Instituto Nacional de Pesquisas da Amazônia
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Diversidade regional de serpentes na Amazônia: uma abordagem multidimensional com implicações para conservação de paisagens naturais

Rafael de Fraga

Manaus, Amazonas
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Orientador: Albertina P. Lima

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Sinopse:
Estudou-se relações de custo-benefício de amostragem de serpentes para avaliações de impacto ambiental na Amazônia e padrões de diversidade regional de serpentes definidas por diferentes medidas de diversidade-beta. Também foram comparados padrões de fluxo gênico entre serpentes com diferentes modos de forrageio.

Palavras-chave: custo-benefício, diversidade beta, fluxo gênico, gradientes ecológicos, impacto ambiental, RAPELD, genômica, SNP

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Resumo

A superexploração de recursos naturais tem ameaçado as florestas tropicais em ritmo alarmante, e estudos integrativos se fazem necessários para o delineamento de ações efetivas de conservação. Nessa tese nós usamos serpentes como um grupo-modelo para estudos em ecologia de comunidades e genômica de paisagens, em três capítulos com objetivos diferentes, mas todos convergindo em embasamento científico para conservação da biodiversidade. No capítulo I nós apresentamos uma avaliação de custo-benefício de amostragem de serpentes para avaliações de impacto ambiental na Amazônia. Nós mostramos que baixas probabilidades de detecção associadas ao alto custo de amostragem de serpentes (U$ 120 por indivíduo) tornam esse grupo pouco adequado para estudos em curto prazo. Alternativamente nós sugerimos que avaliações de impacto ambiental sejam focadas em grupos taxonômicos que simultaneamente reflitam medidas de diversidade robustas, e sejam relativamente fáceis para se obter dados. Os demais capítulos dessa tese resultam de campanhas de monitoramento de serpentes em unidades amostrais padronizadas, distribuídas ao longo de nove anos. No capítulo II nós testamos os efeitos de gradientes ecológicos de densidade de árvores, altitude e porcentagem de argila no solo sobre medidas de β-diversidade taxonômica, funcional e filogenética. Nós mostramos que os três gradientes testados funcionam como filtros à diversidade taxonômica, o que gera padrões regionais de co-ocorrência de espécies. No entanto, diferenças em traços funcionais e filogenias entre unidades amostrais são aleatoriamente distribuídas ao longo da paisagem, independentemente da variação nos gradientes testados. No capítulo III nós usamos milhares de marcadores genéticos (SNPs) para comparar padrões de fluxo gênico entre duas serpentes predadoras senta-e-espera e duas forrageadoras ativas. Nós mostramos que a capacidade de dispersão mais baixa em predadores senta-e-espera gera diferenciação genética entre indivíduos e redução no fluxo gênico por meio de isolamento por distância geográfica e resistência ambiental. Os forrageadores ativos, por sua vez, têm altos níveis de fluxo gênico ao longo de cerca de 880 km de uma paisagem heterogênea, e nenhuma evidência de estruturação genética foi encontrada, independentemente de distância geográfica e resistência ambiental. Nós esperamos que os capítulos dessa tese sejam parte de estudos integrativos de biodiversidade, os quais vêm sendo conduzidos na Amazônia por meio de amostragem padronizada de diferentes grupos de animais e plantas.

Regional snake diversity in the Amazon: a multidimensional approach with implications for conservation of natural landscapes

Abstract

The overexploitation of natural resources have threatened tropical forests at an alarming rate, and integrative studies are necessary for planning effective conservation actions. In this thesis we used snakes as a model-group in studies on community ecology and landscape genomics in three chapters with different goals, but all converging in scientific basis for biodiversity conservation. In the Chapter I we present a cost-benefit evaluation of sampling snakes for environmental impact assessments in the Amazon. We show that the high cost of sampling snakes ($ 120 per individual) associated with the low detection probabilities makes this group inappropriate for short-term studies. Alternatively we suggest that environmental impact assessments are focused on taxonomic groups that simultaneously reflect robust diversity measures and are relatively easy to obtain samples. The remaining chapters of this thesis result from sampling surveys along nine years of monitoring snakes in standardized sampling units. In the Chapter II we tested the effects of ecological gradients of tree density, altitude and soil clay content on measures of taxonomic, functional and phylogenetic β-diversity. We show that the three gradients tested act as filters on the taxonomic diversity, which creates regional patterns of co-occurring species. However, differences in functional traits and phylogenies among sampling units are randomly distributed throughout the landscape, regardless of variation in the gradients tested. In the Chapter III we used thousands of genetic loci (SNPs) to compare patterns of gene flow between two ambush predator snakes and two active foragers. We show that the lower dispersal capability in ambush predators generates genetic differentiation among individuals and reduces the gene flow through isolation by geographic distance and environmental resistance. The active foragers, in turn, have high levels of gene flow over about 880 km of an heterogeneous landscape, and no evidence of genetic structure was found, regardless of the geographic distance and environmental resistance. We expect that the chapters of this thesis are part of integrative studies on biodiversity in the Amazon, which have been conducted through standardized sampling of different groups of animals and plants.

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Introdução geral

As florestas tropicais têm intrigado e fascinado naturalistas e cientistas de biodiversidade ao longo dos séculos. A ciência tem progredido consideravelmente desde as primeiras observações da biodiversidade tropical no século XVIII, de descrições de espécies em poucos parágrafos (*e.g.* Linnaeus, 1758) até a nossa capacidade atual de detectar estágios iniciais de especiação por meio de técnicas de biologia molecular (*e.g.* Kaefer et al., 2013). Contudo, o acesso a áreas remotas de florestas tropicais ao longo dos séculos tem favorecido não somente a ciência, mas também a exploração de recursos naturais que modifica paisagens em ritmo alarmante. As florestas tropicais têm sido devastadas rapidamente por todo o mundo, e hoje a ciência enfrenta o desafio de tentar entender biodiversidade em ambientes altamente complexos, correndo contra a perda de hábitats.

Uma abordagem eficiente para otimizar estudos sobre biodiversidade é a aplicação de métodos integrativos, baseados na padronização de um desenho amostral eficiente para diferentes grupos taxonômicos. Por exemplo, nas florestas tropicais da bacia amazônica a padronização de amostragens pelo método RAPELD (Magnusson et al., 2013) tem contribuído muito para a nossa compreensão sobre mecanismos que geram e mantêm biodiversidade em escalas regionais. Conjuntos de dados padronizados provenientes de diferentes sítios de pesquisa têm sido coletados e publicados em estudos focados em muitos grupos taxonômicos, como plantas (Costa et al., 2005; Drucker et al., 2008), invertebrados (Franklin et al., 2008), peixes de poças (Espírito-Santo et al., 2009), anfíbios (Menin et al., 2007; Rojas-Ahumada et al., 2010; Ribeiro-Jr et al., 2012), lagartos (Lobão, 2008; Moraes, 2008), serpentes (Fraga et al., 2011; Fraga et al., 2013; esta tese), aves de sub-bosque (Bueno et al., 2012), mamíferos terrestres (Mendes et al., 2008) e voadores (Silva e Marques, 2010). Cada um desses estudos é uma contribuição importante para a nossa escassa compreensão sobre interações entre espécies e hábitats em ambientes complexos da bacia amazônica. Pela integração de dados padronizados somos capazes de planejar ações efetivas de conservação, tais como desenho de reservas e avaliação de impactos ambientais antropogênicos.

Amostragem de serpentes geralmente é exigida em avaliações de impacto ambiental para licenciamento de construção e operação de equipamentos industriais e de infraestrutura. Recentemente as agências ambientais têm exigido ou recomendado que essas avaliações sejam padronizadas pelo método RAPELD, porque esse desenho amostral apresenta vantagens em comparação aos métodos tradicionais usados em avaliações de impacto. Por exemplo, o método RAPELD é simultaneamente eficiente para amostragem em curto e longo

prazos, gera dados provenientes de diferentes sítios de pesquisa totalmente comparáveis entre si e otimiza o uso de gradientes ecológicos como variáveis preditoras em modelos inferenciais para testar associações entre espécies e hábitats (Magnusson et al., 2013). No entanto, pelo fato de ser relativamente recente e pouco utilizado para muitos grupos de organismos (*e.g.* serpentes), o método RAPELD deve ser otimizado por ajustes na sua aplicação, como na quantidade de esforço amostral necessária para testar efeitos de impactos ambientais sobre medidas de diversidade. Especificamente para amostragem de serpentes, nós não sabemos quantas observações são necessárias por unidade amostral para detectar padrões ecológicos minimamente enviesados por artefatos de amostragem. Amostragem de serpentes é frequentemente enviesada por falsas ausências causadas por baixas detectabilidades associadas aos hábitos de vida crípticos (Steen, 2010; Fraga et al., 2014), o que pode causar desperdício de tempo e dinheiro em estudos multi-taxa para avaliações de impacto ambiental. No capítulo I desta tese nós apresentamos uma avaliação de custo-benefício de amostragem de serpentes usando o método RAPELD, com foco em tomadas de decisões para avaliações de impacto ambiental no sudoeste da Amazônia (alto Rio Madeira, Rondônia). Nós avaliamos a perda de informações sobre número de espécies e β-diversidade pela redução simulada no esforço amostral (comprimento de trilhas e número de observações).

As terras baixas da Amazônia possuem alta alta diversidade de serpentes, com mais de 150 espécies formalmente descritas. A maioria das espécies é amplamente distribuída por áreas de floresta úmida ao longo da bacia amazônica, o que tem gerado similaridade entre regiões superior a 60 % na composição de espécies (Jorge da Silva e Sites, 1995). No entanto, amplitudes regionais de gradientes ecológicos podem filtrar a composição taxonômica de serpentes, o que resulta em diferentes padrões de co-ocorrência de espécies ao longo da paisagem (Fraga et al., 2011, esta tese). Gradientes ecológicos em escalas regionais também são fatores relevantes influenciando a organização espacial de populações de serpentes na Amazônia (Fraga et al., 2013). Compreender como a variação natural em gradientes ecológicos ao longo de paisagens heterogêneas influencia padrões de diversidade regional é uma questão fundamental em ecologia, porque efetivamente sustenta ações de conservação baseadas em complementaridades e redundâncias bióticas entre locais (Pressey et al., 1993).

As baixas probabilidades de detecção de serpentes podem tornar esse grupo inadequado para estudos em curto prazo e com poucas unidades amostrais, como as avaliações de impacto ambiental tradicionais no Brasil (Fraga et al., 2013). No entanto, por meio de monitoramento padronizado de serpentes em 21 módulos RAPELD distribuídos ao

longo de 880 km, nós obtivemos dados com qualidade suficiente para determinar padrões regionais de diversidade associados à heterogeneidade ambiental nas florestas tropicais da região central ao sudoeste da Amazônia. Gradientes ambientais têm sido amplamente identificados como filtros para diversidade de espécies, mas diferentes abordagens para quantificar diversidade resultam em conclusões diferentes. Por exemplo, medidas de diversidade taxonômica geram estimativas de padrões de co-ocorrência de espécies (*e.g.* Fraga et al., 2011), por meio da identificação de subconjuntos de espécies que ocorrem em diferentes regiões de gradientes ecológicos. No entanto, a definição de diversidade baseada apenas em categorias taxonômicas pode não ser eficiente para investigar o papel de gradientes ecológicos como filtros para traços funcionais e filogenias. Essas dimensões de diversidade são melhor demonstradas por estimativas da distribuição de diversidades funcional e filogenética ao longo de gradientes ecológicos. Medidas de diversidade funcional quantificam a contribuição de cada espécie para o funcionamento global de comunidades (Tilman, 2001), enquanto diversidade filogenética incorpora informações da história evolutiva compartilhada pelas espécies em uma comunidade (Milcu et al., 2013). Por meio de abordagens multidimensionais, as quais englobam diferentes medidas de diversidade, pesquisadores são capazes de refinar o entendimento sobre os mecanismos que geram e mantêm biodiversidade.

No capítulo II desta tese nós quantificamos diversidades taxonômica, funcional e filogenética de serpentes ao longo de 880 km de floresta da região central (Manaus) ao sudoeste da Amazônia (Porto Velho). Nós testamos os efeitos de gradientes ecológicos de densidade de árvores, altitude e proporção de argila no solo como filtros para cada medida de diversidade. Nós usamos medidas de β-diversidade (diferenças entre parcelas de amostragem), porque esta abordagem tem sido altamente recomendada para ecologia e conservação (*e.g.* Meynard et al., 2011; Grass et al., 2015). Medidas de β-diversidade foram identificadas como mais informativas em comparação a α-diversidade (*e.g.* número de espécies), porque quantificam substituição de espécies ao longo da paisagem por meio de complementaridades e redundâncias bióticas entre locais (Magnusson et al., 2013). Em última análise, β-diversidade permite identificar características únicas na diversidade regional, o que torna um local insubstituível e, portanto, prioritário para ações de conservação (Pressey et al., 1993).

Investigar relações entre diferentes medidas de diversidade e gradientes ecológicos tem sido uma abordagem eficaz para determinar mecanismos que geram e mantêm biodiversidade em diferentes escalas. No entanto, padrões de diversidade regional podem ser melhor compreendidos por meio da incorporação de características biológicas das espécies-

alvo em modelos preditivos. Por exemplo, a capacidade de dispersão de uma espécie é um fator chave para a colonização de diferentes ambientes ao longo de gerações. Variação interespecífica na capacidade de dispersão impulsiona diferentes interações entre fluxo gênico, deriva genética e seleção natural. Em geral, capacidade de dispersão alta pode homogeneizar a variabilidade genética, porque permite a entrada de genótipos provenientes de diferentes *pools* gênicos por meio de pouca ou nenhuma resistência ao cruzamento aleatório entre indivíduos (Hellberg, 1996). Por outro lado, dispersão restrita tende a gerar padrões locais de estrutura genética gerados pela redução no fluxo gênico (Wright, 1943). Investigar a influência da capacidade de dispersão sobre o fluxo gênico ao longo da paisagem é relevante para determinar a conectividade entre locais geograficamente distantes e ambientalmente distintos dentro da distribuição geográfica de uma espécie.

No capítulo III desta tese nós usamos milhares de locus gênicos (SNPs) para comparar padrões de fluxo gênico entre quatro espécies de serpentes, as quais diferem na capacidade de dispersão associada ao modo de forrageio. Duas dessas espécies são predadoras de espreita (*Bothrops atrox* e *Corallus hortulanus*), e tendem a passar longos períodos em áreas relativamente pequenas. As outras duas espécies são forrageadoras ativas (*Leptodeira annulata* e *Philodryas georgeboulengeri*), e tendem a ter mais mobilidade. Nós quantificamos diferenças em padrões de fluxo gênico entre os dois modos de forrageio, baseados em isolamento por distância geográfica (IBD) e resistência ambiental (IBR). O primeiro foi quantificado por relações lineares entre distância genética e distância geográfica, e o último foi quantificado por meio de cálculos de probabilidades de um indivíduo dispersar de um local para outro, após ponderar "custos de dispersão" para cada tipo de ambiente e avaliar todas as rotas possíveis (Wang e Bradburd, 2015).

Essa tese é parte de uma base integrativa de dados provenientes de amostragem padronizada entre diferentes grupos taxonômicos. Ela é uma contribuição ao monitoramento integrado de biodiversidade na Amazônia, como proposta inovadora para o progresso da ciência e embasamento científico para ações efetivas de conservação.

Objetivo geral

Produzir embasamento científico para conservação na Amazônia, por meio de metodologia baseada em custo-benefício de amostragem de serpentes e avaliações de associações espécies-hábitats usando diferentes métodos.

Objetivos específicos

1. Avaliar relações de custo-benefício de amostragem de serpentes para estudos de impacto ambiental na Amazônia, testando os efeitos da redução no esforço amostral sobre número e composição de espécies por unidade amostral;
2. Testar os efeitos de gradientes ecológicos de densidade de árvores, altitude e porcentagem de argila no solo sobre medidas de β-diversidade taxonômica, funcional e filogenética;
3. Comparar padrões de fluxo gênico ao longo de 880 km de floresta entre serpentes predadoras de espreita e forrageadoras ativas, com base em estruturação genética e nos efeitos de isolamento por distância geográfica e resistência ambiental sobre a distância genética entre indivíduos.

Capítulo I

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The costs of evaluating species densities and composition of snakes to assess development impacts in Amazonia

Rafael de Fraga[a,1], Adam J. Stow[b], William E. Magnusson[c], Albertina P. Lima[c]

[a] Instituto Nacional de Pesquisas da Amazônia – Programa de Pós-graduação em Ecologia, Av. Efigênio Salles, 2239, Manaus – AM, CEP 69060-020

[b] Department of Biological Sciences, Macquarie University, NSW 2109, Australia

[c] Instituto Nacional de Pesquisas da Amazônia – Coordenação de Biodiversidade, POBOX 2223, Manaus – AM

[1] r.defraga@gmail.com, +55 92 3643-1832

Abstract

Studies leading to decision-making for environmental licensing often fail to provide accurate estimates of diversity. Measures of snake diversity are regularly obtained to assess development impacts in the rainforests of the Amazon Basin, but this taxonomic group may be subject to poor detection probabilities. Recently, the Brazilian government tried to standardize sampling designs by the implementation of a system (RAPELD) to quantify biological diversity using spatially-standardized sampling units. Consistency in sampling design allows the detection probabilities to be compared among taxa, and sampling effort and associated cost to be evaluated. The cost effectiveness of detecting snakes has received no attention in Amazonia. Here we tested the effects of reducing sampling effort on estimates of species densities and assemblage composition. We identified snakes in seven plot systems, each standardised with 14 plots. The 250 m long centre line of each plot followed an altitudinal contour. Surveys were repeated four times in each plot and detection probabilities

were estimated for the 41 species encountered. Reducing the number of observations, or the size of the sampling modules, caused significant loss of information on species densities and local patterns of variation in assemblage composition. We estimated the cost to find a snake as $ 120 U.S., but general linear models indicated the possibility of identifying differences in assemblage composition for half the overall survey costs. Decisions to reduce sampling effort depend on the importance of lost information to target-issues, and may not be the preferred option if there is the potential for identifying individual snake species requiring specific conservation actions. However, in most studies of human disturbance on species assemblages, it is likely to be more cost-effective to focus on other groups of organisms with higher detection probabilities.

Key-words: Detection probability; disturbance; NMDS; RAPELD; spatial distribution

1. Introduction

Obtaining environmental licenses to build and operate infrastructure or industrial facilities typically requires environmental assessment, and this process usually evaluates abiotic features, such as soil and water, and biotic features, such as fauna and flora. In Brazil, taxonomic groups to be included in environmental-impact assessments are defined by environmental agencies, and these usually include “herpetofauna”, which includes snakes. Compared with other surveyed vertebrate groups, snakes are often rarely encountered, and can be more difficult to detect because they are secretive and cryptic [1], [2]. The problem of detecting snakes may be exacerbated by dense vegetation in rainforest areas, such as those in the Amazon Basin. This raises the question of whether collecting data on snakes is a cost-effective means of evaluating the impacts of environmental change in the rainforests of the Amazon Basin.

Describing human impacts on wildlife assemblages and developing conservation strategies can be based on monitoring that repeatedly measures the biotic response to disturbance [3] and provides data with direct application to setting priorities for research and conservation [4], [5]. Complementary phylogenetic and functional diversities have been shown to be more suitable measures to assess assemblage changes, in comparison to species densities and composition [6], because disturbance is usually not acting at the level of species alone, but changing a network of abiotic and biotic factors interacting to filter species assemblies [7]. However defining functional groups depends on accurate estimates of multivariate niche overlap, which has been a challenge in tropical forests. Also, phylogenetic diversity alone does not necessarily reflect functional distance, because evolutionary traits may converge and diverge rapidly [6]. Because this study aimed to test only the effects of sampling design on ecological patterns, we represent snake assemblages using the number of species detected per plot system and species composition.

Recently, the Brazilian environmental agencies have recognized the value of standardizing sampling designs, and require or recommend the use of the RAPELD system [8], [9]. RAPELD is an acronym for rapid assessments (RAP) combined with long term ecological research (PELD; in Portuguese). This method was modified from the 0.1 ha survey method developed by Gentry [10], differing primarily in that the direction of the long axis of each plot is along the altitudinal contour; use of different widths of plot for different taxa; and regular distribution of the plots across the landscape to be sampled [8]. Surveying along the contour line reduces the effects of change in altitude along the plot. Altitude probably does not directly affect organisms in lowland Amazonia, but it is related to other factors influencing plant and animal assemblages, such as edaphic characteristics [8].

The RAPELD system was designed to assess ecological parameters, such as species densities and assemblage composition, across spatially standardized sampling units [8].

Compared to individual or species-based sampling, it offers at least four main advantages: (1) the spatialization of sampling units has been useful both for rapid assessments of biological diversity and long-term monitoring; (2) due to its modular design, data from sites with different sampling intensity can be compared; (3) the sample design allows sampling for taxa of different sizes and mobility in the same sampling units; and (4) because the sampling plots follow the altitudinal contours, more precise measures of habitat factors, such as altitude, vegetation and soil characteristics, can be used as predictor variables in ecological models. The RAPELD system has been used to assess ecological and biogeographic processes that generate patterns of animal (e.g. [11]–[13]) and plant (e.g. [14]–[16]) distributions. However, being a relatively recent approach, its application has to be adjusted, especially in relation to the amount of sampling effort required to answer questions relevant to quantifying disturbance from human activities, and also to improve our knowledge about patterns of species distribution at regional scales.

Sampling effort differs among taxa as a function of detectability, and adjustments are important to generate useful results with the least possible financial investment. In fact, high cost has been identified as a major barrier to the maintenance of biodiversity monitoring programs [17], and exceeding the limits of budgets is typical in multi-taxa studies [18], [19]. Recommendations for adjustments of standardized sampling in order to reduce costs have been provided in the Amazon for ants [20], [21] and mites [22], and some high-performance taxa for biological monitoring have been identified [23].

Although monitoring of snakes is mandatory in most impact assessments associated with major infrastructure projects in the Amazon, there has been no evaluation of the cost-effectiveness of targetting snakes. We used data from RAPELD monitoring of snakes in an area of about 1,500 km^2 covered by primary and secondary tropical rainforest to quantify the

loss of information on differences in species densities and composition under reduced sampling effort.

2. Materials and Methods

2.1 Study Area

We obtained data on snake species-assemblage composition with the support of the Wildlife Conservation Program from Santo Antonio Energia, the concessionaire responsible for building and operating the Santo Antônio Hydroelectric Plant, in the Madeira River in southwestern Brazilian Amazonia (Rondônia State). This dam, which began operations in 2012, flooded about 210 km^2 of mainly primary rainforest, but here we use data collected prior to dam construction.

The study area is covered by "terra-firme" (not seasonally flooded) forest, seasonally flooded forests and "campinaranas" (white-sand forests). In the "terra-firme" areas, the canopy is up to 30 m in height and density of the understory varies according to altitude. In areas covered by "campinarana" the canopy is up to 20 m in height, and the understory is rich in ground bromeliads. The flooded forests are restricted to lowland areas near the banks of the Madeira River. Further details on the physiognomic classification can be found in [24].

The climate is consistently warm with average monthly temperatures between 20^o and 30^o C, and minima and maxima between 18^o and 33^o C in July. The dry season extends from June to September, with rainfall less than 30 mm per month in June and July, and a rainy season from October to May, with up to 330 mm of rain per month in December and January [25]. Tributaries of varying sizes are present in the study area, and the smallest of these often dry completely in the dry season.

2.2 Sampling design

We sampled seven plot systems (Figure 1), each consisting of two parallel 5 km trails, separated by 1 km. Seven 250 m long by 10 m wide plots with centre lines following the altitudinal contours were installed along each trail (14 per system). Plots were established at distances of 0, 500, 1000, 2000, 3000, 4000 and 5000 m from the river bank. Plot systems are called modules hereafter. All modules were installed perpendicular to the river. Four modules were on the left bank of the Madeira River (Madeira - Purus interfluve), two were on the right bank (Madeira - Tapajós interfluve), and one was on the right bank of the Jaci-Paraná River, a tributary of the right bank of the Madeira River.

