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UMA ABORDAGEM MOLECULAR DA FILOGEOGRAFIA E ESTRUTURAÇÃO GENÉTICA DE MORCEGOS FILOSTOMÍDEOS NEOTROPICAIS

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Tese apresentada ao Programa de Pós-Graduação em Genética da Universidade Federal de Pernambuco como parte dos requisitos exigidos para obtenção do título de Doutor em Genética.

Orientadora: Profa. Dra. Neide Santos
Coorientadores: Prof. Dr. Martin A. Montes
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Dedico este trabalho aos meus pais, pois ao longo da vida não mediram esforços para que eu chegasse até aqui.

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RESUMO

A Região Neotropical sofreu uma complexa dispersão de biota, contudo estudos biogeográficos estão auxiliando o entendimento desta dinâmica, facilitados pelo advento das técnicas moleculares. O neotrópico abriga a maior riqueza de quirópteros, embora poucas espécies foram estudadas ao nível genético. Este estudo buscou avaliar a filogeografia da espécie *Artibeus planirostris* e a estrutura populacional no nordeste brasileiro de *A. planirostris* e *Carollia perspicillata*. A partir da análise de sequências do gene Citocromo-B foi possível observar um padrão na espécie *A. planirostris*, que geograficamente possui subgrupos resultantes de eventos de colonização. A datação realizada no estudo coincide com os eventos de formação de ambientes. Ao analisar sete populações de três diferentes regiões fitofisionômicas no nordeste (Floresta seca, Floresta Atlântica e Brejo) foi possível, através de marcadores ISSR, verificar uma forte estruturação populacional de *A. planirostris* e *C. perspicillata*. Contudo, *C. perspicillata* apresentou estruturação ao nível de fitofisionomia, fato este não verificado em *A. planirostris*. As duas espécies têm comportamentos de forrageio diferentes, o que justifica o resultado. Os indivíduos de florestas secas mostraram-se mais distintos em relação aos de outros ambientes e mais homogêneos entre eles. Acreditamos que seja devido à história natural destas florestas e o deslocamento nesses locais ser maior pela necessidade de buscar recursos. Este trabalho contribui para o melhor entendimento da evolução e dinâmica de populações naturais de morcegos filostomídeos na região neotropical.

Palavras-chave: Biogeografia. Chiroptera. Cytb. ISSR. Neotropical.

ABSTRACT

Neotropical Region suffered a complex dispersion of biota, however, biogeographic studies are helping with understanding of this dynamic, facilitated by advent of molecular techniques. Neotropics harbor a greater diversity of Chiroptera, although few species have been studied at genetic level. This study aimed to evaluate phylogeography of the specie *Artibeus planirostris* and population structure in northeastern Brazil of *A. planirostris* and *Carollia perspicillata*. From the sequence analysis of Cytochrome-B gene, it was possible to observe a pattern in *A. planirostris* specie, which geographically has subgroups result of colonization events. Dating performed in the study coincides with the formation events of environments. When analyzing seven populations of three different phytophysionomic regions in the northeast (Dry Forest, Atlantic Forest and Brejo), it was possible, through ISSR markers, to verify a strong population structure of *A. planirostris* and *C. perspicillata*. However, *C. perspicillata* presented structure at level of phytophysiognomy, a fact not verified in *A. planirostris*. The two species have different foraging behaviors, which justifies the result. Individuals from dry forests are more different from the ones from other environments and more homogeneous between themselves. We believe it is due to natural history of these forests and the displacement in these areas is greater because of the need to look for resources. This work contributed to a better understanding of evolution and dynamics of natural populations of phyllostomid bats in Neotropical region.

Key-words: Biogeography. Chiroptera. Cytb. ISSR. Neotropical Region.

SUMÁRIO

1 INTRODUÇÃO

A Região Neotropical possui um mosaico de ambientes, esta característica é uma das razões que faz com que possua uma das maiores biodiversidades do planeta. Em meio a esta riqueza, os mamíferos constituem um dos grupos mais importantes, tanto pela sua ampla distribuição geográfica, quanto pela enorme diversidade de ambientes ocupados pelos seus representantes. O grupo dos morcegos pode representar mais da metade da diversidade em levantamentos mastozoológica na região. Sendo estes organismos de grande importância ecológica e econômica, dispersando sementes, polinizando flores, controlando populações de invertebrados e pequenos vertebrados; além de serem vetores de doenças como raiva, ebola, histoplasmose e salmonelose.

Mesmo com o fato dos quirópteros serem um dos grupos de vertebrados mais representativos entre os mamíferos, o conhecimento sobre estes organismos ainda é incompleto e contraditório entre autores, principalmente em relação à sistemática e evolução do grupo. Contudo, estudos genéticos têm auxiliado no melhor entendimento da origem e evolução das espécies, a relação das populações, os efeitos das mudanças geológicas no padrão de distribuição das espécies e na organização das variações inter e intrapopulacionais. Esta questão tem um significado especial na atualidade devido ao fato de que a diversidade biológica está sendo rapidamente perdida por consequência direta ou indireta das atividades humanas.

Desta forma, estudos ao nível molecular dão o suporte necessário para o melhor entendimento da dinâmica de populações naturais. A espécie *Artibeus planirostris* (Spix, 1823) é uma das espécies de morcegos mais bem estudadas entre os mamíferos neotropicais, também considerada uma das com maior distribuição na Região Neotropical. Embora ainda não exista um estudo preciso e extenso acerca de sua filogeografia. E, no nordeste brasileiro, *A. planirostris* e *Carollia perspicillata* (Linnaeus, 1758) estão entre as espécies mais comuns e abundantes, mas pouco se conhece sobre a estrutura destas populações e como o ambiente pode atuar como mecanismo de diferenciação.

