So, you want to use next-generation sequencing in marine systems? Insight from the Pan-Pacific Advanced Studies Institute. *Bulletin of Marine Science*, **90**, 79-122.

Willing, E.M., Dreyer, C. & van Oosterhout, C. 2012. Estimates of genetic differentiation measured by F(ST) do not necessarily require large sample sizes when using many SNP markers. *PLoS One*, **7**, e42649.

Zimmerman, B.L. & Bierregaard, R.O. 1986. Relevance of the equilibrium theory of island biogeography and species-area relations to conservation with a case from Amazonia. *Journal of Biogeography*, **13**, 133-143.