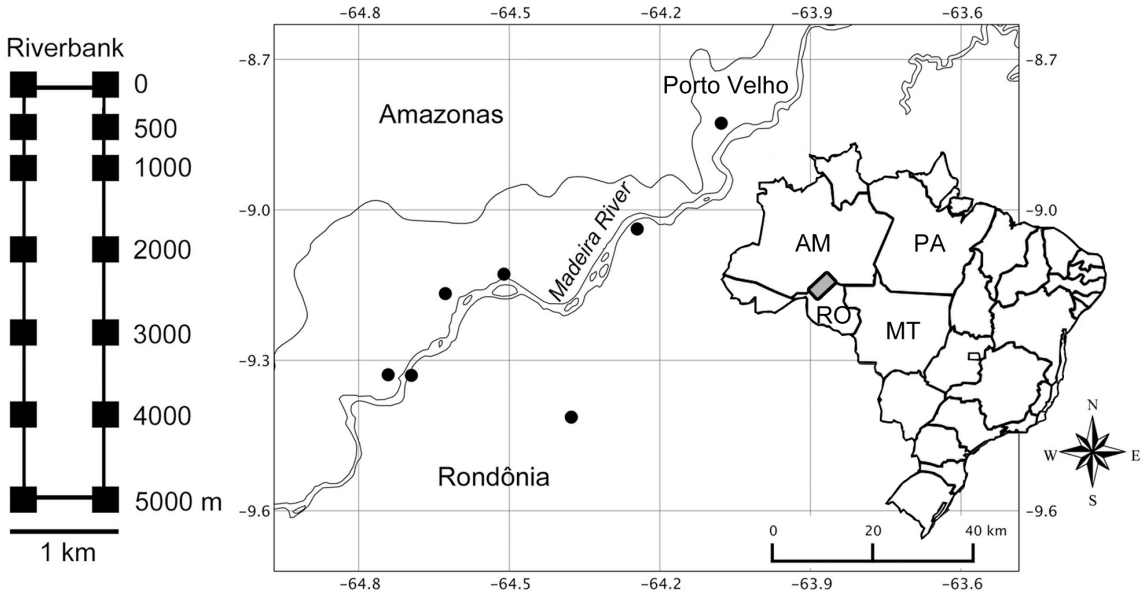


Figure 1. Plot systems in southwestern Amazonia - Sampling systems of 5 km^2 (black circles) located near the banks of the Madeira River in southwestern Brazilian Amazonia (Rondônia state). In detail on the left side, standard configuration of each system, with 14 plots (black squares).

2.3 Snake sampling and sampling effort

We found snakes by visually searching at night, limited by space, with two observers per plot. We undertook four sampling expeditions (March-April 2010, November 2010, January 2011 and May-June 2011). Each expedition lasted about 30 days, and all plots were sampled in each expedition. We standardized the search time to one hour per plot (14 hours per module), but we had an average variation of 15 minutes in total time due to differences in the number of snakes encountered. As we did not search for other snakes while processing captured snakes, the effective search time was about one hour in each plot.

2.4 Data analyses

To test spatial autocorrelation among the modules, we used a Moran's correlogram of geographical distance between pairs of modules and Bray-Curtis dissimilarities in species composition between pairs of modules. We used the correlog function of the Pgirmess package [26] in R V.3.1.0.

We used the number of species detected as an index of the species density in modules. The species-accumulation curves based on rarefaction did not approach asymptotes, so we did not attempt to estimate the total number of species vulnerable to our sampling techniques that use each area. Such methods rarely produce useful information for decisions about megadiverse taxa suring short sampling periods [27]. If the methods are not useful to detect differences in observed species density, they are unlikely to be useful to compare estimates of total number of species, which have far greater standard errors. It was not necessary to use rarefaction to account for differences in search effort between modules because the same temporal and spatial efforts were expended in each module.

We evaluated variation in assemblage composition using a dissimilarity matrix among modules. Dissimilarities were calculated by applying the Bray-Curtis index to the number of

individuals per species, per module. We were interested in obtaining one-dimensional proportions of multivariate Bray-Curtis dissimilarities among modules, in order to use t-tests and general linear models. We reduced the dimensionality of the dissimilarity matrix and reordered the modules using Non-Metric Multidimensional Scaling (NMDS) in one dimension. We have chosen this method rather than alternative ordinations such as PCoA, because NMDS is apparently less sensitive to arch effects generated by heterogeneity in species distributions [28]. Moreover, NMDS has been recognized as the most efficient method to recover original multivariate ecological distances [29]. However, we also ran the analyses using PCoA ordinations and they produced qualitatively similar results not reported here. We used the metaMDS function (arguments k = 1, distance = "bray", trymax=1000) of the Vegan package [30] in R v2.15.2. The NMDS axis captured 60% ($P < 0.0001$) of the variation in species composition among plots (Stress = 0.15). The reduction in dimensionality often causes distortion in relation to the observed dissimilarities [31], although distance can be reduced by rearranging the placement of points along the NMDS axis [32]. We reordered 1,000 times and we used the Shepard diagram drawn by the function stressplot in the Vegan Package in R to show that the observed dissimilarities and the ordination distances were up to 90% correlated ($P < 0.0001$, Figure 2).

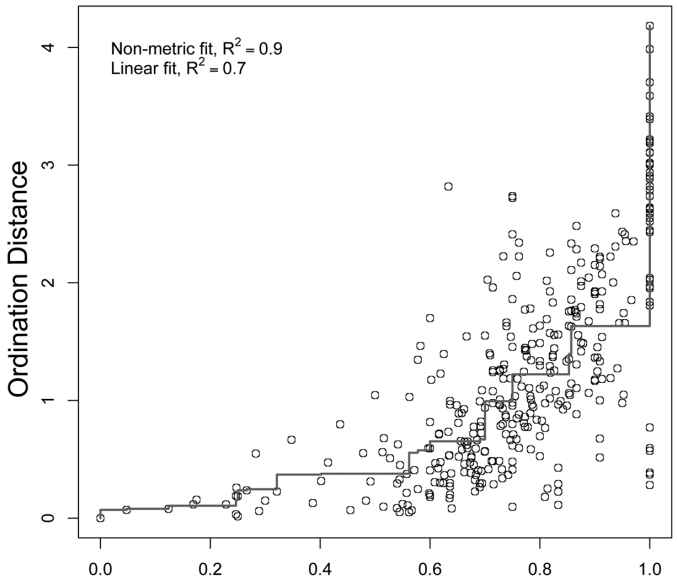


Figure 2. Shepard diagram - Relationship between NMDS ordination distance and original observed distance. NMDS ordination was undertaken on an abundance per species matrix.

We used paired t-tests to investigate the differences among three different sampling intensities on the number of species recorded and on assemblage composition (NMDS scores). In the first test, we used complete modules (5 km^2) to show the changes in number of species and assemblage composition due to increasing the number of observations (campaigns). In the second test, we used the maximum number of observations to pair complete modules with modules constituted by only one 5 km trail (1.25 km^2). In the third test, we used the maximum number of observations to pair complete modules with modules constituted by two 3 km trails (3 km^2 modules). The NMDS ordination produced some negative scores, and they directed the vectors (modules) in opposite directions in paired t-tests, canceling each other. Therefore we added 1 to the NMDS scores to avoid negative numbers. To quantify the similarity of the representation of assemblage composition among modules with different sizes and sampling efforts, we tested for associations among NMDS scores using general linear models.

The major rivers in the Amazon basin are associated with the limits of species distributions of several taxonomic groups (e.g. [33]). Although rivers have not been identified as resulting in vicariance for snakes, we compared number of species per plot and assemblage composition on the opposing river banks of the Madeira River using an analysis of similarities (ANOSIM) with assemblage composition represented by NMDS scores. We used the function anosim of the Vegan package in R.

2.5 Detection probabilities

We quantified detection probabilities for each species using single-season models based on presence-absence data in the Program Presence v.5.2, with 100 bootstrap randomizations [34]. A single-season model provides probabilities of occupancy when detection of the target species is not guaranteed, even in places where they are present. The

estimated occupancy and detection probabilities describe a history of detecting species over a series of surveys in the same locations [34]. Although the Amazon rainforest is apparently homogeneous on satellite images, subtle changes in habitat features across the landscape at a scale of a few kilometers can influence co-occurrence of species in some taxonomic groups, such as frogs [13], understory birds [12] and snakes [11]. Detection probabilities possibly vary slightly among areas within each module as a function of change in habitat features, such as vegetation density along the trails. We expected higher detectability in more open plots and the number of trees was quantified for all plots during the impact assessment for the hydroelectric dam, but differences in the number of trees among modules were negligible (ANOVA $F_{6\text{-}82} = 1.836$, $P = 0.1$). Other environmental factors, such as distance from the streams, can directly affect the composition of snake species in Amazonia [11]. However most of the species recorded here are widely distributed in the study area, and similarity in assemblage composition of up to 60% among modules is expected at scales of tens of kilometers (see [35]). Therefore, we expected the occurrence of all species in all modules, with differences in co-occurrence resulting from the variation in habitat use over a few kilometers. As we were interested in estimating average detectability per species scaled to tens of kilometers, we assumed the same probabilities of occupancy and detection across all modules, and across all campaigns. The average detectability, rather than detectability on any given occasion is what is needed to compare costs of detection of different species in general surveys.

2.6 Cost estimates

To estimate the cost of sampling snakes, we considered fuel for transport among modules, batteries for headlamps, food and field-assistant salaries. We did not include the costs of construction and maintenance of the modules because the same field infrastructure

was used for sampling many other taxa. For fuel, we calculated the cost considering the average consumption per km for a diesel-powered pickup truck for modules accessible by road, and the average consumption per kilometer of a boat powered by a 60 hp gasoline outboard motor for modules accessible by river. To estimate the number of headlight batteries, we considered eight people searching for snakes simultaneously (two per plot), each carrying a headlamp powered by three AA batteries. The batteries were changed every second night. For food, we used $ 8.68 United States Dollars (USD) per day, per person. This is an average value on the local market. For payment of field assistants, we used a daily value of $ 21.71 USD, a work-contract stipulated value.

2.7 Ethics and data availability

Snakes were collected under IBAMA / SISBIO (Ministry of Environment, Government of Brazil) permit n^{o} 02001.000508/2008-99. This permit was subject to approval of all procedures for catching and collecting snakes, and it was allowing us to collect eight specimens per species, per module. However the limit has not been reached for any of the species found.

All data are available for free download on the website of the Programa de Pesquisa em Biodiversidade (PPBio) - http://ppbio.inpa.gov.br/repositorio.

3. Results

3.1 Species densities and assemblage composition

We found 41 species of snakes (Table 1), but the number of species included in each test depended on the module size and number of observations. Neither the variation in number of species detected nor the assemblage composition among modules was spatially

autocorrelated (P > 0.25 in all cases). The number of species detected per module varied from nine to 21, and did not differ when the opposite banks of the Madeira River were compared (ANOVA $F_{1-6} = 0.762$, P = 0.83) and assemblage composition based on NMDS scores was only 4% (P = 0.01) different when comparing either side of the river.

Table 1. Snake species found in seven 5 km^2 sampling systems in the southwestern Brazilian Amazon. N = Number of individuals recorded in the whole study, O.M. = Proportion of modules estimated to be occupied (%), P = species detection probability and confidence intervals (95%) for a single survey of a module.

Taxon	N	O.M.	P
Boidae			
Boa constrictor Linnaeus, 1758	4	43	0.18 (0.03-0.63)
Corallus batesi (Gray, 1860)	2	28.6	0.07 (0.01-0.24)
Corallus hortulanus (Linnaeus, 1758)	10	57.1	0.3 (0.11-0.6)
Eunectes murinus (Linnaeus, 1758)	1	14.3	0.03 (0.005-0.21)
Colubridae (Colubrinae)			
Chironius fuscus (Linnaeus, 1758)	1	14.3	0.03 (0.005-0.21)
Chironius multiventris Schmidt & Walker, 1943	3	42.8	0.1 (0.03-0.28)
Dendrophidion dendrophis (Schlegel, 1837)	1	14.3	0.03 (0.005-0.21)
Drymoluber dichrous (Peters, 1863)	7	42.8	0.22 (0.0009-0.63)
Mastigodryas boddaerti (Sentzen, 1796)	2	14.3	0.07 (0.01-0.24)
Oxybelis aeneus (Wagler, 1824)	3	28.6	0.1 (0.03-0.28)
Pseustes poecilonotus (Günther, 1858)	2	28.6	0.07 (0.01-0.24)
Pseustes sulphureus (Wagler, 1824)	1	14.3	0.03 (0.005-0.21)

Rhinobothryum lentiginosum (Scopoli, 1785)	4	42.8	0.18 (0.03-0.63)
Spilotes pullatus (Linnaeus, 1758)	1	14.3	0.03 (0.005-0.21)
Colubridae (Dipsadinae)			
Apostolepis nigrolineata (Peters, 1896)	1	14.3	0.03 (0.005-0.21)
Dipsas catesbyi (Sentzen, 1796)	11	85.7	0.32 (0.17-0.51)
Dipsas indica Laurenti, 1768	3	42.8	0.1 (0.03-0.28)
Drepanoides anomalus (Jan, 1863)	4	42.8	0.18 (0.03-0.63)
Helicops angulatus (Linnaeus, 1758)	2	28.6	0.07 (0.01-0.24)
Imantodes cenchoa (Linnaeus, 1758)	9	71.4	0.3 (0.11-0.6)
Leptodeira annulata (Linnaeus, 1758)	19	100	0.42 (0.26-0.61)
Liophis reginae (Linnaeus, 1758)	2	28.6	0.07 (0.01-0.24)
Liophis typhlus (Linnaeus, 1758)	2	28.6	0.07 (0.01-0.24)
Oxyrhopus melanogenys (Tschudi, 1845)	5	71.4	0.19 (0.07-0.36)
Oxyrhopus occipitalis (Wied-Neuwied, 1824)	2	14.3	0.07 (0.01-0.24)
Oxyrhopus petolarius (Linnaeus, 1758)	1	14.3	0.03 (0.005-0.21)
Philodryas argentea (Daudin, 1803)	6	42.8	0.19 (0.07-0.36)
Philodryas georgeboulengeri Grazziotin et al., 2012	13	57.1	0.36 (0.14-0.66)
Pseudoboa coronata Schneider, 1801	2	28.6	0.07 (0.01-0.24)
Pseudoboa martinsi Zaher, Oliveira & Franco, 2008	1	14.3	0.03 (0.005-0.21)
Siphlophis compressus (Daudin, 1803)	10	71.4	0.3 (0.11-0.6)
Siphlophis worontzowi (Prado, 1940)	2	28.6	0.07 (0.01-0.24)
Taeniophallus sp.	5	57.1	0.19 (0.07-0.36)
Thamnodynastes pallidus (Linnaeus, 1758)	1	14.3	0.03 (0.005-0.21)
Xenopholis scalaris (Wucherer, 1861)	5	42.8	0.19 (0.07-0.36)

Elapidae			
Micrurus hemprichii (Jan, 1858)	4	42.8	0.18 (0.03-0.63)
Micrurus lemniscatus (Linnaeus, 1758)	5	42.8	0.19 (0.07-0.36)
Micrurus remotus Roze, 1987	4	28.6	0.18 (0.03-0.63)
Micrurus surinamensis (Cuvier, 1817)	1	14.3	0.03 (0.005-0.21)
Viperidae			
Bothrops atrox (Linnaeus, 1758)	18	85.7	0.39 (0.2-0.5)
Bothrops bilineatus smaragdinus Hoge, 1966	1	14.3	0.03 (0.005-0.21)

Using complete modules, species density increased with the number of observations (Figure 3), even increasing between the third and fourth sampling occassions ($t_{1\text{-}6} = 4.459$, $P = 0.004$).

The assemblage composition (Figure 4) differed depending on the number of sampling occasions per module ($t_{1\text{-}6} = 2.497$, $P = 0.04$). However, a general linear model indicated that NMDS scores were correlated between two and three observations ($r^2 = 0.85$, $P = 0.001$), and highly correlated between three and four observations ($r^2 = 0.97$, $P = 0.00001$). The assemblage composition resulting from four surveys per module was based on more species but provided a representation similar to that with only two surveys per module.

Removal of one trail per module (1.25 km^2 modules) significantly reduced ($t_{1\text{-}6} = 7.262$, $P = 0.0003$) the number of species encountered (Figure 5). Assemblage composition (Figure 6) also differed between complete and partial sampling of modules ($t_{1\text{-}6} = 2.404$, $P = 0.05$), but NMDS scores for complete and partial sampling were correlated in a general linear model ($r^2 = 0.62$, $P = 0.02$). Reduction of 2 km in each module (3 km^2) significantly reduced the number of species detected ($t_{1\text{-}6} = 4.289$, $P = 0.005$). However, assemblage composition

did not differ significantly ($t_{1\text{-}6}$ = 1.347, P = 0.21), and NMDS scores were correlated (r^2 = 0.48, P = 0.04). Although the size of the modules influenced the number of species and assemblage composition, conclusions based on similarity in assemblage composition among modules were similar with up to a 50% reduction in the size of the modules.

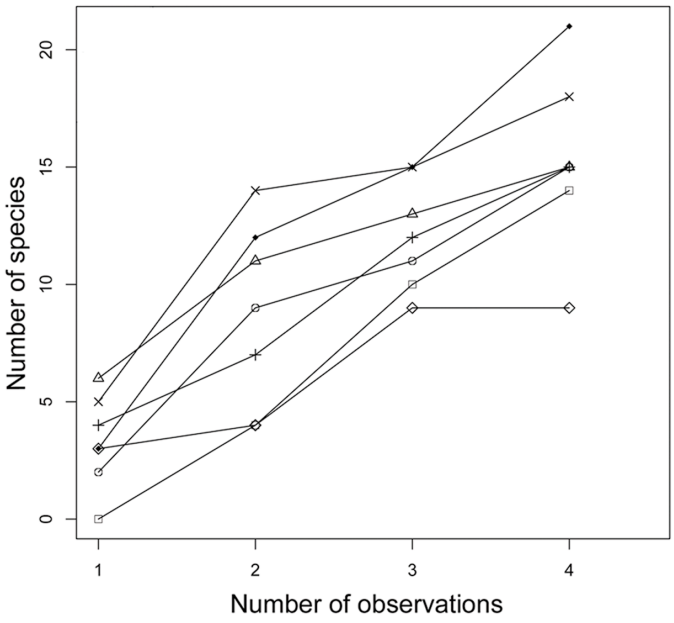


Figure 3. Cumulative number of snake species - Cumulative number of snake species in standardized modules in southwestern Brazilian Amazonia. Modules were sampled 4 times. Different symbols represent different modules.

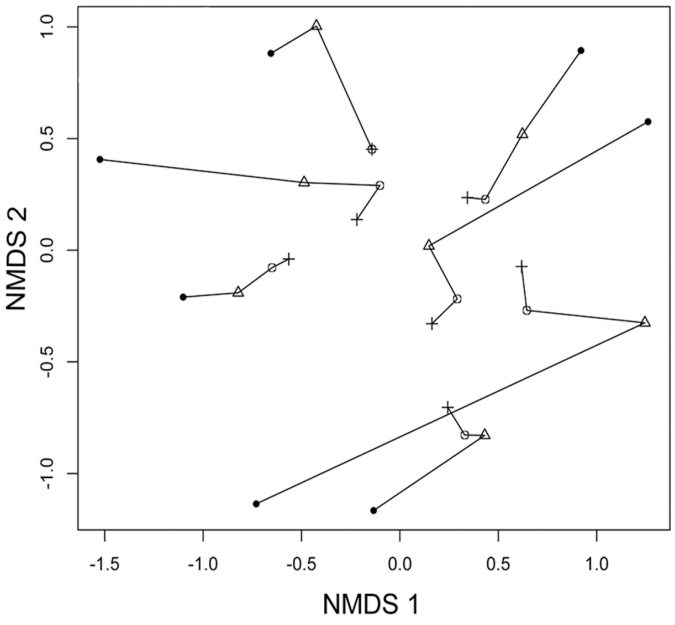


Figure 4. Representation of snake assemblage - Multivariate representation of variation in snake assemblages among modules, based on NMDS scores. Lines connect data for the same module based on different levels of sampling. Black circles = one observation, triangles = two observations, open circles = three observations and crosses = four observations.

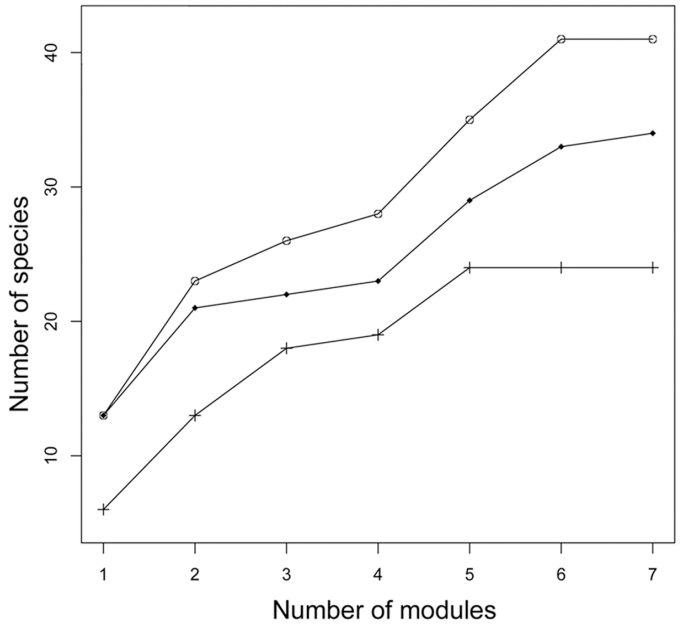
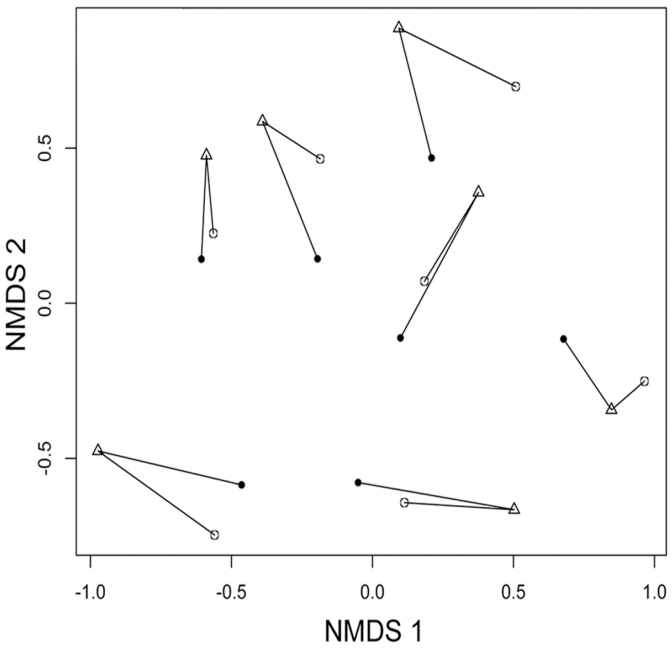


Figure 5. Number of snake species per sample design - Cumulative number of snake species with increasing number of standardized sample modules surveyed in southwestern Brazilian Amazon. Open circles = modules with two 5 km trails (5 km^2), diamonds = modules with one 5 km trail (1.25 km^2) and crosses = modules with two 3 km trails (3 km^2).

Figure 6. Representation of snake assemblage per sample design - Multivariate representation of variation in snake-assemblage composition based on NMDS scores from data obtained in standardized sampling modules in southwestern Brazilian Amazonia. Lines connect the same module sampled at different intensities. Black circles = modules with two 5 km trails (5 km^2), triangles = modules with one 5 km trail (1.25 km^2) and open circles = modules with two 3 km trails (3 km^2).



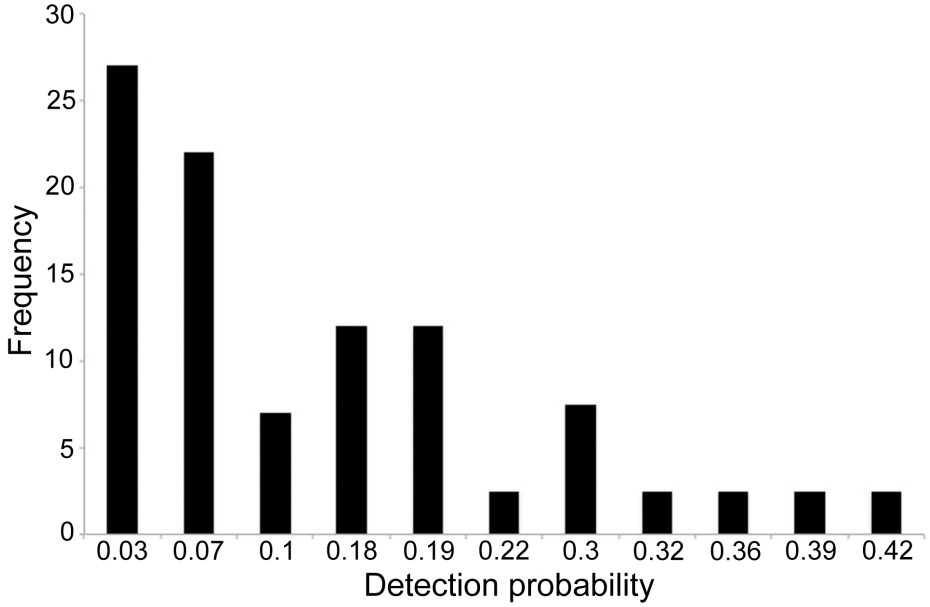
3.2 Detection probabilities

Detection probabilities of species per expedition per module ranged between 0.03 (SE = 0.03) and 0.42 (SE = 0.09), and were below 10% for almost half of the species detected (Figure 7). Confidence intervals for detection probabilities were very wide for most species (Table 1).