Tendo em vista a importância em entender como os eventos naturais da formação da Região Neotropical afetaram a história evolutiva dos morcegos, este trabalho buscou descrever a filogeografia da espécie *A. planirostris* a partir de sequências do gene mitocondrial Citocromo-B. Além disso, o estudo visou avaliar a estruturação populacional desta espécie e de *Carollia perspcillata* em diferentes populações no nordeste do Brasil através de marcadores *Inter-simple sequence repeat* (ISSR).

2 REVISÃO DA LITERATURA

2.1 ORDEM CHIROPTERA

Os morcegos compõem a ordem Chiroptera, do grego *kheir,* mão e *pteron,* asa e são os únicos mamíferos que apresentam voo verdadeiro (Fenton *et al.*, 1992). Os dedos são ligados pela membrana da asa, que consiste de uma dupla camada de pele. Outras adaptações para o voo incluem redução do osso ulna, modificações da cintura escapular, espessamento ou fusão de vértebras torácicas, costelas e de elementos do esterno; além do desenvolvimento de uma quilha no esterno (Gardner, 2007). Algumas espécies podem apresentar fusão de vértebras lombares anteriores e fusão de sétima vértebra cervical com a primeira torácica. Desenvolvimento e especializações de membros traseiros é resultado de adaptações que facilitam as características de voo específicas, como a utilização de locais para dormitório e a coleta de alimentos (Gardner, 2007).

Uma enorme diversidade de hábitos alimentares tem sido encontrada em morcegos, podendo consumir frutos, néctar, pólen, partes florais, folhas, insetos e outros artrópodes (como escorpiões), pequenos peixes, anfíbios (anuros), lagartos, pássaros, pequenos mamíferos (roedores e morcegos) e sangue (Fenton *et al.*, 1992). Estruturas especializadas evoluíram nesse grupo para que pudessem atingir tantas guildas alimentares (Coen, 2002; Sanchez *et al.*, 2012; Gonzales-Terrazas *et al.*, 2016). Esta ampla variedade de hábitos confere aos morcegos grande importância ecológica, pois controlam as populações de insetos, dispersam sementes e polinizam flores (Findley, 1993).

A origem dos quirópteros ocorreu a aproximadamente 65 milhões de anos entre o Cretáceo e o Paleoceno sofrendo uma radiação rápida no Eoceno (Teeling *et al.*, 2005). Esta origem ainda é um tanto obscura pela escassez de registros fosseis, devido ao esqueleto frágil e o ambiente de floresta não ser propício à fossilização (Reis *et al.*, 2011). Os registros fósseis mais antigos são de alguns dentes coletados na França com registro para o Paleoceno que indica uma relação evolutiva com o grupo Eulipotyphla comprovada por análise molecular (Murphy *et al.*, 2001).

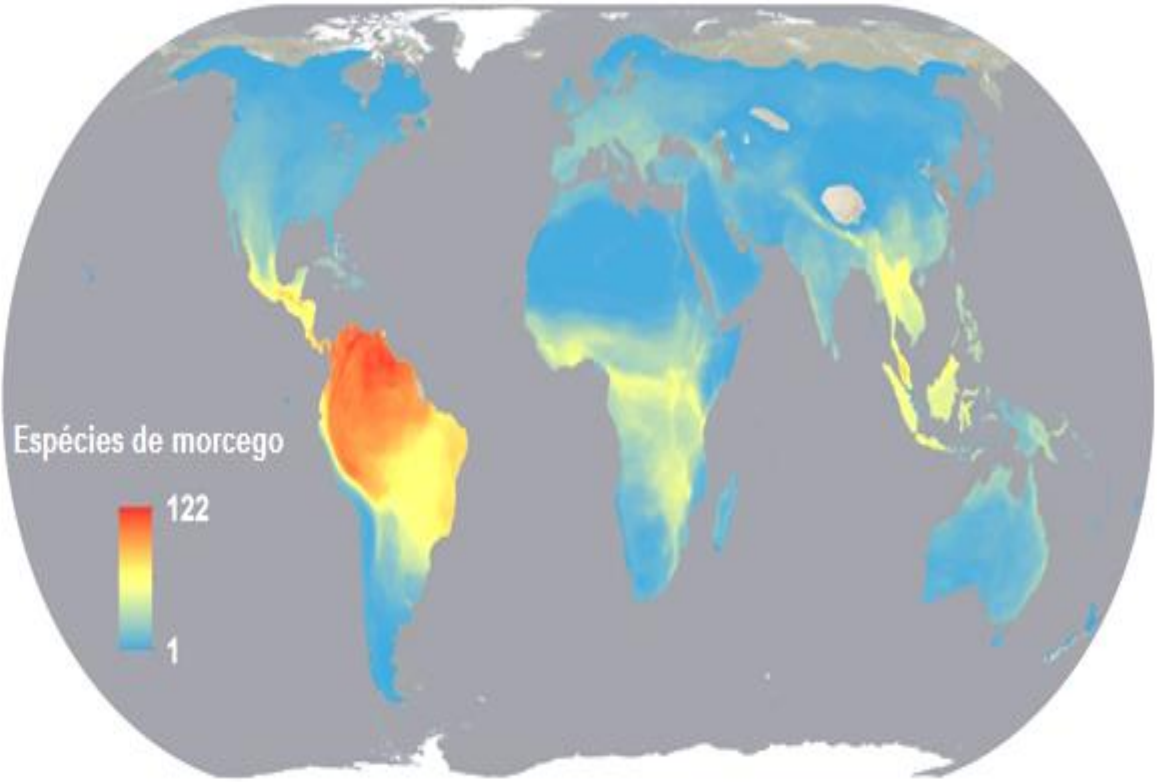
Tradicionalmente, a Ordem Chiroptera subdividia-se em duas subordens: Microchiroptera e Megachiroptera (Koopman, 1993). Os microquirópteros com capacidade de ecolocalização através da emissão de ondas ultrassônicas pela boca ou nariz durante o voo; e os megaquirópetros (morcegos do velho mundo) de maior comprimento, não possuem ecolocalização, mas possuem visão desenvolvida, conhecidos também como “raposas voadoras” (Koopman, 1993). A partir do século XXI trabalhos como o de Jones e Teling (2006) presumiram que a ecolocalização através da laringe seja a forma mais ancestral e que em algum momento esta característica foi perdida (como observado nas “raposas voadoras”), embora outras formas de ecolocalização surgiram por convergência evolutiva. Com base nisso, a evolução dos morcegos foi revista e eles foram divididos em duas subordens, Yinpterochiroptera que abrange as raposas voadoras (superfamília Pteropodidae) e morcegos ecolocalizadores (Superfamília Rhinoliphoidea) e Yangochiroptera com os demais morcegos ecolocalizadores, sendo esta divisão a mais aceita atualmente (Tsagkogeorga *et al.*, 2013).

O sistema de ecolocalização, associado ao voo, é um dos principais responsáveis pela maior diversidade de espécies de morcegos, já que permitiu que explorassem diversos tipos de abrigo e de alimento (Arita e Fenton, 1970; Fenton e Ratcliffe, 2010). Estes organismos habitam diferentes fitofisionomias em todo planeta, além de ser um dos grupos de animais silvestres em maior sinergia com o homem, podendo ser facilmente encontrados em áreas urbanas (Reis *et al.*, 2007).

Esta sinergia tem uma importância socioeconômica, pois os morcegos atuam como reservatórios e vetores de doenças que podem acometer o homem, animais domésticos e silvestres (Bredt e Silva, 1998). Mais de 200 tipos de vírus já foram registrados em morcegos, além de protozoários, fungos e bactérias. Para alguns desses microrganismos foi comprovada a transmissão direta entre morcego e homem como, Raiva, Lyssavirus, Vírus Irkut, Vírus Duvenhage, Ebola, SARS, MERS, Marburg (Allocati *et al.*, 2016). No Brasil é comum a ocorrência de morcegos positivos para o vírus da raiva, independentemente de ser hematófago ou não (Albuquerque *et al.*, 2012; Almeida *et al.*, 2015), do protozoário *Toxoplasma gondii (*Cabral *et al.*, 2014), além dos fungos *Pneumocystis spp*. e *Histoplasma capsulatum* (Veloso *et al.*, 2014).

Dentro da Classe Mammalia, os morcegos são um dos grupos mais diversos, compreendendo aproximadamente 1120 espécies (Simmons, 2005), o que equivale a 22% do total de mamíferos viventes (Wilson e Reeder, 2005). Em muitas áreas das regiões tropicais e subtropicais os quirópteros apresentam uma riqueza de espécies tão significativa que podem representar a maior parte da fauna de mamíferos (Taddei, 1983; Pimm *et al.*, 2014) (Figura 1).

Figura 1 - Distribuição da riqueza de morcegos ao redor do planeta.



Fonte : modificado de Pimm *et al.*, 2014.

De toda a diversidade de quirópteros, a Família Phyllostomidae é a que detém a maior riqueza na Região Neotropical compreendendo seis subfamílias, 44 gêneros e 143 espécies (Gardner, 2007; Tabela I). A etimologia da palavra Phyllostomidae remete a principal característica morfológica que distingue os indivíduos desta Família para outras, que seria a presença de uma membrana nasal em formato de "folha" (Nowark, 1994).

Tabela 1 - Diversidade de subfamílias e gêneros da Família Phyllostomidae na Região Neotropical.

Família	Subfamília	Gênero
Phyllostomidae		
	Carolliinae	
		Carollia
		Rhinophylla
	Desmodontinae	

	Desmodus
	Diaemus
	Diphylla
Glossophaginae	
	Anoura
	Choeroniscus
	Glossophaga
	Leptonycteris
	Lichonycteris
	Scleronycteris
Lonchophyllinae	
	Lionycteris
	Lonchophylla
	Platalina
	Xeronycteris
Phyllostominae	
	Chrotopterus
	Glyphonycteris
	Lampronycteris
	Lonchorhina
	Lophostoma
	Macrophyllum
	Micronycteris
	Mimon
	Neonycteris
	Phylloderma
	Phyllostomus
	Tonatia
	Trachops
	Trinycteris
	Vampyrum
Stenodermatinae	
	Ametrida
	Artibeus
	Centurio
	Chiroderma
	Enchisthenes
	Mesophylla
	Platyrrhinus
	Pygoderma
	Sphaeronycteris
	Sturnira
	Uroderma
	Vampyressa
	Vampyriscus
	Vampyrodes

Fonte: modificado de Gardner, 2007.

Um dos gêneros mais estudados dessa familia é o *Artibeus* (Leach, 1821) considerado um complexo de espécies, pois suas divisões ainda não estão bem resolvidas (Guerrero *et al.*, 2004; Larsen *et al.*, 2007; Redondo *et al.*, 2008). As espécies do gênero estão distribuídas em toda região Neotropical compreendendo pelo menos 10 espécies (Simmons, 2005). Estas espécies têm sido usadas em pesquisas de diversos campos da ciência: biogeografia, ecologia, genética, morfologia, comportamento e fisiologia (Studier *et al.*, 1983; Melo *et al.*, 2012; Puga *et al.*, 2013; Ferreira *et al.*, 2014; Garcia *et al.*, 2014; Gutiérrez *et al.*, 2014a; Carvalho-Neto *et al*., 2016).