Table 2. Costs for sampling snakes in standardized modules in southwestern Brazilian Amazon. The values are in United States Dollars.

	Each survey	**Full study**
Field assistants	2,605.00	10,420.00
Food	2,084.00	8,336.00
Batteries	625.00	2,500.00
Fuel	136.00	544.00
Total per observation	**5,450.00**	
Total for the full study		**21,800.00**

Figure 7. Detection probabilities of Amazonian snakes - Frequency of species of snakes with different probabilities of detection in visual surveys of RAPELD modules.



3.3 Costs

Each full survey of all modules cost $ 5,450 USD, and the full study cost $ 21,799 USD (Table 2). The highest costs were for field assistants, followed by food, headlamp batteries and fuel. We had an encounter rate of 0.89 snakes per hour (total number of individuals / total search time). Considering only the costs of food, field assistants, headlamp batteries and fuel, we calculated the cost to find a snake as $ 120 USD (total cost / total number of snakes found).

4. Discussion

4.1 Sample reduction and decision-making

The number of species per module increased with each additional survey, and there was no tendency for the rate of species accumulation to lessen with the maximum sampling effort. About 95 snake species occur in the region of Porto Velho, state of Rondônia (literature compilation in [36]), more than twice the number of species found in this study. However, this is an estimate based on decades of herpetological collection, an effort generally not viable for biological monitoring applied to assess the impact of human disturbance. Impacts of human activities have been assessed using secondary data, but this method is not appropriate for detecting the influence of habitat factors on the regional distribution of species, because these data usually are based on specimens, and not sites, as sampling units. In addition, secondary data on snakes are generally not comparable due to the lack of sampling standardization and the sampling of snakes in this manner is rare in Brazil (for exceptions, see [11], [37], [38]).

Although there was variation in assemblage composition with increasing number of observations, the general linear models indicated that two surveys were sufficient for

multivariate techniques to capture patterns of species dissimilarities between 5 km^2 modules. Removal of an entire trail from each module (1.25 km^2 modules) caused differences in the assemblage composition between pairs of modules, but not in a general linear model. Similar multivariate patterns were detected even with reduced sampling effort in time and space. However, paired tests are strongly influenced by the spatial configuration of the sampling units and the number of observations and this has traditionally varied among studies. The sampling configuration has often varied in relation to the answers required for the land-use management or improving knowledge about regional patterns of species distribution. A standardized sampling system overcomes some of these issues and allows data to be compared among studies.

We found that assemblage composition varied by up to 40% among modules. Differences in assemblage composition should be expected as a response to environmental gradients that subtly change on a scale of a few kilometers (e.g. [11]). We could have found the same pattern of differences in assemblage composition with a 50% reduction in the costs of food, fuel for transport, batteries for headlamps and field assistants, or a 40% reduction in construction costs of the modules (by reducing of 2 km on each trail). However, these decisions would imply the loss of about 24% of the detected species, and make it difficult to detect local variations in assemblage composition. Deciding on sampling effort should be guided by the target-issues, but such decisions are usually made subjectively by the researchers, rather than in consultation with the regulatory agencies that will make management decisions [39].

4.2 Species detectability

All of the tests were limited by the low detectability of most species and associated wide confidence intervals, despite the relatively high sampling effort in time (980

observers*hours of searching) and space (3,500 ha of plots). We can not discount an effect of variation in the probability of detection of species among the modules because different types of habitat occur in patches throughout each module. However, we do not expect much more than the maximum detectability found in this study (42%), because detection probability for snakes have generally been found to be below 40%, even in areas known to be occupied (e.g. [37], [40]), and species that are not considered rare can be virtually undetectable, showing detection probabilities below 1% [2]. Failure to detect species in occupied habitats can generate erroneous predictions of species responses to natural variation in habitat factors [41], and generating reliable models of habitat use depends on very high sampling effort, and consequently very high financial costs. Thus, conservation programs based on species that are difficult to detect usually prioritize areas where habitat factors favor the detection, and not necessarily the responses of organisms to habitat change [42]. Overcoming these biases for snakes would come at a high monetary cost. In assessments of disturbance, it is generally advantageous to focus on sampling a limited set of high-detectability taxa that reflect the broader patterns of diversity [43], [44].

4.3 Cost-benefit

We calculated the cost to find a snake as $ 120 USD. Mesquita et al. [45] spent $ 0.49 USD per snake found by visual search in a semiarid region of Brazil, more than 230 times cheaper than this study. Differences in costs should be more evident among regions with different climate, terrain, vegetation and logistics, but those authors did not present a detailed description of the spatial distribution of the sites observed, and therefore the independence of sampling units was not clear. Furthermore, they did not estimate costs for food, fuel and field assistants, without which data collection would be impossible in the wide-scale sampling used in this study.

Although snakes are highly diverse in species and lifestyles in the Amazon, ecological models based on snake data are frequently influenced by false absences, resulting from low detectability and lack of specific methods for efficiently catching specimens (see [46]). Complementary methods for detecting snakes, such as passive traps, can increase species lists, especially because they optimize the catching of small litter and fossorial snakes (e.g. [47], [48], but the results usually do not justify the high cost and physical effort (e.g. [45]).

In some cases, a snake species with a limited distribution may be the primary target for impact assessment (e.g. *Bothrops alcatraz* and *Bothrops insularis*, species endemic to islands in southeastern Brazil), and general snake sampling during environmental-impact studies may be useful to generate landscape-scale models of species distribution and to obtain natural-history data, such as those on diet and reproduction. Furthermore, snakes can be found during visual search for other taxa, such as lizards and frogs. However, our simulations show that snakes are generally not good models for impact assessments, because detection of strong ecological patterns requires high financial costs, and cost reduction depends on subjective decisions about the importance of the lost information for management decisions. Also, if visual identification is not sufficient, collecting snakes may involve risks to personnel, and such risks might not be covered under general work insurance.

Limiting studies on human disturbance to a few taxa may be a necessary pragmatic decision in biodiverse regions, because weak cross-taxon congruence in assemblage composition is expected among higher-taxa [49]. Different groups of organisms may respond to habitat changes in different ways, and thus represent only small fractions of the total ecological functionality of an area (see [50], [23]). Therefore we do not expect that there are suitable ecological surrogates for snakes, which makes it a difficult decision to not include them in multi-taxa studies aiming to understand human disturbance on the overall functioning of biodiversity networks. Combining snake sampling with surveys on other taxa, such as frogs

and lizards, could potentially add value to measurements of biological diversity. However, due to the very low probability of detection, the data may not be useful for impact assessments. In any case, no snake species or assemblages are considered endangered in the Brazilian Amazon [51]. Therefore, due to the practical restrictions imposed by limitations of time and money, we recommend that reports on environmental impacts in the Amazon should focus primarily on high-detectability taxa, for which these limitations usually are less of a barrier for decision-making. Strategically appropriate taxa are those that simultaneously reflect useful measures of ecological patterns and are feasibly sampled [23], such as birds [52] and dung beetles [53]. We do not have detailed detectability probabilities for these species. However, standard methods obviously collect more than the mean of 6.5 individuals and 5.75 species of snakes per module per sampling occasion that we found, so it can be expected that they will cost much less than the U.S. $120 per individual that we found for snakes. If the number of taxa is reduced, the resources saved can be used to increase the spatial scale of sampling, which is usually one of the most important restrictions on decision making in the context of environmental impacts [9].

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References

1. Kéry M (2002) Inferring the absence of a species - a case study of snakes. J Wildl Manage 66: 330–338.
2. Steen D (2010) Snakes in the grass: secretive natural histories defy both conventional and progressive statistics. Herpetol Conserv and Biol 5(2): 183–188.
3. Hinds WT (1984) Towards monitoring of long term trends in terrestrial ecosystems. Environ Conserv 11: 11–17.
4. Burbidge AA (1991) Cost constraints on surveys for nature conservation. In: Margules CR, Austin MP, editors. Nature conservation: cost effective biological surveys and data analysis. Melbourne: CSIRO. pp. 3–6.
5. Stork NE, Samways MJ (1995) Inventorying and monitoring: executive summary. In Heywood VH, Watson RT, editors. Global biodiversity assessment. Cambridge: Cambridge University Press. pp. 457–458.
6. Bässler C, Ernst R, Cadotte M, Heibl C, Müller J (2014) Near-to-nature logging influences fungal community assembly processes in a temperate forest. J App Ecol 51: 1–9.
7. Ernst R, Linsenmairs KE, Thomas R, Rödol MO (2007). Amphibian communities in disturbed forests: lessons from the Neo- and Afrotropics. In Tscharntke T, Leuschner C, Zeller M, Guhardja E, Bidin A, editors. The stability of tropical rainforest margins, linking ecological, economic and social constraints of land use and conservation. Berlin: Springer Verlag. pp. 61–87.
8. Magnusson WE, Lima AP, Luizão R, Luizão F, Costa FRC, Castilho CV, Kinupp VF (2005) RAPELD: a modification of the Gentry method of floristic survey for biodiversity surveys in long-term ecological research sites. Biota Neotropica 2: 1–6.

9. Magnusson WE, Braga-Neto R, Pezzini F, Baccaro F, Bergallo H, Penha J, Rodrigues D, Verdade LM, Lima A, Albernaz AL, Marc-Hero J, Lawson B, Castilho C, Drucker D, Franklin E, Mendonça F, Costa F, Galdino G, Castley G, Zuanon J, Vale J, Santos JLC, Luizão R, Cintra R, Barbosa RI, Lisboa A, Koblitz RV, Cunha CN, Ponte ARM (2013) Biodiversity and Integrated Environmental Monitoring. Manaus: Áttema. 356 p.
10. Gentry AH (1982) Patterns of Neotropical plant species. Evol Biol 15: 1–84.
11. Fraga R de, Lima AP, Magnusson WE (2011) Mesoscale spatial ecology in a tropical snake assemblage: the width of riparian corridors in central Amazon. Herpetol J 21: 51–57.
12. Bueno AS, Bruno RS, Pimentel TP, Sanaiotti TM, Magnusson WE (2012) The width of riparian habitats for understory birds in an Amazonian forest. Ecol Appl 22: 722–734.
13. Rojas-Ahumada DP, Landeiro VM, Menin M (2012) Role of environmental and spatial processes in structuring anuran communities across a tropical rainforest. Austral Ecol 37: 865–873.
14. Freitas CG, Costa FRC, Svenning JC, Balslev H (2012) Topographic separation of two sympatric palms in the central Amazon – does dispersal play a role? Acta Oecol 39: 128–135.
15. Pansonato MP, Costa FRC, Castilho CV, Carvalho FA, Zuquim G (2013) Spatial scale or amplitude of predictors as determinants of the relative importance of environmental factors to plant community structure. Biotropica 45(3): 299–307.
16. Schietti J, Emilio T, Rennó CD, Drucker DP, Costa FRC, Nogueira A, Baccaro FB, Figueiredo F, Castilho CV, Kinupp V, Guillaumet JL, Garcia ARM, Lima AP,

Magnusson WE (2013) Vertical distance from drainage drives floristic composition changes in an Amazonian rainforest. Plant Ecol Divers 6: 1–13.

17. Danielsen F, Mendonza MM, Alviola P, Balete DS, Enghoff M, Poulsen MK, Jansen AC (2003) Biodiversity monitoring in developing countries: what are we trying to archieve? Oryx 37: 407–409.
18. Margules CR, Austin MP (1991) Nature conservation: cost effective biological surveys and data analysis. Melbourne: CSIRO. 207 p.
19. Lawton JH, Bignell DE, Bolton B, Bloemers GF, Eggleton P, Hammond PM, Hodda M, Holt RD, Larsen TB, Mawdsley NA, Stork NE, Srivastava DS, Watt AD (1998) Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. Nature 391: 72–76.
20. Souza JLP de, Moura CAR de, Harada AY, Franklin E (2007) Diversidade de espécies dos gêneros *Crematogaster*, *Gnamptogenys* e *Pachycondyla* (Hymenoptera: Formicidae) e complementaridade dos métodos de coleta durante a estação seca numa estação ecológica do estado do Pará, Brasil. Acta Amazonica 37(4): 649–656.
21. Souza JLP de, Moura CAR de, Franklin E (2009) Efficiency in inventories of ants in a forest reserve in Central Amazonia. Pesqu Agropec Bras 44(8): 940–948.
22. Santos EMR, Franklin E, Magnusson WE (2008) Cost-efficiency of subsampling protocols to evaluate Oribatid-Mite communities in an Amazonian Savanna. Biotropica 40(6): 728–735.
23. Gardner TA, Barlow J, Araujo IS, Ávila-Pires TC, Bonaldo AB, Costa JE, Esposito MC, Ferreira LV, Hawes J, Hernandez MIM, Hoogmoed MS, Leite RN, Lo-Man-Hung NF, Malcolm JR, Martins MB, Mestre LAM, Miranda-Santos R, Overal RW, Parry L, Peters SL, Ribeiro-Junior MA, Silva MNF da, Motta C da S, Peres CA

(2008) The cost-effectiveness of biodiversity surveys in tropical forests. Ecol Lett 11: 139–150.

24. Ribeiro JELS, Hopkins MJG, Vincenti A, Sothers CA, Costa MAS, Brito JM, Souza MAD, Martins LHP, Lohman LG, Assunção PACL, Pereira EC, Silva CF, Mesquita MR, Procópio L (1999) Flora da Reserva Ducke: Guia de identificação das plantas vasculares de uma Floresta de Terra Firme na Amazônia Central. Manaus: INPA. 800pp.
25. Horbe AMC, Queiroz MMA, Moura CAV, TORO MAG (2013). Geoquímica das águas do médio e baixo Rio Madeira e seus principais tributários – Amazonas – Brasil. Acta Amazonica 43(4) 489–504.
26. Giradoux P (2013). Miscellaneous functions for analusis and display of ecological and spatial data. Available: http://cran.r-project.org. Accessed June 11 2014.
27. Gotelli NJ, Colwell, RK (2010). Estimating species richness. In Magurran AE, McGill, BJ, editors. Biological Diversity: Frontiers In Measurement and Assessment. Oxford: Oxford University Press. pp 39–54.
28. Ruokolainen L, Salo K (2006). Differences in performance of four ordination methods on a complex vegetation dataset. Ann Bot Fennici 43: 269–275.
29. Austin MP (2013). Inconsistences between theory and methodology: a recurrent problem in ordination studies. J Veg Sci 24: 251 – 268.
30. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2010) Vegan: Community Ecology Package. Available: http://cran.r-project.org. Accessed October 25 2013.
31. James FC, McCulloch CE (1990) Multivariate analysis in ecology and systematics Panacea or Pandora's box? Ann Rev Ecol Syst 21: 129–166.

32. Magnusson WE, Mourão G (2005) Estatística sem matemática – A ligação entre as questões e a análise. Londrina: Planta. pp. 103–122.
33. Colwell RK (2000) A barrier runs through it…or maybe just a river. Proc Nat Acad of Sci 97(25): 13470–13472.
34. 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.
35. da Silva NJ, Sites-Jr JW (1995) Patterns of diversity of Neotropical squamate reptile species with emphasis on the Brazilian Amazon and the conservation potential of indigenous reserves. Cons Biol 9: 873–901.
36. Bernarde PS, Albuquerque S de, Barros TO, Turci LCBT (2012) Serpentes do estado de Rondônia, Brasil. Biota Neotropica 12(3): 1–29.
37. Abrahão C (2007) Influência do tamanho do riacho, chuva, e disponibilidade de alimento na distribuição de *Bothrops atrox* na Reserva Florestal Adolpho Ducke. Masters dissertation. INPA/UFAM, Manaus, Amazonas. Available: http://ppbio.inpa.gov.br/sites/default/files/Dissertacao_Abraao_Carlos.pdf. Accessed 25 October 2013.
38. Fraga R de, Magnusson WE, Abrahão CR, Sanaiotti T, Lima AP (2013) Habitat selection by *Bothrops atrox* (Serpentes: Viperidae) in Central Amazonia, Brazil. Copeia 2013(4): 684–690.
39. Stow AJ, Magnusson WE (2012) Genetically defining populations is of limited use for evaluating and managing human impacts of gene flow. Wildlife Research 39: 290–294.
40. Tibor H, Öllerer K, Farczády L, Moga CI, Băancilă R (2009) Using species detectability to infer distribution, habitat use and absence of a cryptic species: the

smooth snake (*Coronella austriaca*) in Saxon Transylvania. Acta Sci Transylv 17(1): 61–76.

41. Gu W, Swihart RK (2003) Absent or undetected? Effects of non-detection of species occurance on wildlife-habitats models. Biol Conserv 116: 195–203.
42. Pulliam HR (1988) Sources, sinks, and population regulation. Am Nat 132: 652–661.
43. Angermeier PL, Karr JR (1994) Biological integrity versus biological diversity as policy directives – protecting biotic resources. BioSci 44: 690–697.
44. Caro TM, O'Doherty G (1999) On the use of surrogate species in conservation biology. Conserv Biol 13: 805–814.
45. Mesquita PCMD, Passos DC, Cechin SZ (2013) Efficiency of snake sampling methods in the Brazilian semiarid region. An Acad Bras Ciênc 2013: 1–13.
46. Pawar S (2003) Taxonomic chauvinism and the methodologically challenged. BioSci 53: 861–864.
47. Todd BD, Andrews KM (2008) Response of a reptile guild to forest harvesting. Conserv Biol 22: 753–761.
48. Patrick DA, Gibbs JP (2009) Snake occurences in grassland associated with roads versus forest edges. J Herpetol 43: 716–720.
49. Heino J. (2010). Are indicator groups and cross-taxon congruence useful for predicting biodiversity in aquatic ecosystems? Ecol Indicators 10(2010): 112–117.
50. Barlow J, Gardner TA, Araujo IS, Ávila-Pires TCS, Bonaldo AB, Costa JE, Esposito MC, Ferreira LV, Hawes J, Hernandez MIM, Hoogmoed MS, Leite RN, Lo-Man-Hung NF, Malcolm JR, Martins MB, Mestre LAM, Miranda-Santos R, Nunes-Gutjahr AL, Overal WL, Parry L, Peters SL, Ribeiro-Junior MA, Silva MNF, Motta C da S, Peres CA (2007) Quantifying the biodiversity value of tropical primary, secondary and plantation forests. Proc Natl Acad Sci USA 104(47): 18555–18560.

51. IUCN (2013). The IUCN Red List of Threatened Species. Version 2013.2. Available: http://www.iucnredlist.org. Accessed June 11 2014.
52. Bibby CJ (1999) Making the most of birds as environmental indicators. Ostrich 70: 81–88.
53. Spector S (2006) Scarabaeinae dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae): an invertebrate focal taxon for biodiversity research and conservation. Coleoptera Bull 60: 71–83.

Capítulo II

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An assessment of regional snake diversity in the Amazon using a multi-dimensional approach

Rafael de Fraga[a,1], Juliana Schietti[a,c], Thaise Emilio[a,c], Miquéias Ferrão[a], Adam J. Stow[b], William E. Magnusson[c], Albertina P. Lima[c]

[a]Instituto Nacional de Pesquisas da Amazônia – Programa de Pós-graduação em Ecologia, Av. Efigênio Salles, 2239, Manaus – AM, CEP 69060-020

[b]Department of Biological Sciences, Macquarie University, NSW 2109 Australia

[c]Instituto Nacional de Pesquisas da Amazônia – Coordenação de Biodiversidade, POBOX 2223, Manaus – AM

[1]r.defraga@gmail.com, +55 92 3643-1832

Abstract

Mechanisms generating and maintaining biodiversity at regional scales can be evaluated by species turnover along ecological gradients because differences in community assembly result in complementarities and redundancies of organisms between sites. Assemblage turnover can be investigated through multi-dimensional approaches incorporating β-diversity measures, such as taxonomic diversity (TD), functional diversity (FD) and phylogenetic diversity (PD). We used a multi-dimensional approach (TD, FD and PD) to test the effects of ecological gradients on snake diversity at the community level over about 880 km in the central-southern Amazon Basin. We systematically sampled snakes in 116 plots, each 250 m long and 10 m wide distributed across 21 sampling systems (modules), each 5 km^2. We estimated dissimilarities between the plots and modules in TD, FD and PD. Multiple and multivariate multiple linear regressions were used to test for the influence of tree density, elevation and

soil clay content on each diversity measure. Co-occurrence of species as defined by TD did respond to all ecological gradients, but patterns of assembly do not imply in variation in FD and PD different from chance. We show that PD of snakes is a good proxy for FD in the Amazon Basin, but TD resulted in greater complementarity between the plots. Relationships between diversity measures show that evolutionary diversification has generated interspecific phenotypic diversification, and we found no evidence for overdispersion or clustering in FD and PD. Our findings suggest that environmental heterogeneity affects the taxonomic snake assemblages, but functional traits and phylogenies per sampling unit are randomly distributed across the landscape.

Keywords: assemblage; community ecology; environmental filtering; functional diversity; Neotropical snakes; phylogenetic diversity; RAPELD; taxonomic diversity

Introduction

Investigating how environmental gradients influence community structure is crucial to understanding the processes affecting biodiversity at regional scales [1]. Patterns of species co-occurrence associated with ecological gradients may be manifested through complementarities and redundancies among sites. Those differences among sites can be demonstrated on taxonomic, phenotypic and phylogenetic dimensions, and are often described by taxonomic diversity (TD), functional diversity (FD) and phylogenetic diversity (PD). These measures provide different insights into evolutionary and ecological mechanisms underpinning community assembly.

Different measures of diversity may be expected to change over landscapes, because they are often under the influence of biotic and abiotic factors and dispersal ability [2]. These factors include a balance between physiological needs, ecological plasticity and availability of

resources (e.g. [3], [4]), and the degree of gene flow and natural selection (e.g. [5], [6]). Investigating how TD, FD and PD change across the landscape helps disentangle the mechanisms generating and maintaining patterns of biodiversity. For instance, spatial structure in TD, FD and PD may suggest that environmental filtering is limiting the number of species in assemblages, because of different adaptive capacity of each species to environmental conditions [7]. Additionally, the influence of competition on assemblage composition may be evidenced by FD greater than expected based on PD (trait overdispersion), resulting from divergence between species, in contrast to assemblages composed by species that are similar to each other [8]. Alternatively, TD, FD and PD may change randomly across landscapes, and lack of functional or phylogenetic overdispersion suggests that no mechanism is dominant over historical factors that shaped geographic distributions at larger scales [9]. In this case, it is expected that regional diversities are random samples limited only by the species' geographic ranges.

Assemblage turnover in relation to between-site differences in TD, FD and PD are more effective to identifying factors shaping community structure than measures of α-diversity, such as number of species or functional groups at particular sites [10], [11]. This is because mechanisms influencing community assembly act on complementarities and redundancies of organisms between different sites, and not on the number of organism-units within-sites [12], [13]. In general, α-diversity measures fail to capture the contribution of each species to the regional diversity, because different sites may have equal values of diversity (e.g. number of species), even if the species found in each site are taxonomically, functionally or phylogenetically distinct. Identifying mechanisms underpinning assemblage turnover (β-diversity) has clear implications for conservation management. These include identification of unique characteristics in the regional diversity, which makes a site irreplaceable and therefore a priority for conservation actions [14]. This approach has been used to test the efficiency of

protected areas in France [15] and the effects of forest modification on birds and trees in South Africa [16].

Measuring complementarity among sites is valuable for conservation, because it may be used to predict irreplaceable biodiversity loss [14]. For this reason, measuring complementarity is useful in calculating overall diversity of different reserve configurations, and understanding the effects of human disturbance on processes such as extinction and biological invasion [17]. However, there are numerous ways to estimate complementarities in TD, FD and PD, which differ mainly in methodological choices, such as the index to estimate differences between sites and the algorithms used in clustering species [18], [19]. In this study we used PCoA scores to reduce dimensionalities in complementarities between sites. We quantified TD by estimating dissimilarities between sampling units based on presence and absence of species. To quantify FD we estimated dissimilarities between sampling units based on ten traits per species. PD was quantified by estimating dissimilarities between sampling units based on 12S and 16S sequences from GenBank.

Estimates of FD are accessed through trait-based clustering of species, which quantifies the contribution of species to the overall functioning of an assemblage [20]. Function is mediated by phenotypes potentially affecting the species performance and fitness, such as morphological, biochemical, behavioral and phenological traits [21]. Functional diversity may be obtained by summing the branch lengths of a functional tree, in order to provide estimates of complementarity between traits by dispersion of species in phenotypic space [21]. Combinations of environmental gradients occurring at a site may restrict the number of traits that can occupy it, and different combinations may result in different suites of FD in different sites (e.g. [7]).