A espécie *Artibeus planirostris* destaca-se pela ampla distribuição e versatilidade ambiental ocupada. Este táxon é frugívoro e ocorre em grande parte do continente (Argentina, Bolívia, Brasil, Colômbia, Equador, Guiana Francesa, Guiana, Paraguai, Peru, Venezuela e Suriname) além das Antilhas, possui pelagem acinzentada e listas faciais mais claras e discretas possui antebraço entre 62 a 73 mm (Figura 2) (Hollis, 2005; Gardner, 2007; Reis *et al.*, 2007; Garcia *et al.,* 2014; Carvalho-Neto *et al.*, 2016).

Figura 2 - Espécime de *A. planirostris* coletado com rede de neblina em Serra Talhada-PE

Fonte: acervo pessoal.

Além do gênero *Artibeus*, o gênero *Carollia* Gray 1838 exerce uma forte presença em estudos desenvolvidos na região Neotropical em diferentes áreas, como por exemplo, ecologia (Fischer *et al.*, 2014), comportamento (Fasel *et al.*, 2017), estrutura genética (Ripperger *et al.*, 2014), dispersão de sementes (Salazar *et al.*, 2013) e filogenia (Hoffman e Baker, 2003). Sendo a espécie *C. perspicillata* (Linnaeus, 1758) a mais bem distribuída na região ocorrendo do México ao Peur, Bolívia, Paraguai, Brazil e Guianas, além das Antilhas (Gardner, 2007, Reis *et al.*, 2011). É um animal com pelagem amarronzada, seu lábio inferior é em formato de "V" com uma verruga maior no centro e verrugas menores circundando o lábio, com antebraço variando entre 38 e 44 mm. Alimenta-se predominantemente com vegetais complementando a dieta com insetos e néctar (Figura 3) (Ortêncio-Filho *et al.*, 2007).

Figura 3 - Espécime de *Carollia perspicillata* coletado em Igarassu-PE.

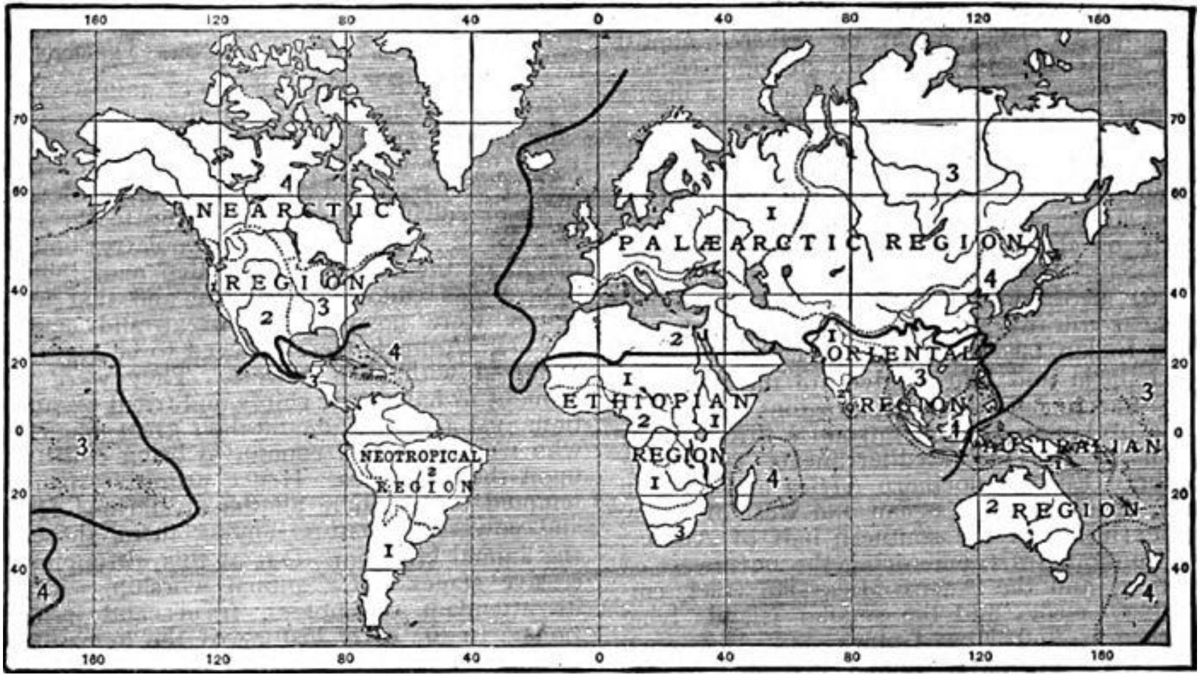
Fonte: acervo pessoal.

2.2 REGIÃO NEOTROPICAL

Discussões e estudos em diversas linhas foram necessários para entender a história da Região Neotropical. Sclater (1858, *apud* Morrone, 2014) a partir de pesquisa com aves, dividiu o planeta em seis sub-regiões que possuíam características faunísticas distintas (Figura 4). Após aplicar esta divisão em outros vertebrados, Wallace (1876) aceita este esquema proposto. De acordo com os dois autores, a Região Neotropical compreende desde o sul do México até a Terra do Fogo, também compreendendo as pequenas Antilhas, Galápagos e Ilhas Malvinas. Contudo, não há um consenso entre autores, pois alguns desconsideram a região sudeste da América do sul afirmando haver ligação com outras regiões do planeta como Austrália e África (Amorim e Tozoni, 1994; Morrone, 2002; Moreira-Munoz, 2007).

Além disso, existe um *senso stricto* que exclui a região dos Andes da Região Neotropical. Sendo esta considerada uma zona de sobreposição entre região Neártica e Neotropical (Morrone, 2006).

Figura 4 - As regiões zoogeográficas globais de acordo com Sclater e Wallace.



Fonte: Ingersoll, 1920.

Segundo Morrone (2014) ocorreram seis eventos históricos marcantes nessa região: (1) a ligação entre América do sul e do norte no período jurássico que permitiu a dispersão da biota neotropical a América do norte e vice-versa; (2)

as Antilhas passaram por um processo de vicariância que se iniciou no período Cretáceo; (3) a separação da Região Neotropical em duas partes que seriam o sudeste e o noroeste da América do sul devido à formação de um grande lago pela junção das bacias dos rios Mamoré, Negro e Amazonas no período Cretáceo; (4) a elevação dos Andes que causou uma vicariância na região da Floresta Amazônica que ocorreu desde o cretáceo ao plioceno; (5) a separação da Amazônia causada pela invasão do mar no Plioceno e consequente formação de um mar continental; e (6) a vicariância entre o Chaco paraguaio com a Floresta Atlântica, estando relacionada com a junção das bacias do Paraná e Parnaíba ocorrida no Paleoceno.

Estes eventos tiveram um papel significativo na dispersão da biota na região atuando diretamente, pois a gama de ambientes heterogêneos foi responsável pelos padrões de distribuição geográfica das espécies (Patterson, 2000).

2.2.1 Formação dos Andes e Diagonal Seca Neotropical

O processo causado por subducção de placas tectônicas foi o responsável pela elevação dos Andes, embora não tenha ocorrido em um momento único, mas, sim, em sucessivos eventos ao longo da história que perduram até hoje (Hoorn *et al.*, 2010). O início da elevação dos Andes ocorreu no Cretáceo, todavia um dos momentos de elevação mais conspícua foi durante o mioceno (23-7 milhões de anos) e no Plioceno (~4,5 milhões de anos) (Hoorn *et al.*, 2010; Mora *et al.* 2010).

A elevação dos Andes reconfigurou a área da Amazônia (Garzione *et al.*, 2008), pois a medida que a elevação superou 2km isso levou a alterações climáticas causando aumento na precipitação da porção leste. Esta mudança climática resultou em maior elevação, erosão e sedimentação. Isto pode ser observado na mudança de registro de deposição para as planícies amazônicas e andinas (Strecker *et al.*, 2009; Poulsen *et al.*, 2010). A maior deposição de sedimento associada ao sistema de rios da região formou áreas úmidas de lagos e pântanos chamados “Pebas”, colonizados por molucos, ostracodes e répteis (Wesseling e Salo, 2006; Hoorn *et al.*, 2010). No final do Mioceno a elevação mais conspícua ocorrida acarretou erosões profundas e como consequência formou o delta aluvial amazônico na costa atlântica (Uba *et al.*, 2007). Com o carreamento dos sedimentos para o Atlântico e a regressão do oceano, o Rio Amazonas pôde se estabelecer em 7 milhões de anos (Figueiredo *et al.*, 2010). Outro processo ligado a formação dos Andes é o estabelecimento do istmo do Panamá (3,5 milhões de anos) que permitiu extensiva migração de fauna proveniente da América do Norte (Morrone, 2005).

Outra importante formação ambiental ocorrida na região Neotropical é a diagonal seca (*diagonal dry belt*) caracterizada por baixas precipitações e alta sazonalidade. Esta formação xérica se estende por três domínios: a Caatinga no Nordeste do Brasil; o cerrado na região central do Brasil; e o Chaco no Paraguai, Argentina, Bolívia (Prado e Gibbs, 1993). Os primeiros estudos ecológicos realizados na região da diagonal seca apontaram para um ambiente de pouca diversidade e baixa taxa de endemismo (Vanzolini, 1963). Contudo, na última década, esta formação xerica mostrou-se significante para a diversidade neotropical (Oliveira e Maquis, 2002; Leal *et al.*, 2005; Nogueira *et al.*, 2011; Garcia *et al.*, 2014). Devido sua localização entre a Floresta Atlântica e Amazônica esta região atua diretamente no fluxo e consequentemente evolução de espécies, atuando como uma barreira ecológica para os mesmos (Martins *et al.*, 2009; Pelegrino *et al.*, 2011; Ferreira *et al.*, 2014).

2.3 FLORESTA ATLÂNTICA E SAZONAL SECA NO NORDESTE DO BRASIL

A Região Nordeste do Brasil possui dois importantes biomas: a Floresta Atlântica, com vários ecossistemas associados, e a Caatinga considerada uma Floresta Tropical Sazonal Seca (Seasonally Dry Tropical Forest - SDTF)((Prado e Gibbs, 1993). Em relação à Floresta Atlântica, resta apenas pouco mais de 7% de floresta original, sendo considerada um dos 25 “hotspots” do mundo. Dentro deste domínio são reconhecidas seis sub-regiões: Brejos Nordestinos, Pernambuco, São Francisco, Diamantina, Bahia e Serra do Mar. Além dessas sub-regiões são reconhecidas duas sub-regiões de transição: a Florestas de Interior e as Florestas de Araucária (Silva e Casteleti, 2003; Galindo-Leal e Gusmão-Câmara, 2005)

A sub-região Pernambuco é uma das mais destruídas, apresentando menos de 5% de sua superfície original (Silva e Casteleti, 2003). Dentro desta sub-região a Agência Estadual de Meio Ambiente e Recursos Hídricos reconhece