Phylogenetic diversity is estimated by summing the branch lengths in a phylogenetic tree [22], and therefore incorporates information from the shared evolutionary history of the

species in an assemblage [23]. Genetic distances between species can be used to measure the extent to which diversity changes among assemblages [24]. Different portions of gradients may favor particular allele frequencies, which results in disruptive selection and structured assemblages (e.g. [7]). Assessing the distribution of PD distances over ecological gradients may reveal the mechanisms generating biodiversity. For example, measures of PD may support the Brownian model, which assumes that ecological differences between species are proportional to the time since they diverged from a common ancestor (e.g. [25]), rapid ecological divergence (e.g. [26]), or convergence between sympatric species as a response to ecological opportunities (e.g. [9]).

We tested for the covariation in PCoA scores (Principal Coordinates Analysis) representing dissimilarities between plots (β-diversity) in TD, FD and PD with ecological gradients, because each measure may reflect different processes influencing community assembly in different portions of gradients. This approach potentially gives insights into mechanisms driving patterns of biodiversity, such as environmental factors that influence variation in species diversity across landscapes [27] and historical mechanisms shaping species' geographic ranges (e.g. [28]). In addition, a multidimensional approach can reflect different aspects of species' ecology [9]. Despite their potential for elucidating patterns in community ecology, multi-dimensional approaches have been poorly explored (but see [7], [15], [29], [31], [30], [31]), and have never before been applied to large scales in the Neotropical region. In fact, to our knowledge the standardized sampling effort used in this study is unprecedented for large-scale studies of this type.

In this paper, we examine the influence of three ecological gradients (tree density, elevation and soil clay content) on three measures of snake β-diversity (TD, FD and PD) depicted by PCoA scores. Snakes are suitable organisms to test for changes in functional and phylogenetic assemblages over landscapes in the Amazon, because they have great variation

in functional traits (e.g. [32]) and clades have distinct evolutionary histories (see [33]). In addition, snakes have been included in estimates of global reptile decline [34], which highlights the importance of assessing mechanisms generating diversity. Although there is much evidence of ecological gradients influencing patterns of community assembly [35], defining which gradients are playing the most important roles is still a challenge for community ecologists.

Most species of snakes recorded in this study are widely distributed throughout the Amazon basin and some occur in other ecosystems in South America. However, although much of the lowlands in the Amazon appear relatively homogeneous in satellite images, ecological studies at mesoscales have shown that subtle changes along ecological gradients influence patterns of co-occurrence of frogs [36], understory birds [37], plants [38] and snakes [39]. Therefore, species do not occupy all sites within their ranges, and different assemblages could be expected even at scales of tens of kilometers (e.g. [39]). In this study, we sampled a continuous gradient of about 880 km of rainforest, from central- to southwest Amazonia. We primarily described patterns of snake β-diversity by measuring TD, FD and PD across the landscape in the Amazon Basin, and secondarily, we aimed to assess the role of the variation in taxonomic, functional and phylogenetic composition on shaping snake diversity. We evaluated the effects of specific environmental variables on the distribution of TD, FD and PD across sampling units to test whether patterns of co-occurring species result from the current environmental heterogeneity. Because we used multiple or multivariate multiple linear regressions, we assume the general null hypothesis for linear models, which predicts that the response variable (measure of diversity) and the predictor variables (tree density, elevation and clay content) are not related. Additionally, we tested for overdispersion in FD and PD to investigate if the observed assemblages are essentially random samples limited only by the species' geographical ranges, and thus shaped by historical factors [40].

Methods

Snake sampling

We sampled snakes in 21 RAPELD plot modules [41], [42], each of which has trails covering 5 km^2 (5 km long by 1 km wide). In each module, we sampled ten 250 m long by 10 m wide plots with center lines following the altitudinal contours. The plots were distributed along two parallel 5 km long trails (five plots per trail) with standardized distance of 1 km between neighboring plots.

The modules were distributed almost linearly over 880 km (Figure 1) from central (Manaus, Amazonas) to southwestern Amazonia (Porto Velho, Rondônia). The study area comprehends three geologically distinct regions, which are Central Amazonia, to the north of the Amazon River, the interfluve between the middle regions of the Madeira and Purus rivers, and the upper Madeira River, in southwestern Amazonia. Three modules were installed at the Ducke Reserve, which is a 100 km^2 fragment of non-flooded primary rainforest, located on the northern outskirts of Manaus. It is one of the most intensively studied rainforest sites in the world [43]. Eleven modules were installed along the federal highway BR-319 that connects Manaus to western Brazil. The highway was largely abandoned in the 1980s. Sampling plots were installed mainly to enable multi-taxa impact assessments of the effect of the road. Along this road, modules were placed in areas covered by primary and old-secondary rainforest, with patches of flooded forest and savannah, but we did not sample savannah areas in this study. The southern Madeira River region contains seven modules. The Madeira River was recently dammed by a large hydroelectric power plant in the Porto Velho region, and the modules were installed along the banks of the river for monitoring the effects of flooding on biodiversity. The data used in this study were collected prior to flooding. The region is covered by primary and secondary rainforest under increasing threat of human occupation.

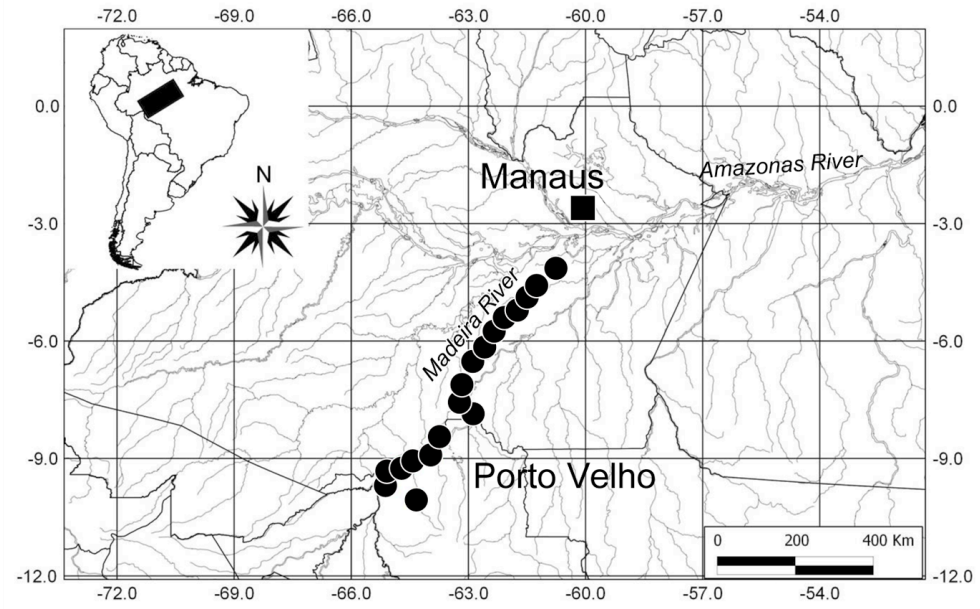

Figure 1. Snake-sampling area in the Brazilian Amazon in 5 km^2 RAPELD modules (black circles and square – the square means three modules). Each module contains ten plots (250 m long and 10 m wide) following the altitudinal contours.

We sampled snakes by nocturnal active visual search, with two observers per plot, and standard searching time of 1 hour per plot per visit. To increase the sampling effort, we undertook four non-consecutive surveys of each plot between 2007 and 2014. The sampling expeditions were distributed throughout the year so that samples are independent of seasonal variables, such as rainfall.

Detection probabilities of snakes are usually low [44], [45], and they may bias the results by generating statistical artifacts. In this study, the large number of zeros in the input matrices generated a circular cloud in some analysis. To avoid statistical artifacts, we discarded data from 94 plots in which we did not record snakes, and our analysis is based on the remaining 116 plots. Alternatively, we used modules as sampling units rather than plots. This results in loss of degrees of freedom, but it increases the predictive power of the analysis because pairs of sampling units usually share more than one species.

We excluded 23 species of snakes from the analysis, due to lack of consistent information on phenotypic traits or sequences available in GenBank. We conducted all of our

analysis on 35 species (Table 1) belonging to four families (Aniliidae, Boidae, Colubridae [including Dipsadinae] and Viperidae).

Table 1. Snake species found in 116 plots distributed in three sampling sites in the Brazilian Amazon, and number of plots per site where each species was found. N = number of sampled plots.

Taxon	Ducke Reserve N = 26	BR-319 N = 41	Madeira River N = 48
Aniliidae			
Anilius scytale (Linnaeus, 1758)	1	0	0
Boidae			
Boa constrictor Linnaeus, 1758	1	1	0
Corallus hortulanus (Linnaeus, 1758)	0	10	8
Epicrates cenchria (Linnaeus, 1758)	0	2	2
Colubridae (Colubrinae)			
Chironius fuscus (Linnaeus, 1758)	3	2	0
Chironius multiventris Schmidt and Walker, 1943	3	2	2
Chironius scurrulus (Wagler, 1824)	1	0	0
Drymoluber dichrous (Peters, 1863)	3	0	3
Leptophis ahaetulla (Linnaeus, 1758)	0	0	2
Oxybelis aeneus (Wagler, 1824)	0	0	3
Rhinobothryum lentiginosum (Scopoli, 1785)	0	0	3
Spilotes pullatus (Linnaeus, 1758)	0	2	0
Colubridae (Dipsadinae)			

Atractus schach (Boie, 1827)	0	1	0
Clelia clelia (Daudin, 1803)	2	5	0
Dipsas indica Laurenti, 1768	0	1	2
Drepanoides anomalus (Jan, 1863)	1	4	4
Helicops angulatus (Linnaeus, 1758)	0	4	0
Imantodes cenchoa (Linnaeus, 1758)	8	13	6
Imantodes lentiferus (Cope, 1894)	0	1	0
Leptodeira annulata (Linnaeus, 1758)	1	6	8
Liophis reginae (Linnaeus, 1758)	0	0	1
Liophis typhlus (Linnaeus, 1758)	1	1	0
Oxyrhopus melanogenys (Tschudi, 1845)	0	8	3
Oxyrhopus occipitalis (Wied-Neuwied, 1824)	1	0	2
Philodryas argentea (Daudin, 1803)	3	0	3
Pseudoboa coronata Schneider, 1801	0	0	2
Siphlophis cervinus (Laurenti, 1768)	0	0	2
Siphlophis compressus (Daudin, 1803)	5	2	6
Taeniophallus brevirostris (Peters, 1863)	3	0	0
Xenodon severus (Linnaeus, 1758)	0	0	1
Xenopholis scalaris (Wucherer, 1861)	0	3	1
Viperidae			
Bothrops atrox (Linnaeus, 1758)	12	12	18
Bothrops brazili Hoge, 1954	0	0	1
Bothrops taeniatus (Wagler, 1824)	0	2	0
Lachesis muta (Linnaeus, 1766)	1	1	2

Ecological gradients

We evaluated the influence of tree density because this variable directly affects the availability of hunting and resting sites for arboreal species, and the amount of sunlight reaching the understory, which may affect thermoregulation in terrestrial and aquatic species. Even nocturnal snakes have been found basking in clearings during the day in the Amazon rainforests [46]. Variation in elevation is subtle in the Amazon lowlands, and it may not directly affect snake assemblages [42]. However, we used elevation because this variable affects other variables, such as humidity, that in turn influence plant and animal assemblages [47]. We selected soil clay content as a variable because soil texture directly affects primary production, which influences the overall trophic network [48].

Ecological gradients of tree density, elevation and clay content were measured per plot in each sampling site as part of the RAPELD protocols. For the analysis using modules as sampling units, we used average values per module. All data, details of collection methods and responsible persons can be found at http://ppbio.inpa.gov.br.

Taxonomic diversity

We represented TD as PCoA scores based on the Forbes' similarity index [49] applied on presence / absence data per species from plots or modules (1 - Forbes to estimate dissimilarities between plots or modules). The Forbes' index has been indicated as robust in the case of incomplete sampling [50], which is common in studies of snakes. In order to reduce the arch effects [51] we used extended dissimilarities [52].

Functional diversity

We constructed a trait matrix using 10 continuous or discrete traits, measured or observed for adult individuals only. These were maximum total length, tail length

proportional to body length, diameter of the eye proportional to head length, life habit (e.g. arboreal, fossorial, aquatic), period of activity (diurnal, nocturnal), foraging strategy (ambushing, active), diet (e.g. frogs, small mammals), defensive behavior (e.g. cloacal discharge, strike), reproductive mode (oviparous, viviparous) and maximum size of offspring. The decision of how many and which traits are used to describe ecological functionality is often arbitrary, based on the available information on the natural history of target-taxa (see [53], [23]). However, all of the traits used in this study have been considered ecologically relevant for snakes (literature compilation in [54]).

The continuous traits were measured for all adult individuals found, and we used averages per species (see [53]). For those species for which we found less than five individuals, we complemented our data with data from literature [32], [55], [56], [57], [58], [59], [60], [61], [62], [63]. We also obtained most of the data for discrete traits per species in the literature, and they were complemented with field observations. The levels of most discrete traits are not mutually exclusive (e.g. species which feed on a wide variety of prey), so we coded discrete traits into independent binary traits as suggested by [19].

We obtained Gower distances between species within each plot in terms of their traits using the *vegdist* function of the Vegan package [65] in R (R Core Team, 2014). We performed hierarchical cluster analysis on the Gower dissimilarity matrices to build an UPGMA functional tree, using the *hclust* function (argument "average") in R. Gower distances are thought to be more appropriate when analyzing mixed continuous and discrete traits, although the results are often strongly correlated with Euclidean distances [19]. Measures of distance and the clustering algorithm should be chosen to maximize the correlation between the dissimilarity matrix in the trait space and the cophenetic distances in the functional tree [18], [19]. We calculated cophenetic distances using the *cophenetic* function in R, and we found a cophenetic correlation of 82 %.

To estimate FD, we used the function *phylorare* in R. We rarefied FD at species level (argument "subsampling = species"), to reduce the effects of species richness on FD estimated per plot. *Phylorare* calculates diversity under rarefaction based on summing the branch lengths of a rooted tree. Details on analytical formula are given in [65]. This function was originally developed to calculate mean rooted phylogenetic diversity, but it works equally well on functional trees, since the two measurements are based on the sum of the branch lengths. *Phylorare* requires the ape package in R [66], and it is available for free download from the website http://davidnipperess.blogspot.com.au/.

Phylogenetic diversity

In the software phyloGenerator [67], we downloaded mitochondrial 12S and 16S sequences from GenBank [68] and we aligned them (300 BP on average), using MAFFT [69], [70]. Problematic regions were removed using trimAl (Capella-Gutiérrez et al 2009). We used these methods because they resulted in fewer gaps for the two markers (see [67]). We constructed a phylogeny using RAxML [71] with 100 searches, and we dated it using PATHd8 to root the phylogenetic tree. We opted for a rooted tree to ensure that plots in which we recorded only one species would have nonzero PD. Adding PD values to single-species plots has been suggested as an appropriate decision for conservation purposes [65]. We constrained the final concatenated tree using an early phylogenetic tree based on 12S to avoid conflicts with well-established clades [68].

The phylogeny reconstructed in this study showed support greater than 80 % for most nodes (1000 bootstraps), and did not conflict with the most complete and well-supported phylogenetic hypotheses for Squamata reptiles [e.g. 33]. The phylogenetic tree was imported to R using the *read.tree* function of the ape package, and we estimated PD per plot using the *phylorare* function (argument "subsampling = species").

Inferential analyses

Except for TD, the methods used in this paper produce measures of α-diversity, which have been appointed as unsuitable for detecting complementarity among sampling plots [42]. Values of α-diversity may be equal between two plots, even if the subsets of species recorded in each plot are completely different each other. Moreover, measures of FD and PD based on pairwise distances between species have been identified as most suitable to remove the assumption of an evolutionary model that is necessarily Brownian [9]. To transform the estimates of FD and PD into β-diversity (complementarity among plots), we calculated the Euclidean distances between the plots or modules using the *vegdist* function of the Vegan package in R, and we summarized the β-diversity measures as PCoA scores. To assess patterns of assembly associated with the ecological gradients, we used multiple or multivariate multiple linear regressions, depending on the variability in the diversity measures across the sampling units captured by the first PCoA axis. If the first PCoA axis captured more than 50 % of the variability, we used multiple linear regression, assuming the formula *PCoA = a + b(tree density) + b(elevation) + b(clay content)*. If it were necessary to use two PCoA axes to capture more than 50 % of the variability, we used multivariate multiple linear regression assuming the formula *PCoA-1, PCoA-2 = a + b(tree density) + b(elevation) + b(clay content)*. For multiple regression models with a single dependent variable, we plotted the partials regressions.

To assess the relationship between the diversity measures, we converted the Forbes distances used to estimate TD into Euclidean distances to calibrate all diversity measures in the Euclidean space. We expressed each diversity measure using PCoA scores and tested for the correlations between them using simple linear regressions. We used the *cophenetic* function in R to calculate cophenetic distances between the plots separately for FD and PD.

To access the correlation between the functional and phylogenetic trees, we applied a mantel test to the cophenetic-distance matrices of FD and PD.

Assessing FD and PD overdispersion

We assessed overdispersion in FD and PD in each plot by comparing mean pairwise distances (MPD) among species and nearest-neighbor mean distances (MNTD) between each species and its closest relative [72]. We obtained values of MPD and MNTD per plot by using respectively the *ses.mpd* and *ses.mntd* functions of the "picante" package in R [74]. We assumed a null model based on an independent swap algorithm (arguments null.model = independentswap, runs = 999, iterations = 1000), maintaining species-occurrence frequency and sample species richness [75]. Estimates of MPD were used in obtaining the Net Relatedness Index (NRI), considering the entire functional and phylogenetic trees, and estimates of MNTD were used in obtaining the Nearest Taxon Index (NTI), considering only relatedness to the closest taxon. We used the formulas given in [76], which assume that positive values of NRI and NTI indicate assemblages formed by functionally or phylogenetically clustered species, and negative values indicate overdispersion [77]. We tested for randomness in the NRI and NTI values using the Wald-Wolfowitz Runs Test [78], available in the "randtests" package [79] in R (*runs.test* function). The null hypothesis predicts that the NRI and NTI values are generated by random association of species in relation to their phylogenetic proximity.

Ethics and data availability

Snakes were collected under IBAMA / SISBIO (Ministry of Environment, Government of Brazil) permits n^{o} 02001.000508/2008-99 and 1377702001.000508/2008-99. These permits were subject to approval of all procedures for catching and collecting snakes.

All data are available for free download on the website of the Programa de Pesquisa em Biodiversidade (PPBio) - http://ppbio.inpa.gov.br/repositorio.

Results

The first PCoA axis in general captured at least 80 % of the variability in the diversity measures across the plots and modules. However, two PCoA axes were required to represent TD, by capturing 57 % of the variability in the dissimilarities among plots. The ecological gradients explained about 12 % of the variation in TD across the plots (PCoA-1, PCoA-2 = 2.1 + 2.89 tree density + (-2.99) elevation + 2.65 clay content; $r = 0.12$, $P < 0.002$). Most of the variation in TD was related to the PCoA axis 2, so we used it to represent graphically the partials from a multiple regression between TD (PCoA 2) and each ecological gradient (Figure 2).

The effects of ecological gradients on TD per plot were consistent with multiple linear regression at the level of modules. However, using modules as sampling units (Figure 3) resulted in a more robust predictive model (PCoA = -0.54 + (-2.02) tree density + 2.09 elevation + (-2.91) clay content; adjusted $r = 0.41$, $P = 0.02$). We found no effect of ecological gradients on FD or PD, for both plots and modules as sampling units ($P > 0.12$ in all cases). The coefficients from all linear models are summarized in the Table 2.

Table 2. Coefficients from multiple or multivariate linear regression models between distance matrices based on taxonomic diversity (TD), functional diversity (FD) and phylogenetic diversity (PD). The results are shown for plots (250 m long, 10 m wide) and modules (5 km^2) as sampling units. PT = Pillai Trace values from multivariate multiple linear regression. r = regression coefficients from multiple linear models.

	Plots as sampling units					
	TD		FD		PD	
	Regression	P	Regression	P	Regression	P
Tree density	PT 0.07	0.01	r 0.07	0.93	r 0.07	0.54
Elevation	PT 0.07	0.01	r 0.009	0.34	r 0.02	0.21
Clay content	PT 0.06	0.03	r 0.009	0.38	r 0.02	0.25
	Modules as sampling units					
Tree density	r 0.41	0.05	r 0.2	0.28	r 0.18	0.42
Elevation	r 0.41	0.05	r 0.2	0.71	r 0.18	0.72
Clay content	r 0.41	0.009	r 0.2	0.12	r 0.18	0.13

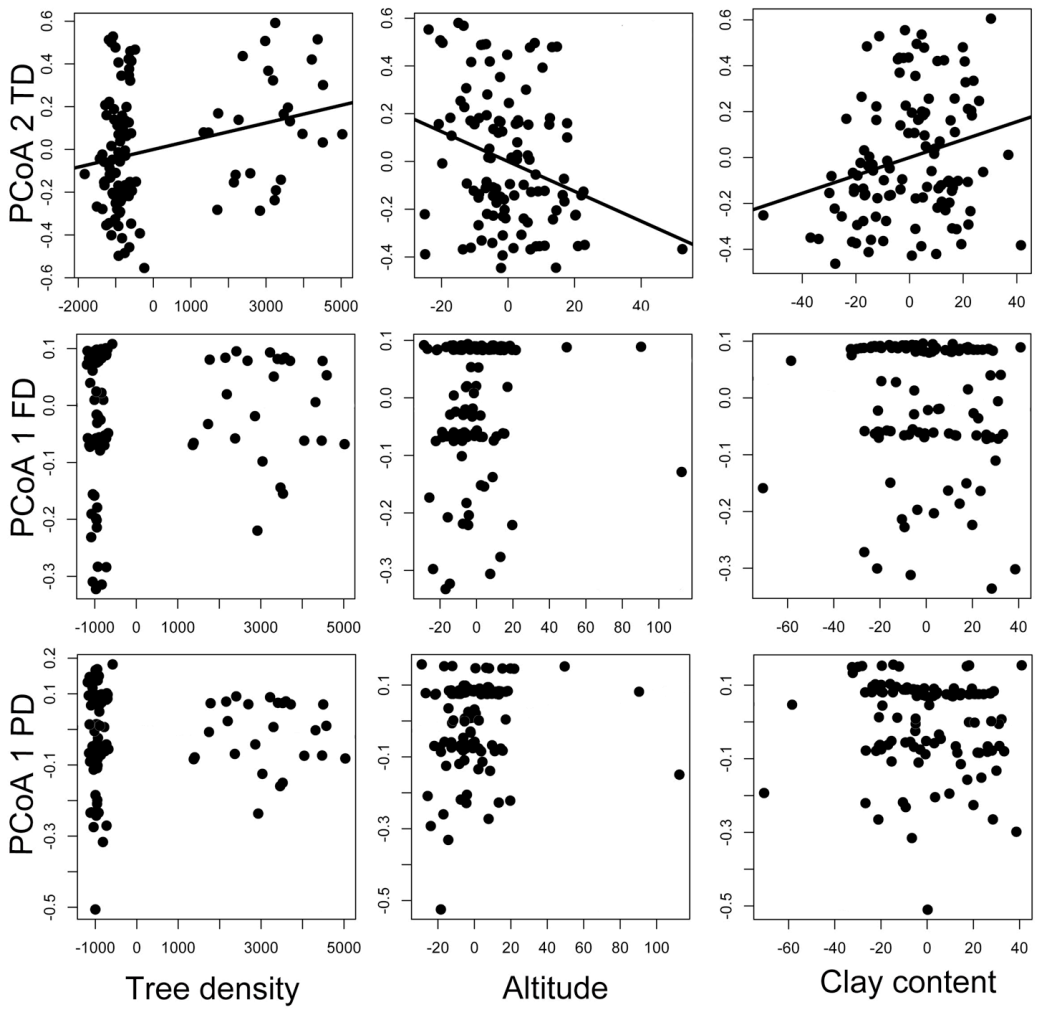

Figure 2. Partials from multiple linear regression models. PCoA scores represent taxonomic (TD), functional (FD) or phylogenetic (PD) diversities were response variables and ecological gradients were predictor variables. All the diversity measures were estimated based on presence / absence data of snakes from 115 plots (each 250 m long, 10 m wide) in a transect along the Amazon basin.