25 unidades de conservação de proteção integral para a Mata Atlântica, com extensões que variam entre pouco mais de 10 ha e quase 1.100 ha, a maioria delas apresentando uma superfície de aproximadamente 100 ha (CPRH, 2006). Inseridos na Caatinga podemos encontrar os Brejos Nordestinos que são “ilhas” de Mata Atlântica. Aproximadamente 25% da Mata Atlântica da região Nordeste do Brasil está nos Brejos. No Nordeste são reconhecidos 43 Brejos, dos quais 23 estão no Estado de Pernambuco. Dentro dos Brejos localizados em Pernambuco, apenas quatro deles apresentam algum tipo de reserva ecológica destinada a sua proteção: Parque Nacional do Catimbau, Reserva Biológica de Serra Negra, Parque Ecológico João Vasconcelos Sobrinho e RPPN-Fazenda Bituri (Pôrto *et al.*, 2004).

A crença de que as florestas secas são um ambiente pobre levou por muito tempo a exclusão desta região das políticas públicas para o estudo e a conservação da biodiversidade do país. No entanto, vários estudos vêm mostrando que estas abrigam uma alta biodiversidade (Rodal, 1992; Garda, 1996; Silva e Oren, 1997; Oliveira *et al.*, 2003, Garcia *et al.* 2014; Carvalho-Neto *et al.* 2016). Aproximadamente 40% da cobertura florestal tropical e subtropical, correspondem a florestas secas (Pennington *et al.*, 2000).

As SDTFs se estendem de zonas equatoriais a regiões subtropicais do planeta, onde durantes vários meses sofrem severo período de seca com pluviometria anual não ultrapassando de 1600 mm, distingue-se das savanas pela presença de árvores dominadoras, compreendendo, ocasionalmente, um sistema de dosséis (Bullock *et al.*, 1995; Kauffman *et al.*, 2003). A vegetação é predominantemente decídua durante a estação seca, embora nas florestas mais secas haja um aumento acentuado em espécies suculentas (Mooney *et al.*, 1995).

Muitas das florestas secas são reconhecidas como *hotspots* de biodiversidade devido à alta riqueza de espécies, alta taxa de endemismo e destruição de Habitat (Myers *et al.*, 2000; Trejo e Dirzo, 2000). Apesar disso, apresenta menos atenção por parte dos pesquisadores em relação às florestas úmidas. Menos de 20% dos artigos que avaliaram florestas tropicais trabalharam com Florestas secas (Sanchez-Azofeifa *et al.*, 2005).

As SDTFs da região nordeste do Brasil são consideradas criticamente ameaçadas (Trejo e Dirzo, 2000), até mais que as florestas tropicais chuvosas (Gentry, 1995). Podendo o cenário ser ainda pior tendo em vista que avaliações quantitativas do estado de conservação desses remanescentes são extremamente escassas (Trejo e Dirzo, 2000). As causas mais comuns da destruição desses ambientes é a conversão das áreas florestais em pastos e plantações (Maass, 1995).

2.4 MARCADORES MOLECULARES UTILIZADOS EM FILOGENIA E FILOGEOGRAFIA

Os estudos genéticos podem ajudar no conhecimento da diversidade que as espécies possuem e, assim, auxiliar a manter as espécies como entidades dinâmicas capazes de se adaptar às mudanças ambientais. Esta questão é muito importante na atualidade, devido à diversidade biológica está sendo rapidamente perdida por consequência direta ou indireta das atividades humanas. A genética ecológica, como todos os componentes da biologia da conservação, tem como objetivo reduzir a taxa de extinção e preservar a biodiversidade (Frankham *et al.*, 2002). O estudo da biodiversidade, ao nível molecular, de vários organismos em diferentes ambientes nos permite estabelecer que locais possuam maior diversidade genética. Dessa forma, determinando valor de cada ambiente e sua importância como área de conservação do patrimônio genético das espécies investigadas (Galindo-Leal e Gusmão-Câmara, 2005).

Na década de 70 houve o surgimento de metodologias capazes de decifrar as sequências de DNA, como eletroforese, reações de hibridação e sequenciamento, aliadas à implantação da técnica de reação em cadeia da polimerase (PCR) (Mullis e Faloona, 1987). Com isto, os pesquisadores tiveram acesso a uma variedade de tecnologias para utilizar as variações das sequências de DNA do núcleo ou de organelas como marcadores informativos para os mais diversos estudos (Mattevi, 2003).

O estudo de genes nucleares são usado para análise de variação alélica (Schad *et al.*, 2012), padrões de seleção e estrutura populacional (Schad *et al.*, 2011), variação genética em populações (Ricanova *et al.*, 2011), associação com infecção viral e parasitária (Froeschke e Sommer, 2012), análises filogenéticas (Musolf *et al.*, 2004) e estudos de associação com doenças (Ueda *et al.*, 2003). Apesar disso, ao longo das décadas, foram desenvolvidas outras formas de utilização do DNA nuclear como, por exemplo, Inter-Simple Sequence Repeat

(ISSR). Técnica fundamentada no anelamento de oligonucleotídeos iniciadores dentro de microssatélites e amplificação, pela técnica de PCR, das regiões entre microssatélites. A técnica de ISSR apresenta alta taxa de replicabilidade, uma vez que os oligonucleotídeos iniciadores apresentam um local fixo de ligação e uso de âncoras que impedem o mau pareamento, onde se anela no limite do microssatélite (ISSR ancorados). Outra vantagem apresentada por esta técnica é que permite analisar a variabilidade total do genoma sem a necessidade do conhecimento prévio do mesmo (Bornet e Blanchard, 2001).