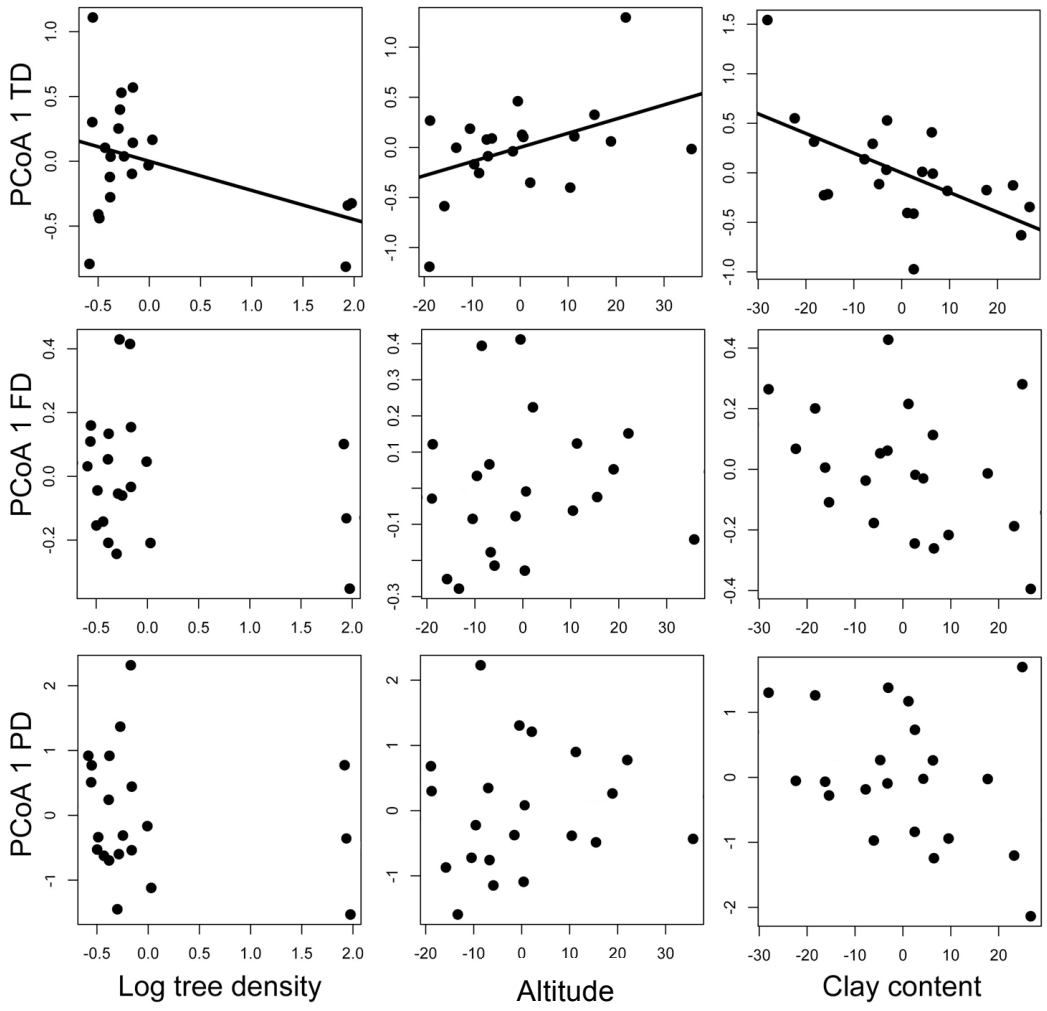

Figure 3. Partials from multiple linear regression models. PCoA scores represent taxonomic (TD), functional (FD) or phylogenetic (PD) diversities were response variables and ecological gradients were predictor variables. All the diversity measures were estimated based on presence / absence data of snakes from 21 sampling modules (each 5 km^2) along the Amazon basin.

The PCoA scores based on pairwise Euclidean distances for each diversity measure (Figure 4) were little correlated between TD and PD ($R^2 = 0.01$, $P = 0.08$). However, plots in which we recorded phylogenetically closely related species were more similar in functional traits ($R^2 = 0.93$, $P < 0.001$), and the functional and phylogenetic trees were correlated ($R^2 = 0.26$, $P < 0.001$).

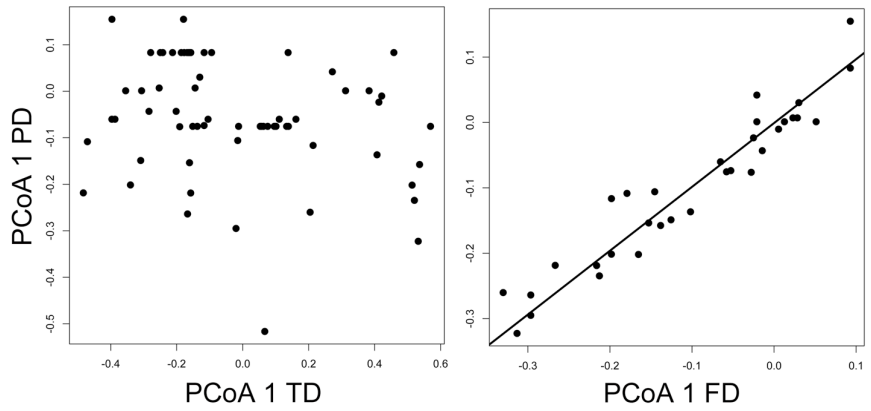

Figure 4. Relationship between PCoA scores representing taxonomic diversity (TD), functional diversity (FD) and phylogenetic diversity (PD) between snake-sampling plots in the Brazilian Amazon.

We were not able to distinguish NRI and NTI values from those generated for random association ($P > 0.26$ in all cases). Therefore, we conclude that the regional snake assemblages in the study area did not show clustering or overdispersion in FD or PD (Figure 5).

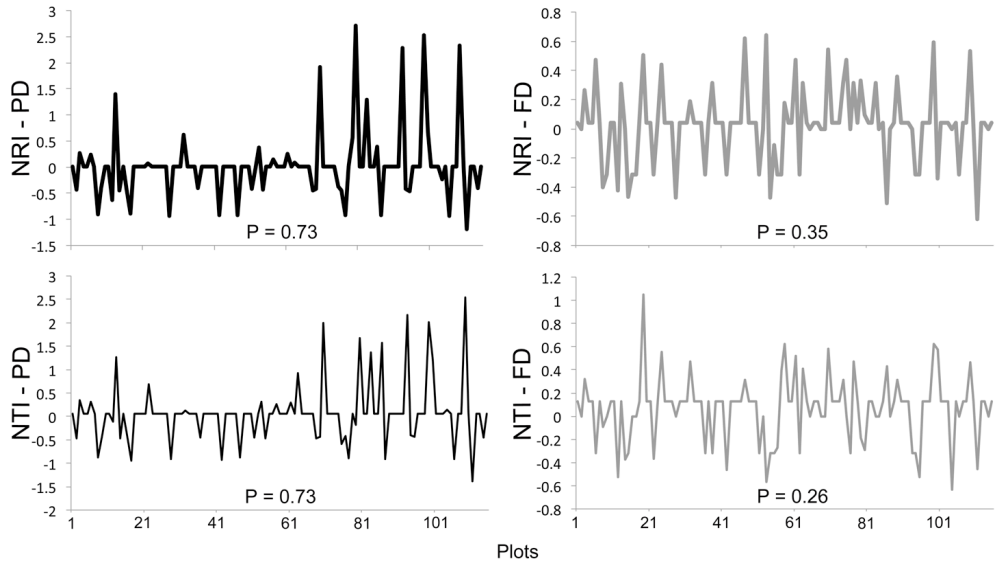

Figure 5. Distribution of Net Relatedness Index (NRI, thick lines) and Nearest Taxon Index (NTI, thin lines) among plots. Values were estimated for phylogenetic diversity (PD, black lines) and functional diversity (FD, gray lines). The P values refer to the Wald-Wolfowitz Runs Test for randomness.

Discussion

Our data indicate that ecological gradients filter snake assemblages based on taxonomic diversity in central-southern Amazonia, but functional and phylogenetic diversities are essentially randomly distributed across the landscape. These findings contrast with snake assemblages in the United States, which are structured by phylogenetic and trait variability [54]. Furthermore, our data contrast with a recent study in Brazil, which found variation in phylogenetic and phenotypic composition of snake assemblages associated with environmental gradients [31]. However, the variation found in that study was due primarily to differences between forested and open habitats, which was expected. In this study, we tested the effects of subtle variations in environmental gradients on snake diversity within a biome, which is more challenging, but the results could give non-obvious insights into ecology and conservation. The limited effects of environmental barriers on the variation in functional traits and phylogenies are indicated by the strong positive correlation between FD and PD, which shows that closely related species contribute similarly to regional habitat functionality. However, despite the fact that snakes often move between different habitats in the landscape, the assemblages in central-southern Amazonia are taxonomically distinct at scales of plot (250 long and 10 m wide) and module (5 km^2).

Ecological gradients filtering taxonomic species composition have been found in many groups of organisms in the Amazon, such as frogs [36], understory birds [37], plants [38] and snakes [39]. In general, it is expected that species occupy portions of gradients in a way to optimize the balance between physiological needs and availability of resources [80]. Such responses are expected when comparing very different habitats with each other (e.g. [31]), but comparing plots on a finer-scale requires a sampling design which includes gradients with large amplitudes, such as those tested in this study (about seven degrees of latitude of variation among plots). A previous study [39] did not find an influence of soil clay content on

snake taxonomic composition in a 25 km^2 study site in central Amazonian rainforest, probably because the study area was not large enough to detect species turnover associated with the variation in soil structure, but there was a statistically significant association in the present large-scale study.

Plots with higher FD had higher PD, and this shows that closely related species are ecologically similar. Slow evolutionary diversification has generated interspecific phenotypic diversification, which suggests that snake assemblages based on FD and PD are not ultimately driven by any dominant mechanism, such as biotic and abiotic interactions [9]. Biotic interactions structuring functional traits are generally evidenced by assemblages more functionally distinct than expected by chance [7], which results from mechanisms limiting distributions of certain species through biotic interactions, such as interspecific competition [81]. In turn, abiotic interactions structuring functional traits may be evidenced by assemblages more functionally similar than expected by chance, because different regions of ecological gradients can select for the persistence of different traits [26]. We found conservative functional traits, but no evidence that assemblages show trait or phylogenetic overdispersion caused by competition or environmental filtering (see [82]). We assume that snakes often cross boundaries between areas with different environmental conditions in the Amazon, which causes unstable patterns of regional community assembly based on FD and PD (see [9]).

Our findings are unlikely to be biased by the spatial distribution of sampling units (see [83]), because the RAPELD system provides regular distribution of plots across the landscape regardless of logistical issues [42]. However, snakes usually have low detection probabilities [44], [45], which have been estimated at less than 10 % for surveys of many Amazonian species in RAPELD plots [45]. Low detection probabilities often cause false absences of species from plots, and this may generate misinterpretation of how species respond to

landscape change [84]. We are unable to discount effects of low detectability on our results. However, noise generated by the possible false absences would result in type II errors and it is unlikely to have been responsible for the significant relationships we detected.

The strong positive correlation between FD and PD indicates that PD is a good proxy for FD for snake assemblages in the Amazon. The phenotypic traits used in estimating FD captured the phylogenetic signal, and therefore they carry relevant information for regional FD estimates. This indicates that it would be possible to effectively describe assemblages using only one of the two measures, because they carry redundant information. However, identifying the best measure may be subjective, because the two methods depend on many steps and choices. Estimates of FD are highly sensitive to the choice and number of traits [53], and a single set of traits may not be able to capture all ecological attributes underpinning differentiation among species or sites in the multivariate space [85]. Therefore, potentially important traits may be overlooked, or irrelevant traits may be included in the trait matrix, which reduces ability to explain patterns of community assembly [9]. Estimates of PD may contain ecological information which can not be accessed through directly measurable traits [82], [86], which makes them attractive to assess complementarity between assemblages. However, phylogenetic hypotheses may differ according to selection of genes, clustering algorithms and calibration of branch lengths [9]. Moreover, the best way currently available to increase the efficiency of phylogenetic hypotheses depends on prior knowledge of the phylogenetic relationships between the target-taxa that can be used to constrain the phylogeny ([67], but see [16]). An alternative method may be to reduce the limitations of each measure by simultaneously estimating phylogenetic and functional diversity using composite indices (e.g. [9]). However, because different sampling methods and diversity estimates can generate different relationships between FD and PD, composite indices changing along ecological gradients may not be easily interpreted for practical applications, such as for conservation.

Using PD as a proxy for FD directs the focus of biodiversity monitoring programs and reserve planning to long-term interpretations, because this measure reflects the maintenance of ecosystem processes operating over long timescales [87]. Functional diversity is estimated based on sets of traits that reflect environmental tolerances and requirements [87], which in turn determine where species can live [88] and interact with each other in assemblages [88]. Therefore, loss of evolutionarily distinct species may result in irreversible loss of functions for ecosystems [90]. At larger scales it may be difficult to decide which diversity measure should be prioritized in conservation, and a multi-dimensional approach may be more appropriate [29].

In general, greater distances between plots in TD were not associated with greater distances in FD and PD. TD is an index based on species per plot, but taxonomic units at higher levels, such as genus and tribe are more clearly associated with community assembly measured by functional and phylogenetic diversity. In fact, snakes in the Amazon tend to be similar in lifestyles (e.g. diet, microhabitat) at the level of genus and tribes, although some species are dietary-specialists (e.g. [91]). Decision-making in conservation has been historically based on species level, so TD should not be overlooked in conservation plans. As TD is associated with environmental predictors, it is more likely to reflect associations with other taxonomic groups. Another advantage of TD is that it is possible to include all species, independent of information on traits of phylogenetic relationships.

We used an unprecedented standardized sampling effort to show that the environmental heterogeneity causes species turnover in snake assemblages in the Amazon, but the local and regional patterns of species co-occurrence do not imply patterns of assembly through functional or phylogenetic similarities among plots. The contributions of regional assemblages to overall ecosystem function are under effects of slow evolutionary diversification generating interspecific phenotypic divergence. This highlights the importance

of investigating different diversity measures in ecological studies and conservation actions, because multi-dimensional approaches are clearly more informative than simple metrics, such as how many species occur in a site.

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References

1. Keddy PA (1992) Assembly and response rules: two goals for predictive community ecology. - J Veg Sci 3: 157–164.
2. MacArthur RH (1972) Geographical ecology: patterns in the distribution of species. Princeton: Princeton University Press. pp 269.

3. Hangartner S, Laurilla A, Räsänen K (2012) Adaptive divergence in moor frog (*Rana arvalis*) populations along an acidification gradient: inferences from QST – FST correlations. Evolution 66: 867–881.
4. Mariac C, Jehin L, Saïdou AA, Thuillet AC, Couderc M, Sire P, Judgé H, Adam H, Bezançon G, Pham JL, Vigouroux Y (2011) Genetics basis of pearl millet adaptation along an environmental gradient investigated by a combination of genome scan and association mapping. Mol Ecol 20: 80–91.
5. Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of rainforest faunas: an integrated molecular approach. Annu Rev Ecol Evol S 31: 533–563.
6. Kozak KH, Wiens JJ (2007) Climatic zonation drives latitudinal variation in speciation mechanisms. P Roy Soc Lond B Bio 274: 2995–3003.
7. Dehling DM, Fritz SA, Töpfer T, Päckert M, Estler P, Böhning-Gaese K, Schleuning M (2014) Functional and phylogenetic diversity and assemblage structure of frugivorous birds along an elevational gradient in the tropical Andes. Ecography 37: 1047–1055.
8. Astor T, Strengbom J, Berg MP, Lenoir L, Marteinsdóttir B, Bengtsson J (2014) Underdispersion and overdispersion of traits in terrestrial snail communities on islands. Ecol Evol 4(11): 2090–2102.
9. Cadotte M, Albert CH, Walker SC (2013) The ecology of differences: assessing community assembly with trait and evolutionary distances. Ecol Lett 16: 1234–1244.
10. Cadotte M, Cavender-Bares J, Tilman D, Oakley TH (2009) Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. PloS ONE 4: e5695.

11. Flynn DFB, Mirotchnick N, Jain M, Palmer MI, Naeem S (2011) Functional and phylogenetic diversity as predictors of biodiversity-ecosystem function relationships. Ecology 92: 1573–1581.
12. Diaz S, Cabido M (2001) Vive la difference: plant functional diversity matters to ecosystem processes. Trends Ecol Evol 16: 646–655.
13. McGill BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol 21: 178–185.
14. Pressey RL, Humphries CJ, Margules CR, Vane-Wright RI, Williams PH (1993) Beyond opportunism: key principles for systematic reserve selection. Trends Ecol Evol 8: 124–128.
15. Meynard CN, Devictor V, Mouillot D, Thuiller W, Jiguet F, Mouquet N (2011) Beyond taxonomic diversity patterns: how do α, β and γ components of bird functional and phylogenetic diversity respond to environmental gradients across France? Global Ecol Biogeogr 20: 893–903.
16. Grass I, Brandl R, Botzat A, Neuschulz EL, Farwig N (2015) Contrasting taxonomic and phylogenetic diversity responses to forest modifications: comparisons of taxa and successive plant life stages in South African scarp forest. PloS ONE 10(2): e0118722.
17. Winter M, Devictor V, Schweiger O (2012) Phylogenetic diversity and nature conservation: where are we? Trends Ecol Evol 2012: 1–6.
18. Blackburn TM, Petchey OL, Cassey P, Gaston KJ (2005) Functional diversity of mammalian predators and extinction in island birds. Ecology 86(11): 2916–2923.
19. Petchey OL, Gaston KJ (2007) Dendrograms and measuring functional diversity. Oikos 116: 1422–1426.
20. Tilman D (2001) Functional diversity. In Levin SA, editor. Encyclopedia of Biodiversity. Amsterdam: Academic Press. pp 109–121.

21. Petchey OL, Gaston KJ (2002) Functional diversity (FD), species richness and community composition. Ecol Lett 5: 402–411.
22. Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biol Conserv 61: 1–10.
23. Milcu A, Allan E, Roscher C, Jenkins T, Meyer ST, Flynn D, Bessler H, Buscot F, Engels C, Gubsch, König S, Lipowsky A, Loranger J, Renker C, Scherber C, Schmid B, Thébault E, Wubet T, Weisser WW, Scheu S, Eisenhauer (2013) Functionally and phylogenetically diverse plant communities key to soil biota. Ecology 94(8): 1878–1885.
24. Nipperess DA, Matsen-IV FA (2013) The mean and variance of phylogenetic diversity under rarefaction. Meth Ecol Evol 4: 566–572.
25. Webb CO, Ackerly DD, McPeek MA, Donoghue M J (2002) Phylogenies and community ecology. Annu Rev Ecol Evol S 33: 475–505.
26. Seehausen O (2006) African cichlid fish: a model system in adaptive radiation research. P Roy Soc Lond B Bio 273: 1987–1998.
27. Graham CH, Fine PVA (2008) Phylogenetic beta diversity: linking ecological and evolutionary processes across space in time. Ecol Lett 11: 1265–1277.
28. Ribas CC, Aleixo A, Nogueira ACR, Miyaki CY, Cracraft J (2011) A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. P Roy Soc Lond B Bio DOI: 10.1098/rspb.2011.1120.
29. Devictor V, Mouillot D, Meynard C, Jiguet F, Thuiller W, Mousquet N (2010) Spatial mismatch and congruence between taxonomic, phylogenetic and functional diversity: the need for integrative conservation strategies in a changing world. Ecol Lett 13: 1030–1040.

30. Burbrink FT, Myers EA (2015) Both traits and phylogenetic history influence community structure in snakes over steep environmental gradients. Ecography 38: 001–013.
31. Cavalheri H, Both C, Martins M (2015) The interplay between environmental filtering and spatial processes in structuring communities: the case of Neotropical snake communities. PloS ONE 10(6): e0127959. doi: 10.1371/jornal.pone.0127959.
32. Martins M, Oliveira ME (1999) Natural history of snakes in forests of the Manaus region, Central Amazonia, Brazil. Herpetol Nat Hist 6: 78–150.
33. Pyron RA, Burbrink FT, Wiens JJ (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol Biol 13: 1–53.
34. Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winnie CT (2000) The global decline of reptiles, déjà vu amphibians. BioScience 50(8): 653–666.
35. Whitakker RH (1965) Dominance and diversity in land communities. Science 147: 250–260.
36. Rojas-Ahumada DP, Landeiro VL, Menin M (2012) Role of environmental and spatial processes in structuring anuran communities across a tropical rainforest. Austral Ecol 37: 865–873.
37. Bueno AS, Bruno RS, Pimentel TP, Sanaiotti TM, Magnusson WE (2012) The width of riparian habitats for understory birds in an Amazonian forest. Ecol Appl 22: 722–734.
38. Drucker DP, Costa FRC, Magnusson WE (2008) How wide is the riparian zone of small streams in tropical forests? A test with terrestrial herbs. J Trop Ecol 24: 65–74.

39. Fraga R, Lima, AP, Magnusson WE (2011) Mesoscale spatial ecology in a tropical snake assemblage: the width of riparian corridors in central Amazon. Herpetol J 21: 51–57.

40. Jackson DA, Peres-Neto PR, Olden JD (2001) What controls who is where in freshwater fish communities: the roles of biotic, abiotic, and spatial factors. Can J Fish Aquat Sci 58: 157–170.

41. Magnusson WE, Lima AP, Luizão R, Luizão F, Costa FRC, Castilho CV, Kinupp VF (2005) RAPELD: a modification of the Gentry method of floristic survey for biodiversity surveys in long-term ecological research sites. Biota Neotropica 2: 1–6.

42. Magnusson WE, Braga-Neto R, Pezzini F, Baccaro F, Bergallo H, Penha J, Rodrigues D, Verdade LM, Lima A, Albernaz AL, Hero J-M, Lawson B, Castilho C, Drucker D, Franklin E, Mendonça F, Costa F, Galdino G, Castley G, Zuannon J, Vale J, Santos JLC, Luizão R, Cintra R, Barbosa RI, Lisboa A, Koblitz RV, Cunha CN, Pontes ARMP (2013) Biodiversity and Integrated Environmental Monitoring. Santo André: Áttema. pp 63–135.

43. Pitman NCA, Widmer J, Jenkins CN, Stocks G, Seales L, Paniagua F, Bruna EM (2011) Volume and geographical distribution of ecological research in the Andes and the Amazon, 1995–2008. Trop Conserv Sci 4: 64–81.

44. Steen D (2010) Snakes in the grass: secretive natural histories defy both conventional and progressive statistics. Herpetol Conser Biol 5(2): 183–188.

45. Fraga R, Stow AJ, Magnusson WE, Lima AP (2014) The costs of evaluating species densities and composition of snakes to assess development impacts in Amazonia. PloS ONE 9(8): e105453.

46. Moraes LJCL, Fraga R (2015) Climbing behavior in the aquatic snake Helicops hagmanni Roux 1910 (Serpentes: Dipsadidae). Herpetol. Notes. 8: 19–20.

47. Ribeiro-Jr JW, Lima AP, Magnusson WE (2012) The effect of riparian zones on species diversity of frogs in Amazonian forests. Copeia 2012(3): 375–381.
48. Cintra BBL, Schietti J, Emillio T, Martins D, Moulatlet G, Souza P, Lecis 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.
49. Forbes SA (1907) On the local distribution of certain Illinois fishes: an essay in statistical ecology. Bull Illinois St Lab Nat Hist 7: 272–303.
50. Alroy J (2015) A new twist on a very old binary similarity coefficient. Ecology 96: 575–586.
51. Gauch HG-Jr (1982) Multivariate analysis in community ecology. Cambridge: Cambridge University Press. pp 109–172.
52. De'ath G (1999) Extended dissimilarity: a method of robust estimation of ecological distances from high beta diversity data. Plant Ecol 144(2): 191–199.
53. Petchey O L, Gaston KJ (2006) Functional diversity: back to basics and looking forward. Ecol Lett 9: 741–758.
54. Burbrink FT, Myers E A (2015) Both traits and phylogenetic history influence community structure in snakes over steep environmental gradients. Ecography 38: 001–013.
55. Beebe W (1946) Field notes on the snakes of Kartabo, British Guiana, and Caripito, Venezuela. Zoologica 31: 11–52.
56. Belluomini HE, Hoge AR (1958) Contribuição à biologia de *Eunectes murinus* (Linnaeus 1758) (Serpentes). Observações sobre hábitos alimentares de “sucuris” em cativeiro. Mem I Butantan 28: 207–216.

57. Duellman WE (1978) The biology of an equatorial herpetofauna in Amazonian Ecuador. U Kansas Mus Nat Hist Misc Pu 65: 1–352.
58. Cunha OR, Nascimento FP (1983) Ofídios da Amazônia XIX - As espécies de *Oxyrhopus* Wagler, com uma subspécie nova, e *Pseudoboa* Schneider, na Amazônia oriental e Maranhão. (Ophidia, Colubridae). B Mus Paraense Emílio Goeldi Zool 122: 1– 42.
59. Dixon JR, Soini P (1986) The reptiles of the upper Amazon Basin, Iquitos region, Peru. Milwaukee: Milwaukee Public Museum. pp 160.
60. Michaud EJ, Dixon JR (1989) Prey items of 20 species of the Neotropical colubrid snake genus *Liophis*. Herpetol Rev 20: 39–41.
61. Cunha OR, Nascimento FP (1993) Ofídios da Amazônia: As cobras da região leste do Pará. B Mus Paraense Emílio Goeldi Zool 9: 1–191.
62. Starace F (1998) Guide des Serpents at Amphisbènes de Guiane. Matoury Cedex: Ibis Rouge Éditions. pp 452.
63. Fraga R, Lima AP, Prudente ALC, Magnusson WE (2013) Guia de cobras da região de Manaus – Amazônia central / Guide to the snakes of the Manaus region – central Amazonia. Manaus: INPA. pp 303.
64. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H (2015) Vegan: Community Ecology Package. http://cran.r-project.org.
65. Nipperess DA, Matsen-IV FA (2013) The mean and variance of phylogenetic diversity under rarefaction. Meth Ecol Evol 4: 566–572.
66. Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics. 20: 289–290.

67. Pearse WD, Purvis A (2013) phyloGenerator: an automed phylogeny generations tool for ecologists. Meth Ecol Evol 4: 692–698.

68. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2009) Genbank. Nucleic Acids Res. 37: D26–D31.

69. Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33: 511 – 518.

70. Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9: 286–298.

71. Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

72. Webb CO (2000) Exploring the phylogenetic structure of ecologial communities: an example for rain forest trees. Am Nat 156: 145–155.

73. Webb CO, Ackerly DD, McPeek, MA, Donoghue, MJ (2002) Phylogenies and community ecology. Annu Rev Ecol Evol S 33: 475–505.

74. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO (2010) Picante: R tools for intergrating phylogenies and ecology. Bioinformatics 26: 1463–1464.

75. Gotelli NJ, Entsminger GL (2003) Swap algorithms in null model analysis. Ecology. 84: 532–535.

76. Gotelli NJ, Graves GR (1996) Null models in ecology. Washington DC: Smithsonian Institution Press. pp 139–147.

77. Santorelli S-Jr, Magnusson W, Ferreira E, Caramaschi E, Zuanon J, Amadio S (2014) Phylogenetic community structure: temporal variation in fish assemblage. Ecol Evol 4(11): 2146–2153.

78. Wald A, Wolfowitz J (1940) On a test whether two samples are from the same population. Ann Math Stat 11: 147–162.

79. Caiero F, Mateus A (2014) randtests: Testing randomness in R. R package version 1.0. http://CRAN.R-project.org/package=randtests.

80. Jankowski JE, Ciecka AL, Meyer NY, Rabenold KN (2009) Beta diversity along environmental gradients: implications of habitat specialization in tropical montane landscapes. J Anim Ecol 78: 315–327.

81. MacArthur RH, Levins R (1967) The limiting similarity, convergence, and divergence of coexisting species. Am Nat 101: 377–385.

82. Cavenders-Bares J, Kozak KH, Kembel SW (2009) The merging of community ecology and phylogenetic biology. Ecol Lett 12: 693–715.

83. González-Caro S, Parra JL, Graham CH, McGuire JA, Cadena CD (2012) Sensitivity of metrics of phylogenetic structure to scale, source of data and species pool of hummingbird assemblages along elevational gradients. PloS ONE 7(4): e35472.

84. Gu W, Swihart RK (2003) Absent or undetected? Effects of non-detection of species occurrence on wildlife-habitats models. Biol Conserv 116: 195–203.

85. Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, ter-Steege H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H (2013) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust J Bot 51: 335–380.

86. Mouquet N, Devictor V, Meynard CN, Munoz F, Bersier LF, Chave J, Couteron P, Dalecky A, Fontaine C, Gravel D, Hardy OJ, Jabot F, Lavergne S, Leibold M, Miullot D, Münkermüller, Pavoine S, Prinzing A, Rodrigues ASL, Rohr RP, Thébault E, Thuiller W (2012) Ecophylogenetics: advances and perspectives. Biol Rev 87: 769–785.

87. Cadotte M, Carscadden K, Mirotchnick N (2011) Beyond species: functional diversity and the maintenance of ecological processes and services. J Appl Ecol 48: 1079–1087.
88. Lavorel S, (1997) Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends Ecol Evol 12: 474–478.
89. Davies TJ, Meiri S, Barraclough TG, Gittleman JL (2007) Species co-existence and character divergence across carnivores. Ecol Lett 10: 146–152.
90. Bracken MES, Low NHN (2012) Realistic of rare species disproportionately impact higher trophic levels. Ecol Lett 15: 461–467.
91. Gaiarsa MP, Alencar LRV, Valdujo PH, Tambosi LR, Martins M (2015) Setting conservation priorities within monophyletic groups: an integrative approach. J Nat Conserv DOI 10.1016/j.jnc.2015.01.006.

Capítulo III

Fraga R, Lima AP, Magnusson WE, Ferrão M, Stow AJ (2016) Contrasting patterns of gene flow for ambush predator and active forager snakes in the Amazon. *Molecular Ecology* (em preparação).

Contrasting patterns of gene flow for ambush predator and active forager snakes in the Amazon

Rafael de Fraga[a,1], Albertina P. Lima[b], William Magnusson[b], Miquéias Ferrão[a], Adam J. Stow[c]

[a] Instituto Nacional de Pesquisas da Amazônia – Programa de Pós-graduação em Ecologia, Av. Efigênio Salles, 2239, Manaus – AM, CEP 69060-020

[c] Instituto Nacional de Pesquisas da Amazônia – Coordenação de Biodiversidade, POBOX 2223, Manaus – AM

[b] Department of Biological Sciences, Macquarie University, NSW 2109, Australia

[1] r.defraga@gmail.com, +55 92 3643-1832

Abstract

Rarely has spatial genetic structure been described for snakes, in part owing to difficulties in obtaining sufficient sample sizes. Here we test the hypothesis that actively foraging snakes will have higher levels of gene flow than ambush predators. We evaluated four co-distributed species of snake in the Brazilian Amazon, two active foragers and two ambush predators. We analyzed thousands of SNPs to compare patterns of neutral gene flow based on isolation by geographic distance (IBD) and environmental resistance (IBR). Snakes were sampled along an 880 km transect in the central to southwestern Amazon basin which covered a mosaic of vegetation types and seasonal differences in climate. We show that IBD and IBR were evident in ambush predators, implying lower levels of dispersal than the active foragers, where gene flow was high enough to prevent the buildup of genotypic structure,

regardless of geographic distance and environmental heterogeneity. Nonetheless, it was also apparent that gene flow was relatively high for all snake species over surprisingly large distances, consistent with the widespread distributions of each of these snake species.

Key-words: isolation by distance; isolation by environment; landscape genomics; SNPS

Introduction

In recent years measures of gene flow have been described for a large range of organisms, but there are only a few datasets for snakes (e.g. Blouin-Demers & Weatherhead 2001; Bittner & King 2003; Harper & Pfennig 2008). Knowledge of the spatial distribution of genetic variation has provided insights to the environmental factors influencing dispersal and gene flow (e.g. Stow *et al*. 2001; Dudaniec *et al*. 2013) and consequently the mechanisms that generate components of biodiversity (genetic diversity and species diversity). Information on genetic variation can also be valuable for conservation because it informs on how habitat loss influences gene flow, and levels of intraspecific genetic variation captured within reserve systems (Stow *et al*. 2004; Bell & Okamura 2005).

Snakes constitute approximately 6 % of global vertebrate diversity (see Uetz & Hošek 2015), are known to have key ecological functions, and make other numerous contributions to human societies in culture, medicine and economics (Konar & Monak 2010). Nonetheless, the cryptic life style of snakes (Steen 2010; Fraga *et al*. 2014) means that fundamental ecological traits, such as dispersal characteristics at landscape scales, are difficult to obtain. In particular, obtaining sufficient numbers of individuals for genetic analysis of gene flow has been challenging. Recently more powerful analyses based on thousands of SNPs have become accessible, and these allow reliable estimates of genetic structure from fewer individuals (e.g. Willings *et al*. 2012).

The relationship between genetic structure, geographic distance and environmental heterogeneity can provide insight to the role of dispersal-dependent processes in shaping species distributions (Lowe & Allendorf 2010; Wang & Bradburd 2015). Isolation by geographic distance (hereafter IBD) and isolation by environmental resistance (hereafter IBR) are among the most well-known factors driving genetic variability (e.g. McRae 2006; Koen *et al*. 2012; Marrote *et al*. 2014). The first is estimated simply by testing for correlations between genetic distance and geographic distance. The latter may be quantified by calculating the probability of an individual dispersing from one location to another after weighting the 'resistance' to dispersal in each of the environments, and then assessing all possible pathways (Wang & Bradburd 2015; Manthey & Moyle 2015).

To evaluate IBR, the disciplines of landscape ecology, population genetics and spatial statistics are applied to create a resistance surface (Peterman 2014). By optimizing resistance surfaces, researchers are able to evaluate ecological processes (e.g. levels of dispersal) underlying gene flow and the degree of connectivity through different habitats. Environmental resistance-based models can assume that gene flow is influenced by mechanisms such as non-random migration, population size, dispersal capability, life history and the geographic features of the study area. These models therefore provide a mechanism to patterns of gene flow for different species (Dudaniec *et al*. 2013).

The Amazon lowlands have an exceptionally high number of snake species (e.g. Bernarde *et al*. 2012), but the mechanisms generating and maintaining snake diversity over landscapes are poorly known. Recent studies have shown that environmental gradients may filter taxonomic snake assemblages at regional scales (Fraga *et al*. 2011, Fraga *et al*. *in prep.*) and influence habitat connectivity at local scales (Fraga *et al*. 2013a). However, there isn't any knowledge of genetic structuring of snakes in the Amazon.

In this study we describe the effects of IBD and IBR on gene flow of four co-distributed snake species, two of which are ambush predators and the other two are active foragers. Contrasting different foraging modes is relevant to the understanding of species-habitat associations (Shine *et al*. 2004). This is because foraging modes are often linked to numerous species attributes, such as activity patterns (Cooper *et al*. 2001), habitat use (Fedriani *et al*. 1999), digestive physiology (Hilton *et al*. 1999), seasonal patterns of reproduction (Colli *et al*. 1997) and mortality (Willette *et al*. 1999). We hypothesize that ambush predators will disperse less and therefore show less gene flow at the landscape scale than the two species of snakes that actively forage.

Materials and Methods

Target species

The Common lancehead *Bothrops atrox* is a nocturnal pitviper (Crotalinae), widely distributed throughout the Amazon Basin. The species is primarily terrestrial, but individuals can climb vegetation up to 2 m from the ground, especially juveniles. It is an ambush predator that is found in a variety of habitats (Martins & Oliveira 1999) with temporary increases in density of individuals in areas near to streams (Fraga *et al*. 2013a).

The Garden treeboa *Corallus hortulanus* is a nocturnal boid (Boinae), widely distributed through different forested habitats in South America. The species is an ambush predator, arboreal, and it tends to be sedentary, spending long periods in small areas (Henderson 1997). The species shows marked polychromatism, with up to five sympatric color morphs (Duarte *et al*. 2015).

The Cat-eyed banded Snake *Leptodeira annulata annulata* is a nocturnal dipsadid (Dipsadinae), widely distributed in northern South America. The species is an active forager,

which often moves and hunts at ground level, although it may climb up on the vegetation to sleep protected from terrestrial predators (Martins & Oliveira 1999). The species is a habitat-generalist, and individuals may be found even in disturbed areas.

The Southern sharpnose snake *Philodryas georgeboulengeri* (Dipsadinae) has the smallest geographic range of the species investigated in this study, being limited to the south-southwestern Amazonia (Prudente *et al*. 2008). The biological traits of this species are poorly known, but it appears to have similar habits to the closely related *Philodryas argentea* (see *Xenoxybelis argentea* in Martins & Oliveira 1999). It is an active predator, diurnal and arboreal.

Study area and snake sampling

We sampled snakes along approximately 880 km from the central (Manaus; 03°00'29" N; 60°02'29" W) to the southwest (Porto Velho; 09°20'05" N; 64°44'05" W) regions of the Amazon lowlands. Environmental gradients generates changes in the landscape over the study area (e.g. vegetation cover type and climatic seasonality; Figure 1). In the north of our study area we sampled the Ducke Reserve, which is covered by 100 km^2 of primary non-flooded forest and bathed by perennial streams. In the central region of our study area (interfluve Purus - Madeira) we sampled primary and old secondary forests crossed by a federal highway (BR-319) which was partially abandoned in the 80s. This region is characterized by seasonally flooded forests (streams overflowing) and patches of arboreal *Campinarana*, which is forest growing on white sand. In the south of our study area (Madeira River, Porto Velho) we sampled primary and old secondary forests, characterized by a drier climate compared to the rest of the study area, where most streams completely dry in the dry season.

Snakes were sampled from 21 RAPELD modules (Magnusson *et al*. 2013), each of which are 5 km^2, with an average distance of 40 km between neighboring modules. Each

module contains 10 plots, 250 m long and 10 m wide each, following the altitudinal curves, and distributed on two parallel trails (5 plots per trail), with standardized distance of 1 km between neighboring plots.

Snakes were found by actively searching the modules at night, limited by space (plot area) and time (one hour per plot), with two observers per plot. We sampled each plot four times during independent survey expeditions from 2007 to 2015.

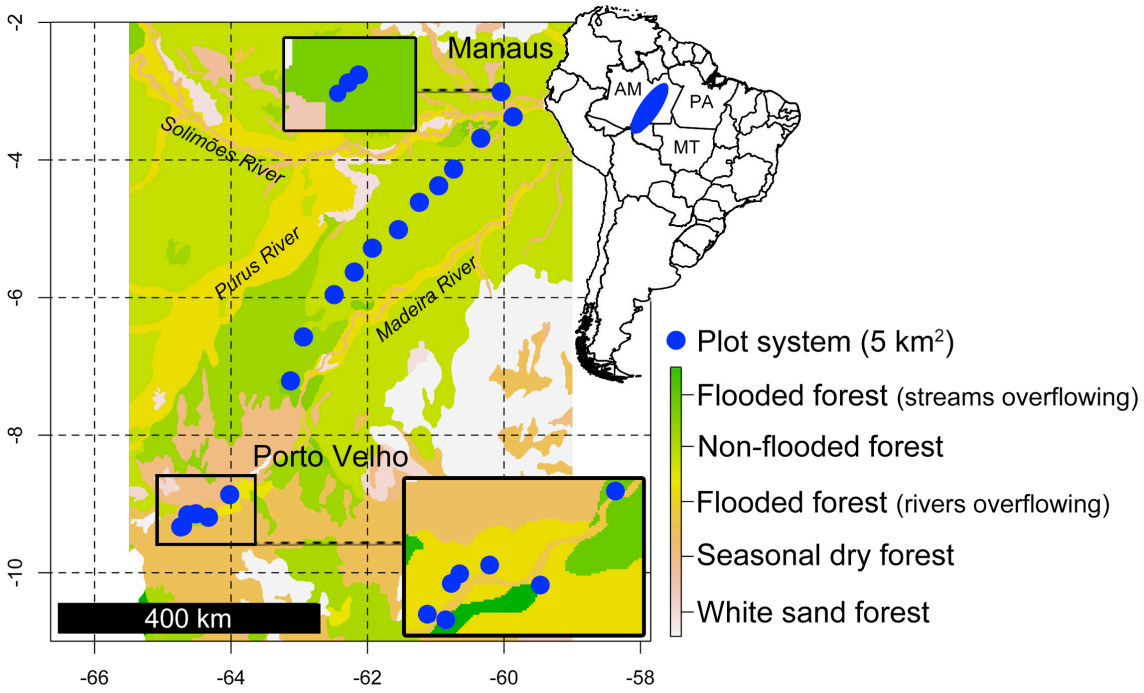


Figure 1. Sampling modules (blue circles) across the Amazon basin, that have been used to contrast patterns of gene flow among four snake species. The study area comprises different habitats based on vegetation cover types and climate seasonality. The rectangles zoom in areas with overlapping modules. The blue ellipse on the map of South America shows the study area on a continental scale, and the acronyms are the Brazilian states of Amazonas (AM), Pará (PA) and Mato Grosso (MT).

SNP data

We extracted and purified DNA from muscle tissue samples using the GenCatch™ Genomic DNA Extraction Kit. We mostly followed the protocols suggested by the manufacturer, but we added an extra hour of incubation for protein digestion. We checked

DNA quality visually using 0.8 % agarose gel. Subsamples of 0.5 µg of high quality DNA samples were sent to Diversity Arrays Technology Pty. Ltd. (Canberra, Australia), where SNPs were discovered and genotyped using the standard DartSeq™ protocol (Petroli *et al*. 2012; Jaccoud *et al.* 2001, Kilian *et al*. 2012) which makes use of next-generation Illumina sequencing (Sansaloni *et al*. 2011). We present a brief description of the DartSeq™ protocol in S1 (Appendix 1).

Diversity Arrays genotyped more than 14,000 SNPs for each species. We filtered this dataset by excluding SNPs with a read depth < 10, call rate < 70 % and repeatability < 90 %. Additionally we excluded sequences with more than one SNP to reduce statistic bias due to physical linkage.

To assess whether the inclusion of loci putatively influenced by selection influenced our results, we first used two methods to identify loci that deviate from neutrality. These were an FST outlier test based on Bayesian modeling (BAYESCAN; Foll & Gaggiotti 2008) and the Latent Factors Mixed Models (LFMM) approach, which tests for correlations between genetic polymorphisms and environmental variables. The environmental variables used were the PCoA scores from cost distance matrices per species (see methods below). The LFMM approach was performed using the LEA package (Frichot & François 2015) in R (R Core Team, 2015). We set both BAYESCAN and LEA with False Discovery Rate of 0.01. We did not identify any locus with a strong signal of adaptive selection. Therefore, we do not expect that the patterns of gene flow shown in this study are biased by adaptive selection.

Investigating genetic structure

For each species, samples were pooled and each locus tested for Hardy-Weinberg Equilibrium (HWE). For any locus deviating from Hardy-Weinberg equilibrium we noted whether this was a result of a heterozygote excess or deficit. A possible explanation for

species with larger proportions of loci with a heterozygote deficit is a spatial Wahlund effect (Wahlund 1928). Wahlund effect is the apparent deficit of heterozygotes that occurs when genetically subdivided samples are pooled.

Because the number of individuals sampled at any one locality was generally small, this excluded analysis based on locational differences in allele frequency. We therefore based our analysis of spatial genetic structure on inter-individual differences.

Measuring genetic differentiation

We calculated pairwise genetic distances between individuals based on genotypic relatedness which is better suited to detecting subtle genetic variation. This is because genotypes are shuffled at each generation, therefore genotypic structure derived from genotypic similarity between individuals can be influenced by short-term processes such as the spatial distribution of close relatives (see Stow *et al*. 2001). We used the related R-package, which allows estimating pairwise relatedness based on seven different indices, in order to identify the best index for the dataset (Pew *et al*. 2015). The Ritland index (Ritland 1996) was identified as the best estimator for each of the species.

Spatial autocorrelation analysis

Because the relationship of genetic distance with geographic distance is often non-linear we analyzed for spatial autocorrelation of a genetic similarity measure at several different distance classes using GenAlEx (Peakall & Smouse 2012). To test the overall relationship (e.g. the shape of the correlogram) we used a heterogeneity test, which rejects the null hypothesis of no genotypic isolation-by-distance at $P < 0.01$ (Banks & Peakall 2012). We

set the distances classes used in the spatial correlograms to approximately equalize numbers of pairwise comparisons in each geographic distance class.

Isolation by environment

The study area is characterized by a mosaic of different vegetation and climate over a latitudinal gradient of about seven degrees (S1, Appendix 2). To capture environmental heterogeneity we selected vegetation cover type as a discrete variable, seasonality in temperature and seasonality in rainfall as continuous variables. The environmental variables were obtained in raster format in the public repository Ambdata (Amaral *et al*. 2013; www.dpi.inpe.br/Ambdata). Ambdata provides environmental data from the entire Amazon basin, and the raster files have a resolution of 1 km. We cropped the raster files to our study area and reduced the resolution to 12 km given the available computing power. The raster files were modified using the raster R-package (Hijmans *et al*. 2015).

Most of the methods used to build resistance surfaces require *a priori* definition of maximum resistance values to environmental variables. Factors limiting dispersal can be conspicuous, such as low altitudes to mountain goats in northern USA (Shirk *et al*. 2010). However, here we were interested in investigating factors influencing gene flow across subtle variation in vegetation cover type and climatic seasonality in the Amazon rainforests. Therefore we weighted resistances using genetic algorithms that combine genotypes, environmental layers and estimations of the fittest individuals to survive and reproduce from each generation (Peterman 2014). This approach overcomes the challenge of complex environmental heterogeneity and there is no *a priori* information needed on the influence of habitat type on dispersal. In addition, the method used in this study allows optimizing multiple resistance surfaces, based on more than one environmental variable. Therefore the

environmental resistance on gene flow may result from interactions between different factors (Peterman 2014).

We ran IBR models by optimizing multiple resistance surfaces per species, each composed by vegetation cover type, seasonality in temperature and seasonality in rainfall. We used the ResistanceGA R-package (Peterman 2014), which integrates sampling locations, genetic distances and continuous or discrete environmental variables (raster layers). Mixed linear models are then used to identify patterns of resistance to gene flow, through circuit theory - CS (Circuitscape; McRae & Beier 2007; McRae *et al*. 2008; McRae & Shah 2009) and least cost path - LCP (e.g. Driezen *et al*. 2007; Wang *et al*. 2009) and fitted by corrected Akaike information criterion (Peterman 2014). We obtained pairwise cost distance matrices per species from the resistance scores obtained in each multiple resistance surface.

To assess the statistical coefficients of the linear relationships between the distance matrices and decouple the effects of log-geographic distance (IBD) and cost distance (IBR) on genetic distance, we used PCoA scores (axis 1) from each distance matrix in multiple linear regressions. We assumed the general formula *PCoA1-genetic distance = a + b(PCoA1-geographic distance) + b(PCoA1-cost distance)* and we plotted the partials from each model.

Results

Hardy-Weinberge equilibrium

Ambush predators consistently had higher proportions of loci that significantly deviated from HWE, compared to active foragers (Table 1). Additionally, for most loci significantly deviated from HWE, the expected heterozygosity was higher than the observed heterozygosity in ambush predators , which suggests stronger genetic structure (S1, Appendix 3).

Table 1. Summary of genetic structure of snakes from the Amazon based on deviation from Hardy-Weinberg equilibrium (HWE). The table shows the proportions of loci significantly deviated from HWE and proportions of loci for which the expected heterozygosity (He) is greater than the observed heterozygosity (Ho). Genetic structure has been compared between ambush predators (AP) and active foragers (AF). S = species (1. *Bothrops atrox*, 2. *Corallus hortulanus*, 3. *Leptodeira annulata*, 4. *Philodryas georgeboulengeri*). FM = foraging mode, Avg = average, values in brackets = standard errors.

S	FM	N	Filtered loci	Avg He	Avg Ho	HWE ($P \leq 0.05$)	% HWE	He > Ho	% He > Ho
1	AP	32	3,169	0.26 (0.003)	0.27 (0.003)	466	14.89	329	10.51
2	AP	15	7,805	0.22 (0.002)	0.20 (0.002)	898	11.50	853	10.92
3	AF	23	2,173	0.07 (0.001)	0.08 (0.001)	32	1.47	28	1.28
4	AF	19	720	0.07 (0.002)	0.07 (0.002)	2	0.27	1	0.27

Measures of environmental resistance

All PCoA ordinations of distance matrices (genetic, geographic and environmental cost) returned axis 1 representing at least 70 % of the variation in the original distances observed. The IBR distance matrices based on LCP and CS were at least 86 % correlated ($P < 0.001$ in all cases), therefore we arbitrarily show only the results from LCP. Resistance surfaces based on LCP and CS may be found at S1, Appendix 4.

Estimates of genetic structure

We found different effects of IBD on genetic distance between ambush predators and active foragers. The linear models explained 35 % ($P = 0.001$) and 82 % ($P > 0.001$) of the genetic distance between individuals respectively to the Common lancehead (Figure 2) and the Garden treeboa (Figure 3). However, the influence of IBR on genetic distance was restricted to the sedentary Garden treeboa ($P < 0.001$). Forests flooded by streams and rivers overflowing had greater resistance scores, showing that flooded habitats are not optimal

routes for Garden treeboa's dispersal. We found no significant effects of IBD or IBR on the genetic distance from the active foragers, the Cat-eyed banded (Figure 4) and Southern sharpnose (Figure 5) snakes ($P > 0.1$ in all analyses). The coefficients from the multiple linear regressions are summarized in the Table 2.