Nas últimas duas décadas esta técnica tem sido amplamente utilizada em estudos ecológicos e evolutivos para estimar a diversidade genética de diferentes espécies animais e vegetais, determinar estruturação genética, avaliar hibridização e no campo da genética da conservação (Lu et al., 2006; Vijayan et al., 2006; Antunes et al., 2010; Lin et al., 2012; Manrique-Poyato et al., 2013; Quezada et al. 2014; Rodríguez-Rodríguez et al., 2015).

Além de marcadores nucleares, a análise do DNA mitocondrial (DNAmt) vem sendo amplamente realizada. O DNAmt caracteriza-se por ser haplóide, circular, e apresentar tamanho pequeno, aproximadamente 16.500 pares de bases em eucariotos(Shimko *et al.*, 2001). Maior parte do genoma mitocondrial é codificante e acumula substituições de bases, inserções e deleções com uma taxa média de 5 a 10 vezes mais rápida do que o DNA nuclear (Shenkar *et al.*,1996).

Um dos principais genes mitocondiais é *Citocromo B* (CytB), amplamente utilizado em estudos genéticos de espécies naturais. A larga preferência do uso deste gene é justificada pelo fato de que a dinâmica evolutiva e as características do seu produto protéico são bem conhecidos, além de ser um dos genes de vertebrados mais extensamente sequenciado (Johns e Avise, 1998). Os níveis de divergência genética associados a espécies irmãs, de mesmo gênero, gêneros de mesma família e, ainda, grupos da mesma espécie estão em uma faixa em que o gene *CytB* é filogeneticamente informativos. Não sendo comprometido por efeitos de saturação envolvendo substituições de nucleotídeos sobrepostos (Meyer, 1994). Este gene vem sendo utilizado em estudos filogeográficos, filogenéticos, taxonômicos e estruturação populacional (Clancy *et al.*, 2011; Nicolas *et al.*, 2012; Ferreira *et al.*, 2014).

2.5 FILOGEOGRAFIA E ESTRUTURAÇÃO GENÉTICA DE MORCEGOS FILOSTOMÍDEOS

A Biogeografia de espécies, indivíduos ou múltiplas espécies co-distribuídas, avaliada através de métodos moleculares tem sido produtiva e amplamente utilizada para elucidar a diversificação da biota da Terra (Edwards e Beerli, 2000). O Termo Filogeografia, proposto por Avise *et al.* (1987), é o estudo que aborda os princípios e processos que governam a distribuição geográfica de linhagens genealógicas ao nível intra e interespecífico, tendo como objetivo caracterizar essas linhagens. A filogeografia é uma área da Biogeografia em que o estudo se dá por análise de clados monofiléticos inferidos a partir de estudos filogenéticos (Avise, 1994). Estudos nesse campo envolvem o uso de um ou mais marcadores moleculares que possuam caracteres informativos e um dos mais utilizados são os genes por adequar-se bem a examinar variação geográfica genética e fornecem maior resolução a padrões intraespecíficos (Avise, 2000). Com esses dados é possível fazer inferência sobre processos históricos de demografia e migração, importantes para identificar padrões de dispersão (Hickerson *et al.*, 2010). Além disso, informações deste tipo são primordiais na manutenção de projetos de finalidade conservacionistas (Avise, 2009). Contudo, este ainda é um campo de estudo recente que não possui densidade significativa de trabalhos, o que leva a presumir que esta é uma área fértil a novas descobertas.

Organismos que possuem uma distribuição ampla e grande capacidade de dispersão mostram-se verdadeiros desafios para os pesquisadores (Rissler *et al.,* 2010). Os morcegos, devido ao voo, têm uma capacidade de se dispersar por longas distâncias, isso gera um interesse por parte dos biólogos evolucionistas. Potencialmente, eles podem exibir padrões filogeográficos diferentes de outros organismos não alados. Em geral, os morcegos apresentam baixa divergência genética e pouca estrutura genética quando comparada a outros mamíferos, chegando a ser 10 vezes menor do que o valor encontrado para os roedores e marsupiais, para distâncias que são 10 vezes maiores. Este fato estar relacionado à grande capacidade de vagilidade dos morcegos (Ditchfield, 2000).

Segundo Holanda *et al.* (2012) a família Phyllostomidade, em geral, tem um mesmo padrão de dispersão no continente americano. Contudo os vários acidentes geográficos e complexa radiação de espécies que a região possui pode ter causado, em alguns grupos, um padrão específico. Anteriormente a este estudo, Ditchfield (2000) descreveu um padrão filogeográfico geral e estruturação populacional para os morcegos na região neotropical. Neste estudo ele encontrou baixa variação intraespecífica o que foi corroborado por estudos posteriores (Hoffmann e Baker, 2003; Lamb *et al.*, 2012). Mutação, deriva genética e a seleção natural favorecem adaptações as condições ambientais o que acarreta diferenciação intraespecífica. Esta modificação local reflete a estrutura genética de uma espécie. Existem métodos diretos para estimar a estruturação baseada em dados moleculares (Slatikin 1987). Em morcegos não é clara a estruturação genética de várias espécies, mas espécies de morcegos filostomídeos apresentam um padrão de adaptação ligado ao tipo de ambiente ou por isolamentos devido a eventos históricos (Hoffman e Baker, 2003; Martins *et al.*, 2009; Larsen *et al.*, 2010).