The spatial correlograms were generally consistent with the linear models (Figures 2–5). They showed a continuous decrease in genetic similarity over geographic distance classes in the ambush predators ($P < 0.01$ in all cases), and no pattern in genetic similarity over geographic distance classes for the Cat-eyed banded ($P = 0.01$). However, the Southern sharpnose showed significant spatial correlogram ($P = 0.003$).

Table 2. Coefficients from multiple linear regressions using PCoA scores representing genetic distance as response variable and PCoA scores representing geographic distance (IBD) and environmental cost (IBR) as predictor variables. IBD = isolation by geographic distance, IBR = isolation by environmental resistance.

Species	Predictor	Residuals	Standard error	t-value	P
Lancehead	IBD	0.004	0.001	3.97	<0.001
	IBR	-0.0002	0.0003	-0.77	0.44
Treeboa	IBD	0.005	0.002	2.64	0.02
	IBR	0.08	0.013	6.35	<0.001
Cat-eyed	IBD	5.065	0.03	1.73	0.1
	IBR	-0.01	0.03	-0.35	0.72
Sharpnose	IBD	-0.11	0.17	-0.64	0.52
	IBR	0.09	1.12	-0.08	0.93

Figure 2. Patterns of gene flow in the Common lancehead *Bothrops atrox* based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the bars show errors in genetic similarity at 95 % confidence intervals, the dashed lines show 95 % confidence intervals for the null hypothesis (no spatial structure) and the values in brackets show numbers of pairwise comparisons per class. The scatterplots show the partials from a multiple linear regression.

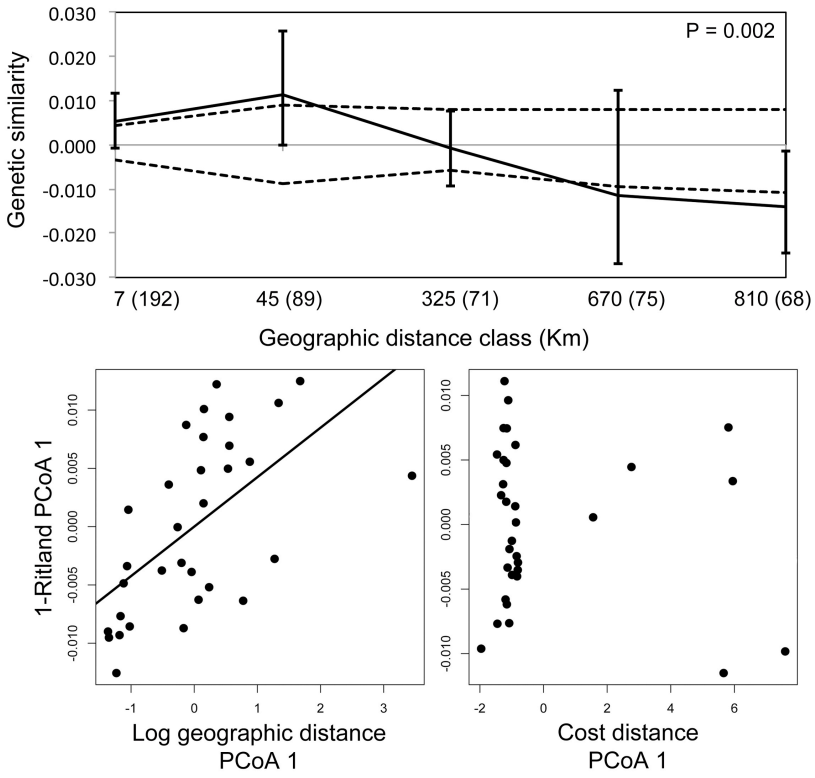


Figure 3. Patterns of gene flow in the Garden treeboa *Corallus hortulanus* based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the bars show errors in genetic similarity at 95 % confidence intervals, the dashed lines show 95 % confidence intervals for the null hypothesis (no spatial structure) and the values in brackets show numbers of pairwise comparisons per class. The scatterplots show the partials from a multiple linear regression.

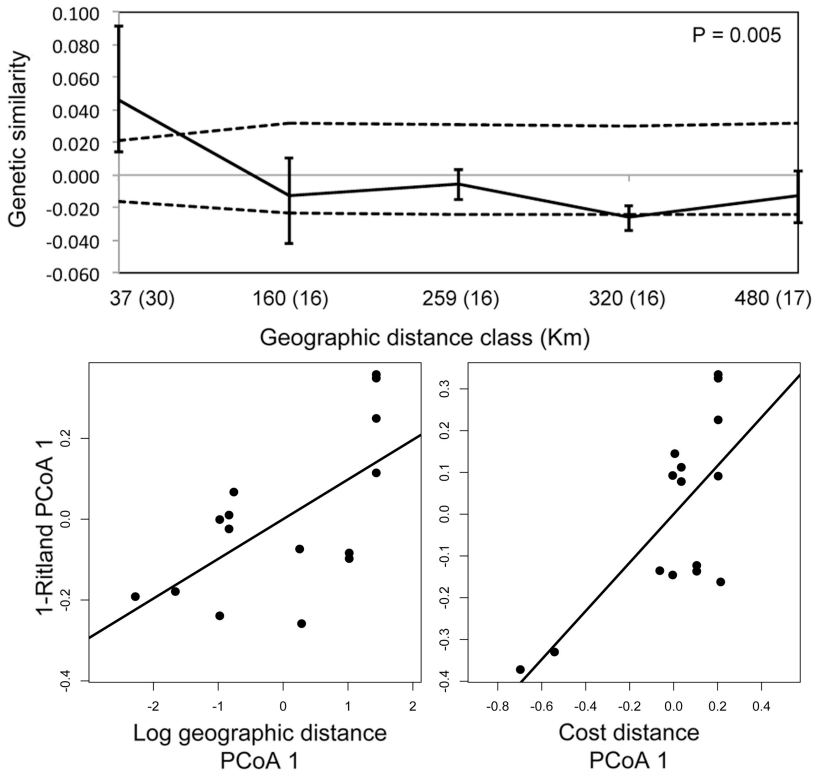


Figure 4. Patterns of gene flow in the Cat-eyed banded snake *Leptodeira annulata annulata* based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the bars show errors in genetic similarity at 95 % confidence intervals, the dashed lines show 95 % confidence intervals for the null hypothesis (no spatial structure) and the values in brackets show numbers of pairwise comparisons per class. The scatterplots show the partials from a multiple linear regression.

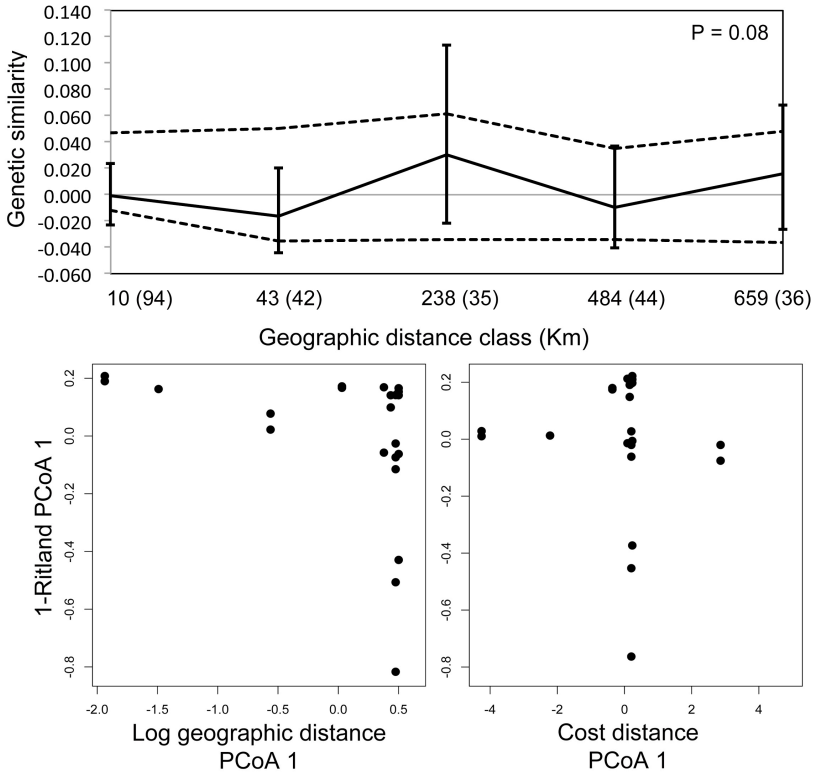
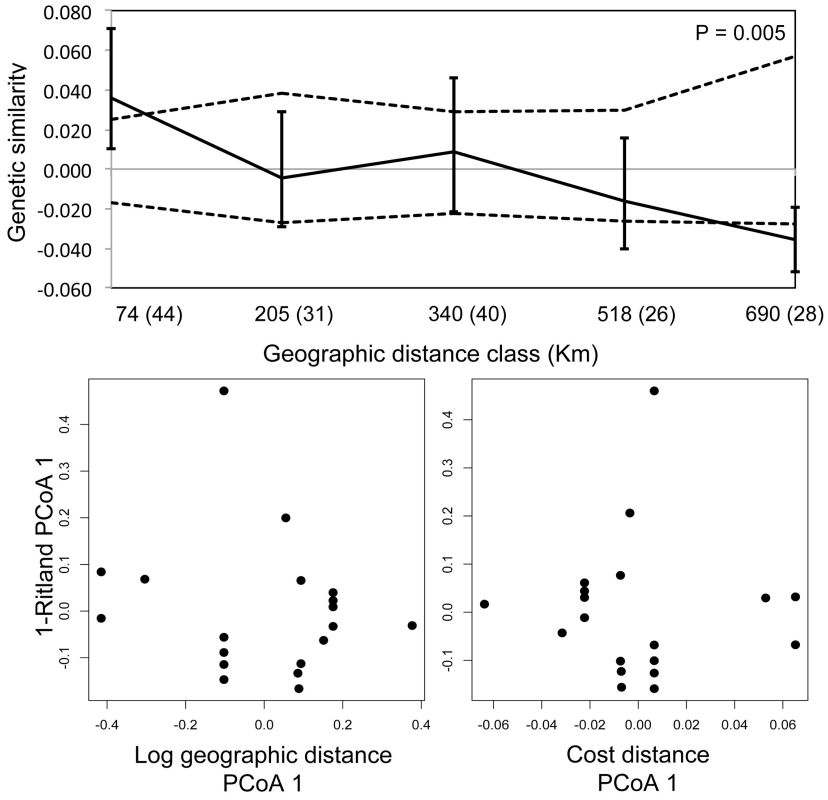


Figure 5. Patterns of gene flow in the Southern sharpnose *Philodryas georgeboulengeri* based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the bars show errors in genetic similarity at 95 % confidence intervals, and the dashed lines show 95 % confidence intervals for the null hypothesis (no spatial structure) and the values in brackets show numbers of pairwise comparisons per class. The scatterplots show the partials from a multiple linear regression.



Discussion

Our genetic data from four snake species co-distributed across ~880 km in the central-southwestern Amazon suggest that foraging mode is associated with the level of genetic differentiation among individuals. The two ambush predators tended to show greater genotypic partitioning than the active foraging species. These data imply higher levels of gene flow across the sampled landscape for the active foragers. Genetic differentiation within populations was previously found as a result from dispersal associated to biological traits such

as water loss (Peterman *et al*. 2014). In this study, despite the apparent overall little genetic structure for all species, we found that the foraging mode drives the probability of interbreeding between individuals, which has caused different patterns of gene flow between ambush predators and active foragers.

Knowledge of gene flow in snakes is severely limited by the ability to obtain large enough sample sizes, because detectability is relatively low (Steen 2010; Fraga *et al*. 2014). Despite lower than typical sizes used in population genetic studies the sample sizes obtained here were sufficient to detect different patterns among species. The significant association of genotypic distance with geographic or cost distances for the two sit-and wait predators matches with our prediction that dispersal limited by a sedentary lifestyle directs non-random mating, while active foraging causes higher levels of gene flow.

Ambush hunting requires spending long periods in relatively small areas, and this has been demonstrated in the Garden treeboa (Henderson 1997) and the Common lancehead (Fraga *et al*., 2013a). Low dispersal has been linked to fitness advantages, such as retreat site familiarity (Davis & Stamps 2004) or matching organisms and background colors (Rosenblum & Harmon 2011). While both the Garden treeboa and Common lancehead are polychromatic, different colormorphs are often sympatric at local scales (Fraga *et al*. 2013b; Duarte *et al*. 2015). Consequently it seems that fitness benefits, other than color matching are associated with higher levels of phylopatry in these species.

Surprisingly, at the scale of the study area, the active foraging species showed little genotypic divergence, even among locations that are geographically distant (> 800 km) and environmentally distinct from each other. The Cat-eyed banded showed a lack of genotypic structure in all tests, and for the Southern sharpnose no relationship between genotypic and geographic or environmental distances could be detected. Less genetic structure in the active

foragers than the ambush predators cannot be explained by sample sizes which were more or less equivalent for each of the species tested at a range of spatial scales.

The only species for which we found significant effects of IBR on gene flow is the Garden treeboa. In this species, gene flow occurs primarily through non-floodable rainforests. A pattern of IBR has been linked to adaptive selection in several organisms, such as grasses (Freeland *et al*. 2010), fishes (Smith *et al*. 2005), amphibians (Dudaniec *et al*. 2012; Peterman *et al*. 2014), birds (Smith *et al*. 2005; Manthey & Moyle 2015) and invertebrates (Funk *et al*. 2011). However, because we didn't identify loci with selection signal, the effect of IBR on gene flow in Garden treeboa is better explained by non-random dispersal across heterogeneous environments (Stevens *et al*. 2005; Feder & Forbes 2007; Davis & Stamps 2004). Garden treeboas have mainly been found in the understory in the Amazon (Martins & Oliveira 1999), which is relatively open in flooded forest compared to non-floodable forests in the Amazon. This is because most plant species cannot survive flooding for long periods. Open habitat provides greater exposure to predators and prey and this might explain why flooded forests are not optimal habitats for dispersal.

Despite the differences in gene flow between ambush predators and active foragers, our study assesses genotypic partitioning at regional scale, and comprises only three interfluves and a very complex mosaic of different habitats. In the Amazon, mechanisms generating and maintaining biological diversity have been explained by multiple factors, often operating across larger distances than assessed here (e.g. Wallace 1852; Haffer 1969; Ayres & Clutton-Brock 1992; Hoorn *et al*. 2012). We therefore are not suggesting that the patterns of IBD and IBR reported here can be extrapolated to larger scales (e.g. the whole Amazon basin), which would incorporate greater geographic isolation and environmental heterogeneity (e.g. Ribas *et al*. 2011).

Implications for conservation

High levels of gene flow along 880 km in active foragers and habitat-specific dispersal in ambush predators suggest habitat connectivity as essential to maintain genotypic diversity in snakes from the central-southwestern Amazon basin. Habitat disconnection is particularly critical to snakes, because reproductive isolation, genetic variability and fitness traits are often correlated (Madsen *et al*. 1996). Reducing heterozygosity in snakes raises relevant consequences for conservation, for example by increasing the frequency of scale and skeleton anomalies (Schwaner 1990).

Identifying corridors of gene flow has wide application in conservation, for example by optimizing reserves design and supporting long-term biodiversity monitoring (Wang & Bradburd 2015). By combining genetic data, geographic space and environmental layers, conservation scientists are able to identify restricted or isolated gene flow, which is undetectable through demographic studies (Prior *et al*. 1997). Much of our study area is currently threatened by the rapid growth of urban areas in Manaus (Ducke Reserve), construction of roads (BR-319) and artificial flooding by hydroelectric plants (Madeira River). Sites geographically distant from each other and environmentally distinct will be disconnected in the near future, and we expect that patterns of heterozygosity in snakes will be severely affected.

Conclusions

We showed that gene flow in snakes from central-southwestern Amazon rainforests is associated with foraging mode. It was apparent that gene flow is relatively high for all snake species over surprisingly large distances, which is consistent with the widespread distributions of each of these snake species studied. However, patterns of genotypic variation showed IBD and IBR as mechanisms reducing gene flow in ambush predators. Low dispersal across

generations causes genetic structure driven by regionally isolated genetic drift and demographic histories. Contrarily, high dispersal in active foragers causes high levels of gene flow, regardless geographic distance and environmental heterogeneity at the scale applied in this study. Our findings have relevant implications for landscape genomics and ecology, because they show patterns of gene flow dependent of biological traits and habitat-specific dispersal. Moreover, our findings have broad application in conservation of natural landscapes in the Amazon, because they highlight the importance of connectivity between different habitats for biodiversity protection.

Acknowledgements

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References

Amaral S, Costa CB, Arasato LS *et al.* (2013) AMBDATA: Variáveis ambientais para Modelos de Distribuição de Espécies (MDEs). In: *Anais do XVI Simpósio Brasileiro de Sensoriamento Remoto (SBSR)*. pp. 6930–6937, Foz do Iguaçu. Available at http://urlib.net/3ERPFQRTRW34M/3E7GH36

Ayres JMC, Clutton-Brock T H (1992). River boundaries and species range size in Amazonian primates. *American Naturalist,* **140**, 531–537.

Banks SC, Peakall R (2012) Genetic spatial autocorrelation can readily detect sex-biased dispersal. *Molecular Ecology*, **21**, 2092–2105.

Bell JJ, Okamura B (2005) Low genetic diversity in a marine nature reserve: re-evaluating diversity criteria in reserve design. *Procedures of the Royal Society B*, **27**, 1067–1074.

Bernarde PS, Albuquerque S de, Barros TO, Turci LCBT (2012) Serpentes do estado de Rondônia, Brasil. *Biota Neotropica*, **12(3)**, 1–29.

Bittner TD, King RB (2003) Gene flow and melanism in garter snakes revisited: a comparison of molecular markers and island vs. coalescent models. Biological *Journal of the Linnean Society*, **79**, 389–399.

Blouin-Demers G, Weatherhead PJ (2002) Implications of movement patterns for gene flow in black rat snakes (*Elaphe obsoleta*). *Canadian Journal of Zoology*, **80**, 1162–1172.

Colli G, Peres A, Zatz M (1997) Foraging mode and reproductive seasonality in tropical lizards. *Journal of Herpetology*, **31**, 490–499.

Cooper W, Vitt L, Caldwell J, Fox S (2001) Foraging modes of some American lizards: relationships among measurement variables and discreteness of modes. *Herpetologica*, **57**, 65–76.

Davis JM, Stamps JA (2004) The effect of natal experience on habitat preferences. *Trends in Ecology and Evolution*, 19, 411–416.

Driezen K, Adriaensen F, Rondinini C *et al.* (2007) Evaluating least-cost model predictions with empirical dispersal data: A case-study using radiotracking data of hedgehogs (*Erinaceus europaeus*). *Ecological Modelling*, **209**, 314–322.

Duarte MO, Freitas TMS, Prudente ALC (2015) Polychromatism of populations of *Corallus hortulanus* (Squamata: Boidae) from the Southern Amazon Basin, Brazil. *Acta Amazonica*, **45(4)**, 373–382.

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.

Dudaniec RY, Rhodes JR, Worthington Wilmer J *et al*. (2013) Using multilevel models to identify drivers of landscape-genetic structure among management areas. *Molecular Ecology*, **22**, 3752–3765.

Feder JL, Forbes AA (2007) Habitat avoidance and speciation for phytophagous insect specialists. *Functional Ecology*, **21**, 585–597.

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.

Fraga R, Lima AP, Magnusson WE (2011) Mesoscale spatial ecology of a tropical snake assemblage: the width of riparian corridors in central Amazonia. *Herpetological Journal*, **21**, 51–57.

Fraga R, Magnusson WE, Abrahão CR et al. (2013b) Habitat selection by Bothrops atrox (Serpentes: Viperidae) in central Amazonia, Brazil. *Copeia*, **2013(4)**, 684–690.

Fraga R, Lima AP, Prudente ALC, Magnusson WE (2013a) Guide to the snakes of the Manaus region – central Amazonia. Manaus: INPA. pp 303.

Fraga R, Stow AJ, Magnusson WE *et al*. (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/jornal.pone.0105453.

Fraga R, Schietti J, Emílio T, Ferrão M, Stow AJ, Magnusson WE, Lima AP (*in press*) An assessment of regional snake diversity in the Amazon using a multi-dimensional approach. *PloS ONE*, submitted.

Freeland JR, Biss P, Conrad KF, Silvertown J (2010) Selection pressures have caused genome-wide population differentiation of Anthoxanthum odoratum despite the potential for high gene flow. *Journal of Evolutionary Biology*, **23**, 776-782.

Frichot E, François O (2015) LEA: an R package for landscape and ecological association studies. *In press*.

Funk DJ, Egan SP, Nosil P (2011) Isolation by adaptation in Nechlamisus leaf beetles: host-related selection promotes neutral genomic divergence. *Molecular Ecology*, **20**, 4671-4682.

Haffer J (1969) Speciation in Amazonian forest birds. *Science*, **165**, 131–137.

Harper GR, Pfennig DW (2008) Selection overrides gene flow to break down maladaptive mimicry. *Nature*, **451**, 1103–1107.

Henderson, RW (1997). A taxonomic review of the *Corallus hortulanus* complex of neotropical tree boas. *Caribbean Journal of Science*, **33**, 198–221.

Hijmans RJ (2015). raster: Geographic Data Analysis and Modeling. R package version 2.3-40. Available at http://CRAN.R-project.org/package=raster

Hilton G, Houston D, Barton N, Furness R, Ruxton G (1999) Ecological constraints on digestive physiology in carnivorous and piscivorous birds. Journal of *Experimental Zoology*, **283**, 365–376.

Hoorn C, Wesselingh FP, ter Steege H *et al*. (2010) Amazonia trough time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, **330**, 927–931.

Jaccoud D, Peng K, Feinstein D *et al.* (2001) Diversity Arrays: a solid state technology for sequence information independente genotyping. *Nucleic Acids Research*, **29(4)**, e25.

Kilian A, Wenzl P, Huttner E *et al*. (2012) Diversity Arrays Technology: a generic genome profiling technology on open plattforms. In: *Data production and Analysis in population genomics – Methods and protocols* (eds Pompanon F, Bonin A). pp. 67–89, Humana Press, Grenoble.

Koen EL, Bowman J, Garroway CJ *et al*. (2012) Landscape resistance and American marten gene flow. *Landscape Ecology*, **27**, 29–43.

Konar AK, Monak BK (2010) Socialising snake society: an indian instance. *Social Change*, **40(2)**, 157–174.

Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity. *Molecular Ecology*, **19**, 3038–3051.

Madsen T, Stille B, Shine R (1996) Inbreeding depression in an isolated population of adders *Vipera berus*. *Biological Conservation*, **75**, 113–118.

Magnusson WE, Braga-Neto R, Pezzini F *et al*. (2013) *Biodiversity and Integrated Environmental Monitoring*. Áttema, Manaus, 356 pp.

Manthey JD, Moyle RG (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 RR, Gonzales A, Millien V (2014) Landscape resistance and habitat combine to provide an optimal model of genetic structure and connectivity at the ramge margin of a small mammal. *Molecular Ecology*, **23**, 3983–3998.

Martins M, Oliveira ME (1999) Natural history of snakes in forests in the Manaus region, Central Amazonia, Brazil. *Herpetological Natural History*, **6(2)**, 78–150.

McRae BH (2006) Isolation by resistance. *Evolution*, **60**, 1551–1561.

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences*, **104**, 19885–19890.

McRae BH, Dickson BG, Keitt TH *et al.* (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology*, **89**, 2712–2724.

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

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excell. Population genetic software for teaching and research-an update. *Bioinformatics*, **28**, 2537–2539.

Peterman WE (2014) ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms. *bioRxiv*, doi 10.1101/007575.

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.

Petroli CD, Sansaloni CP, Carling J *et al*. (2012) Genomic characterization od DArT markers based on high-density linkage analysis and physical mapping to the *Eucalyptus* genome. *PLoS ONE*, **7**, e44684.

Pew J, Muir, PH, Wang J *et al*. (2015) related: an R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology*, **15**, 557–561.

Prior KA, Gibbs HL, Weatherhead, PJ (1997) Population genetic structure in the Black Rat Snake: implications for management. *Conservation Biology*, **5**, 1147–1158.

Prudente ALC, Silva MAA, Rocha WA *et al*. (2008) Morphological variation in *Xenoxybelis boulengeri* (Procter, 1923)(Serpentes, Xenodontinae, Philodryadini). *Zootaxa*, **1743**, 53–61.

R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ribas CC, Aleixo A, Nogueira ACR, Miyaki CY, Cracraft J (2011) A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society of London*, doi: 10.1098/rspb.2011.1120.

Ritland K (1996) Estimators for pairwise relatedness and inbreeding coefficients. *Genetical Research*, **67**, 175–186.

Rosenblum EB, Harmon LJ (2011) "Same same but different": replicated ecological speciation at White sands. *Evolution*, **65**, 946–960.

Sansaloni C, Petroli C, Jaccoud D *et al*. (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**, P54.

Schwaner TD (1990) Geographic variation in scale and skeleton anomalies of tiger snakes (Elapidae: *Notechis scutatus-ater* complex) in Southern Australia. *Copeia*, **1990**, 1168–1173.

Shirk AJ, Wallin DO, Cushman SA *et al*. (2010) Inferring landscape effects on gene flow: a new model selection framework. *Molecular Ecology*, **19**, 3603–3619.

Smith TB, Calsbeek R, Wayne RK, Holder KH, Pires D, Bardeleben C (2005) Testing alternative mechanisms of evolutionary divergence in an African rain forest passerine bird. *Journal of Evolutionary Biology*, **18**, 257–268.

Steen D (2010) Snakes in the grass: secretive natural histories defy both conventional and progressive statistics. *Herpetological Conservation and Biology*, **5(2)**, 183–188.

Stevens VM, Polus E, Wesselingh RA, Schtickzelle N, Baguette M (2005) Quantifying functional connectivity: experimental evidence for patch-specific resistance in the Natterjack toad (*Bufo calamita*). *Landscape Ecology*, **19**, 829–842.

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.

Uetz P, Hošek J (eds.)(2015) The reptile database – http://www.reptile-database.org. Acessed April 15, 2016.

Wahlund S (1928) Zusammensetzung von Population und Korrelationserscheinung vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas* **11**, 65–106.

Wallace AR (1852) On the monkeys of the Amazon. *Proceedings of the Zoological Society of London*, **20**, 107–110.

Wang IJ, Bradburd GS (2015) Isolation by environment. *Molecular Ecology*, **23**, 5649–5662.

Wang IJ, Savage WK, Shaffer B (2009) Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (*Ambystoma californiense*). *Molecular Ecology*, **18**, 1365–1374.

Willette T, Cooney R, Hyer K (1999) Predator foraging mode shifts affecting mortality of juvenile fishes during the subarctic spring bloom. Canadian Journal of Fisheries and Aquatic Sciences, **56**, 364–376.

Willings 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(8)**, e42649, doi: 10.1371/jornal.pone.0042649

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

Supplementary material (S1)

Appendix 1. Methods for DartSeq™ used by Diversity Arrays Technology (Canberra, Australia) to discover and genotype SNPs of snakes from the Amazon.

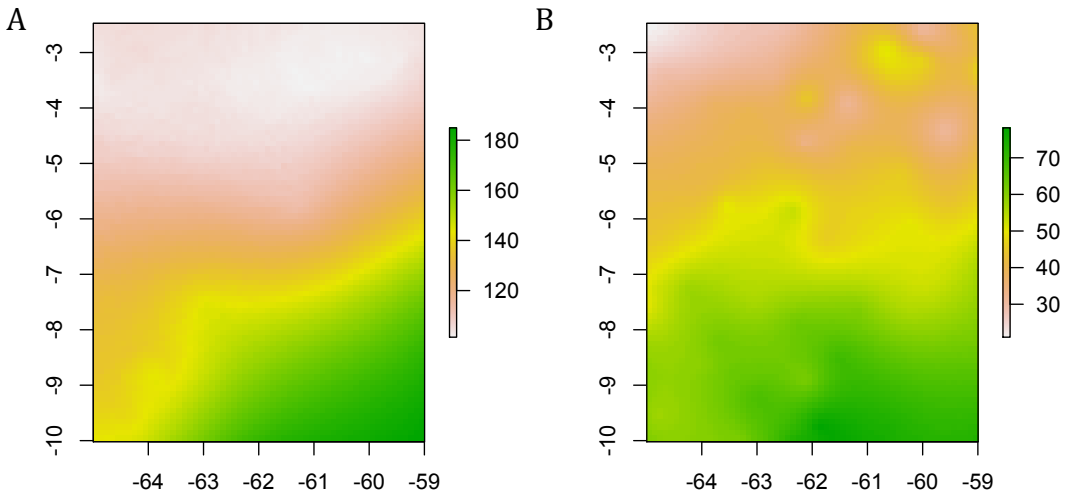
Template DNA (0.5µl) was incubated in a 1X solution of Multi-CoreTM restriction enzyme (RE) buffer (Promega) at 37°C for 2 hours to check genomic DNA quality. Approximately 100ng per µL of each sample was digested with a combination of PstI and SphI restriction enzymes. PstI and Sphl adapters and unique barcodes were ligated to each sample. Each sample was amplified using a PCR primers specific to barcode and adaptor sequences. The PCR conditions were as follows: 1 min initial denaturation at 94 C°, followed by 30 cycles of 20 sec denaturation (94 °C), 30 sec annealing (58 °C) and 45 sec extension (72 °C), and a final extension of 7 min at 72 °C. Using approximately 10 µL of each sample, all samples were pooled, diluted and denatured using NaOH in preparation for hybridization to the flow cell. The library was sequenced on an Illumina HiSeq®2500 platform (single read) using 77 cycles, resulting in fragments 77bp long. A proportion of the samples (>40%) were processed again through the whole library preparation protocol and downstream analysis to create the set of technical replicates which were used to assess the reproducibility of SNPs calls.

Quality control and initial SNP calling

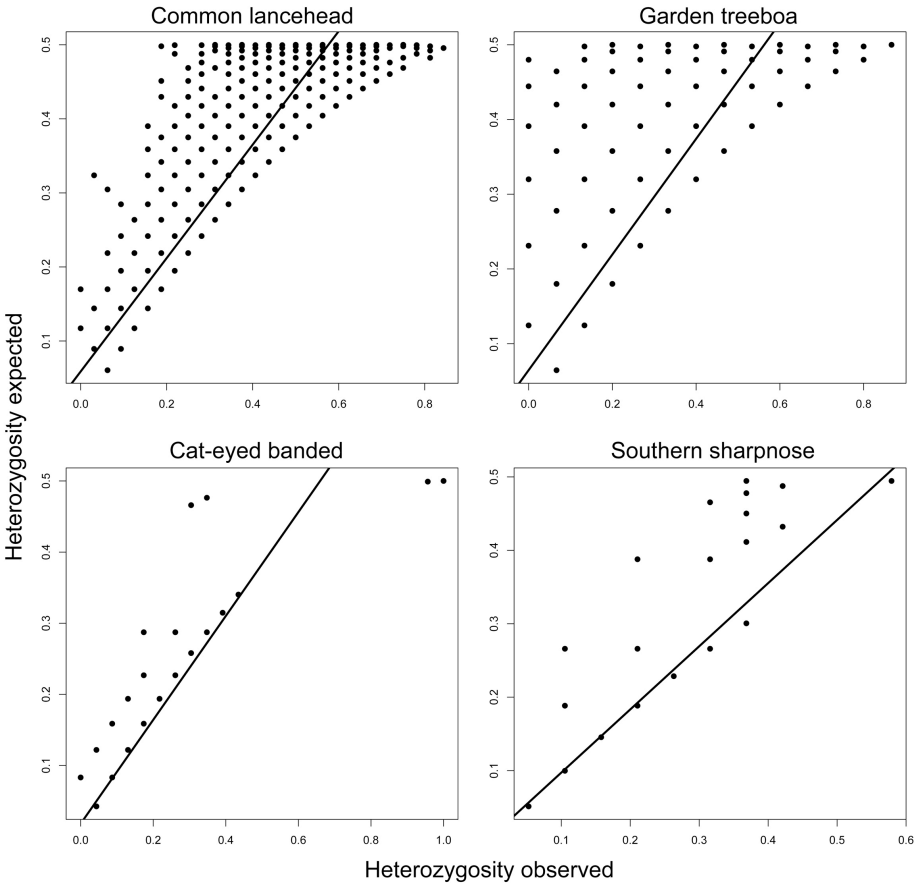
Libraries sequenced were converted to .fastq files, using the Illumina HiSeq®2500 software, and individuals were de-multiplexed based on the ligated barcode. Each read was assessed using PHRED (Ewing and Green, 1998) quality scores (Q-scores), and any read containing Q-scores <25 was removed. All reads were checked against the DArT database

and GenBank viral and bacterial sequences to identify potential contaminations. Following this primary workflow, SNPs were identified and called following the standard procedure in DArT proprietary pipeline DArTSoft14TM (Diversity Arrays Technology). The pipeline workflow is technically similar to the commonly used STACKS pipeline (Catchen et al., 2013), yet it differs from it as sequence clusters are first called for all pooled samples prior to be called for each individual. The SNPs were called only if they were present in both homozygous and heterozygous forms. DArT pipeline also removes any locus with very high read depth, retained only SNP with high balance between allele read depth and depth read depth (average ratio of read depth between alleles= 0.75), reproducibility of >90% and minimum read depth of 5.

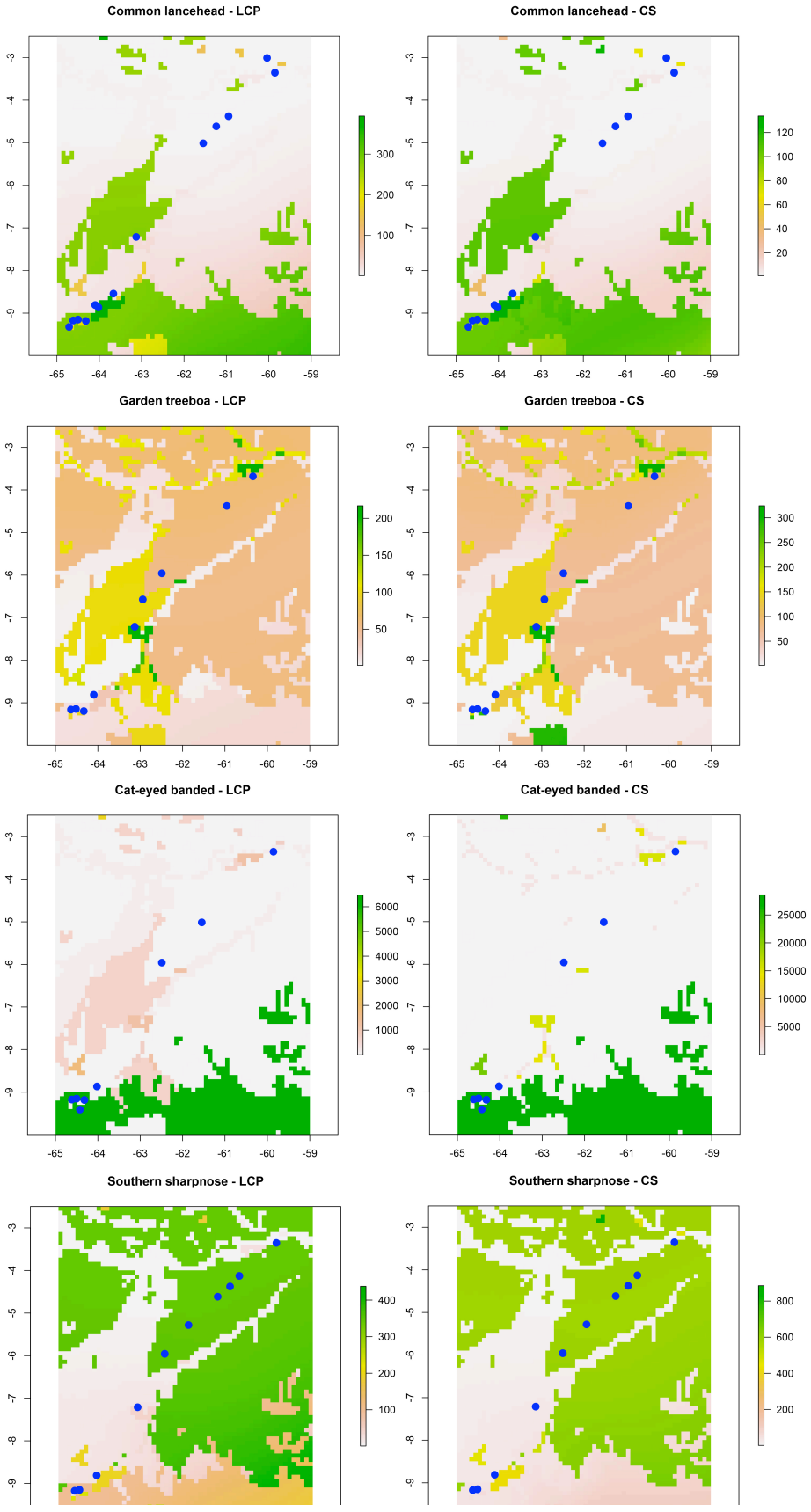
Appendix 2. Climatic variables used to optimize multiple resistance surfaces for four snake species from the central-southwestern Amazon basin, Brazil. A – seasonality in temperature, B – seasonality in rainfall. Available at www.dpi.inpe.br/Ambdata



Appendix 3. Relationships between heterozygosity expected (He) and heterozygosity observed (Ho) for four snake species from the Amazon. The scatterplots on top show He/Ho in ambush predators and the bottom scatterplots show He/Ho in active foragers.



Appendix 4. Multiple resistance surfaces optimized in ResistanceGA based on vegetation cover type, seasonality in temperature and seasonality in rainfall. Resistance scores were obtained by Least Cost Path (LCP) and Circuitscape (CS). Blue circles show sampling sites.



Conclusões gerais

Embora amostrar serpentes para avaliações de impacto ambiental seja frequentemente obrigatório, os altos custos (U$ 120 por indivíduo) associados às baixas probabilidades de detecção da maioria das espécies, bem como a subjetividade na tomada de decisões em relação à redução no esforço amostral, tornam esse grupo pouco eficiente para estudos que sustentam avaliações de impacto ambiental. Porque esses estudos são geralmente baseados em amostragem de curto prazo e com número relativamente baixo de unidades amostrais, é esperado que os padrões ecológicos determinados sejam fortemente enviesados por falsas ausências de espécies em unidades amostrais (Gu e Swihart, 2003). Alternativamente, nós sugerimos que estudos de impacto ambiental em curto prazo sejam focados em grupos de organismos que simultaneamente reflitam medidas robustas de diversidade e sejam relativamente fáceis de amostrar, como aves de sub-bosque (Bibby, 1999) e besouros rola-bosta (Spector, 2006). O dinheiro economizado pode ser investido em aumentar a escala espacial do estudo, o que geralmente é um fator limitante na tomada de decisões sobre conservação (Magnusson et al., 2013).

Estimativas de complementaridades e redundâncias bióticas entre unidades amostrais (β-diversidade) mostraram que a diversidade taxonômica de serpentes é filtrada ao longo da área de estudo (Manaus a Porto Velho) por gradientes de densidade de árvores, altitude e porcentagem de argila no solo. Apesar de que a maioria das espécies encontradas nesse estudo é de ampla distribuição na bacia Amazônica (veja Jorge da Silva e Sites, 1995), a variação em elementos de paisagens heterogêneas gera padrões regionais de co-ocorrência de espécies. No entanto, unidades amostrais distribuídas ao longo de 880 km de floresta são essencialmente redundantes entre si em termos de diversidade funcional e filogenética, independentemente da variação em amplitudes regionais de gradientes ecológicos. Esses resultados são contrastantes com estudos anteriores (Burbrink et al., 2015; Cavalheri et al., 2015), mas são fortemente sustentados pela alta correlação entre medidas de diversidade funcional e filogenética, e nenhuma evidência de superdispersão em traços funcionais e filogenias. Esses resultados mostram que fatores abióticos (*e.g.* gradientes ecológicos) e bióticos (*e.g.* competição interespecífica) cumprem um papel menor na composição de comunidades de serpentes, definida por diversidade funcional e filogenética. Em última análise, os resultados desse estudo destacam a importância de investigar diferentes medidas de diversidade em estudos sobre ecologia e conservação, porque abordagens multidimensionais são claramente mais informativas que as tradicionais medidas de α-diversidade como número de espécies por área.

Padrões de diversidade regional podem ser melhor interpretados pela incorporação de traços biológicos em modelos preditivos, como a capacidade de dispersão (Peterman et al., 2014). Por exemplo, pela comparação de proporções de locus gênicos que desviam do equilíbrio de Hardy-Weinberg e modelos lineares relacionando distância genética, distância geográfica e custo ambiental, nós mostramos que o modo de forrageio é um fator relevante determinando estruturação genética em serpentes, e consequentemente mecanismos que geram componentes de diversidade em escala regional. Predadores senta-e-espera tendem a se deslocar menos, e portanto têm dispersão limitada ao longo de gerações, o que gera estruturação genética determinada por deriva gênica e demografia localmente isoladas por distância geográfica e resistência ambiental. Contrariamente, nós não encontramos nenhuma evidência de estruturação genética em forrageadores ativos, os quais têm altos níveis de fluxo gênico entre indivíduos, independentemente de distância geográfica e heterogeneidade ambiental. Esses resultados têm ampla aplicação em conservação, porque sustentam a relevância da conectividade entre hábitats para a manutenção de níveis heterozigosidade associados ao modo de forrageio. Redução em heterozigosidade é um resultado direto da perda de hábitat, e pode ter consequências drásticas para serpentes, como aumento na frequência de doenças (Schwaner, 1990).

Os estudos desta tese são parte de uma nova tendência em ecologia de serpentes (*e.g.* Fraga et al., 2011; Fraga et al., 2013; Cavalheri et al., 2015), a qual investiga relações espécies-habitats usando métodos mais informativos do que a tradicional abordagem de contagem de espécies por área. Considerando que os estudos apresentados aqui são baseados em dados padronizados, esta tese é parte de objetivos maiores em ecologia e conservação na Amazônia, os quais propõem a integração de conjuntos de dados para diferentes grupos taxonômicos (veja Magnusson et al., 2013). Esse parece ser um caminho eficiente para a ciência cumprir o seu papel na tomada de decisões sobre o uso da terra, que por sua vez tem crescentemente ameaçado a vida selvagem nas florestas tropicais.

Referências

Bibby CJ (1999) Making the most of birds as environmental indicators. *Ostrich* 70: 81–88.

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: 722–734.

Burbrink FT, Myers EA (2015) Both traits and phylogenetic history influence community structure in snakes over steep environmental gradients. Ecography 38: 001–013.

Cavalheri H, Both C, Martins M (2015) The interplay between environmental filtering and spatial processes in structuring communities: the case of Neotropical snake communities. *PloS ONE* 10(6): e0127959. doi: 10.1371/jornal.pone.0127959.

Costa FRC, Magnusson WE, Luizão R (2005) Mesoscale distribution patterns of undertorey herbs in relation to topography, soil and watersheds. *Journal of Ecology* 93: 863–878.

Drucker DP, Costa FRC, Magnusson WE (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.

Espírito-Santo HMV, Magnusson WE, Zuanon J, Mendonça FP, Landeiro VL (2009) Seasonal variation in the composition of fish assemblages in small Amazonian forest streams: evidence for predictable changes. *Freshwater Biology*, 54: 536–548.

Fraga R, Lima AP, Magnusson WE (2011) Mesoscale spatial ecology of a tropical snake assemblage: the width of riparian corridors in central Amazonia. *Herpetological Journal* 21: 51–57.

Fraga R, Magnusson WE, Abrahão CR, Sanaiotti T, Lima AP (2013) Habitat selection by *Bothrops atrox* (Serpentes: Viperidae) in central Amazonia, Brazil. *Copeia* 2013(4): 684–690.

Fraga R, Stow AJ, Magnusson WE, Lima AP (2014) The costs of evaluating species densities and composition of snakes to assess development impacts in Amazonia. *PloS ONE* 9(8): e105453.

Franklin E, Aguiar NO, Soares EDL (2008) Invertebrados do solo. **In:** Oliveira ML, Baccaro FB, Braga-Neto R, Magnusson WE (eds). *Reserva Ducke: A biodiversidade através de uma grade*. Áttema Design Editorial, Manaus. Pp 109–122.

Grass I, Brandl R, Botzat A, Neuschulz EL, Farwig N (2015) Contrasting taxonomic and phylogenetic diversity responses to forest modifications: comparisons of taxa and successive plant life stages in South African scarp forest. *PloS ONE* 10(2): e0118722.

Gu W, Swihart RK (2003) Absent or undetected? Effects of non-detection of species occurance on wildlife-habitats models. Biol Conserv 116: 195–203.

Hellberg ME (1996) Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution* 50(3): 1167–1175.

Jorge da Silva N, Sites JW (1995) Patterns of diversity of Neotropical squamate reptile species with emphasis on the Brazilian Amazon and the conservation potential of indigenous reserves. *Conservation Biology* 9(4): 873–901.

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. *Evolution Biology* 40: 228–245.

Linnaeus C (1758) *Systema naturæ per regna tria naturaæ, secundum classes, ordines, genera, species, cum characteribus, differentilis, synonymis, locis.* Tomus I, editio decima, reformata. Laurentii Salvii, Holmiæ. 10th ed: 824pp.

Lobão PSP (2008) *Associações no uso de hábitat por cinco espécies de lagartos amazônicos*. Master thesis. Instituto Nacional de Pesquisas da Amazônia, Manaus.

Magnusson WE, Braga-Neto R, Pezzini F, Baccaro F, Bergallo H, Penha J, Rodrigues D, Verdade LM, Lima A, Albernaz AL, Hero J-M, Lawson B, Castilho C, Drucker D, Franklin E, Mendonça F, Costa F, Galdino G, Castley G, Zuannon J, Vale J, Santos JLC, Luizão R, Cintra R, Barbosa RI, Lisboa A, Koblitz RV, Cunha CN, Pontes ARMP (2013) *Biodiversity and Integrated Environmental Monitoring*. Áttema Design Editorial, Manaus.

Mendes-Pontes AR, Sanaiotti T, Magnusson WE (2008) Mamíferos de grande porte. **In:** Oliveira ML, Baccaro FB, Braga-Neto R, Magnusson WE (eds). *Reserva Ducke: A biodiversidade através de uma grade*. Áttema Design Editorial, Manaus. Pp 51–61.

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: 539–547.

Meynard CN, Devictor V, Mouillot D, Thuiller W, Jiguet F, Mouquet N (2011) Beyond taxonomic diversity patterns: how do α, β and γ components of bird functional and phylogenetic diversity respond to environmental gradients across France? *Global Ecology and Biogeography* 20: 893–903.

Milcu A, Allan E, Roscher C, Jenkins T, Meyer ST, Flynn D, Bessler H, Buscot F, Engels C, Gubsch, König S, Lipowsky A, Loranger J, Renker C, Scherber C, Schmid B, Thébault E, Wubet T, Weisser WW, Scheu S, Eisenhauer (2013) Functionally and phylogenetically diverse plant communities key to soil biota. *Ecology* 94(8): 1878–1885.

Moraes LFP (2008) *Diversidade beta em comunidades de lagartos em duas ecorregiões distintas na Amazônia*. Masters thesis. Instituto Nacional de Pesquisas da Amazônia, Manaus.

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.

Pressey RL, Humphries CJ, Margules CR, Vane-Wright RI, Williams PH (1993) Beyond opportunism: key principles for systematic reserve selection. *Trends in Ecology and Evolution* 8: 124–128.

Ribeiro-Jr JW, Lima AP, Magnusson WE (2012) The effect of riparian zones on species diversity of frogs in Amazonian forests. *Copeia* 2012(3): 375–381.

Rojas-Ahumada DP, Landeiro VL, Menin M (2012) Role of environmental and spatial processes in structuring anuran communities across a tropical rainforest. *Austral Ecology* 37: 865–873.

Schwaner TD (1990) Geographic variation in scale and skeleton anomalies of tiger snakes (Elapidae: Notechis scutatus-ater complex) in Southern Australia. *Copeia* 1990: 1168–1173.

Silva AP, Marques SR (2010) Morcegos. **In:** Fernandes IM, Signor CA, Penha J (eds). *Biodiversidade no Pantanal de Poconé.* Áttema Design Editorial, Manaus.

Spector S (2006) Scarabaeinae dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae): an invertebrate focal taxon for biodiversity research and conservation. *Coleoptera Bulletin* 60: 71–83.

Steen D (2010) Snakes in the grass: secretive natural histories defy both conventional and progressive statistics. *Herpetological Conservation and Biology* 5(2): 183–188.

Tilman D (2001) Functional diversity. **In:** Levin SA (ed). *Encyclopedia of Biodiversity*. Academic Press, Amsterdam. Pp 109–121.

Wang IJ, Bradburd GS (2015) Isolation by environment. *Molecular Ecology* 23: 5649–5662.

Wright S (1943) Isolation by distance. *Genetics* 28: 114–138.