Algumas espécies mostraram uma forte estruturação populacional em gradientes ambientais, como por exemplo, *Desmodus rotundus, Artibeus obscurus* e *Carollia perspicillata* (Hoofman e Baker *et al.*, 2003; Martins *et al.*, 2009; Ferreira *et al.*, 2014). Várias espécies se dispersaram para novos habitats em busca de alimento, como ocorreu com o gênero *Carollia* (Hoffman e Baker, 2003). Estudos genéticos serviram para mostrar que espécies como *Artibeus jamaicensis* se dispersaram para o leste, provavelmente por causa do melhor habitat com o surgimento de refúgios florestais durante o Pleistoceno (Holanda *et al.*, 2012). Contudo, o entendimento das diferenciações genéticas ao longo do gradiente espacial das espécies da região ainda não é claro. Além do mais, a compreensão deste tema auxiliará não só no conhecimento da evolução das espécies, mas também a formação histórica da região.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar a diversidade genética das espécies de morcegos *Artibeus planirostris* e C*arollia perspicillata* ao nível inter e intrapopulacional.

3.2 OBJETIVOS ESPECÍFICOS

1. Descrever a filogeografia e avaliar a história demográfica da espécie *A. planirostris*.
2. Entender como a formação dos ambientes neotropicais afetaram a diversificação de *A. planirostris.*
3. Realizar datação dos eventos de vicariância sofridos pela espécie *A. planirostris.*
4. Mensurar a estruturação genética populacional em diferentes ambientes para os morcegos *A. planirostris* e *C. perspicillata.*
5. Relacionar caracteristicas biologicas de *A. planirostris* e *C. perspicillata* com a estruturação genética das populações.

4 CAPÍTULO I

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Running Heading: Phylogeography of the *A. planirostris*

Phylogeography, population genetic structure and demographic history of flat-faced fruit-eating bat *Artibeus planirostris*

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Bats are among the most representative mammals in the Neotropical region, and have gone through various natural history events that promoted the complex dispersal of their species. This group may present distinct evolutive histories in comparison with other representatives of this class. *Artibeus planirostris* is one of the most common bat species in the Neotropical region, with widely geographic distribution. The genetic structure of several species living in that territory was affected by geo-climatic events, such as the Andean uplift, the separation of the Antilles, and the rise of the South American diagonal dry belt, which split the Amazon and the Atlantic Forests in the past. This study evaluated the phylogeographic patterns of *A. planirostris* based on the analysis of 110 cytochrome *b* gene sequences from 41 locations across their entire geographic range. Phylogeographic trees divided the species into two major groups: one in west portion of the Amazon Forest, which separated probably 3.44 Ma.; and another in the eastern region subdivided in two smallest groups along the diagonal dry belt, separated about 2.12 Ma. This geographic structure was strongly supported statistically. The data obtained did not afford to identify vicariance events that separated the Antilles from the South American continent, and the samples collected in these islands were closer genetically to the representatives from the eastern Amazon Forest. AMOVA analysis revealed that 54% of variation is due to differentiation between groups. Our study suggests that the genetic differentiation of *A. planirostris* reflects a colonization model, and such pattern was caused by the geo-climatic modifications of the Andean uplift and interglacial cycles in the Neotropics.

Os morcegos estão entre os animais mais representativos da região Neotropical, tendo sofrido diferentes eventos históricos naturais responsáveis pela complexa dispersão de suas espécies e podem apresentar histórias evolutivas distintas em comparação com outros representantes desta classe. Na região Neotropical *Artibeus planirostris* é uma das espécies de morcegos mais comuns e com maior distribuição geográfica. Neste território várias espécies tiveram sua estrutura genética afetada por eventos geoclimáticos como a elevação da Cordilheira dos Andes, a separação das Antilhas e a diagonal seca na América do Sul, que separou as Florestas Amazônica e Atlântica. Este estudo avaliou os padrões filogeográficos de *A. planirostris* pela análise de 110 sequências do gene mitocondrial citocromo *b* em 41 localidades, cobrindo toda a distribuição geográfica da espécie. As árvores filogeográficas foram suportadas estatisticamente e mostraram uma divisão em dois grupos principais, um incluindo a porção oeste da Floresta Amazônica e os outros no lado leste com as demais amostras, com provável separação há 3.44 Ma. O grupo do lado leste se subdividiu em dois pela diagonal seca há aproximadamente 2.12 Ma. Os dados não permitiram identificar o evento de vicariância que separou as Antilhas do continente sul americano, sendo as amostras destas ilhas geneticamente mais próximas de representantes do leste da Floresta Amazônica. A AMOVA revelou que 54% da variação devem-se à diferenciação entre grupos. Nosso estudo sugere que a diferenciação genética de *A. planirostris* reflete um modelo de colonização causado pelas modificações geoclimáticas da elevação dos Andes e ciclos interglaciais.

Key words**:** ABC, Biogeography, CytB, Neotropical region, mtDNA.

Widely distributed species that have adapted to different environments are suitable models in studies that aim to elucidate diversification and biogeographic patterns. According with Perez et al. (2016), one of the most intriguing questions that remain unanswered in biogeography for the last decades is the duality between vicariance and colorization. Bioclimatic and landscape features may play an important role and can affect drastically the biology of many species, forcing their dispersion (Rolland et al. 2015). Additionally, territories posing physical obstacles that may have restricted gene flow between populations in the past are especially interesting in this kind of studies (Martins et al. 2009).

In this context, bats are an interesting group for biogeographic investigations. Chiroptera order, comprehend these animals is one of the planet's most abundant and diversified groups of mammals, living in all continents of the globe. This order includes 18 families, 202 genera, and approximately 1,120 species (Simmons 2005). Chiropterans represent roughly one fourth of the world's mammal species (Wilson and Reeder 2005), and about half of this class's richness lives in Neotropical forests (Simmons and Voss 1998). These organisms play a key role in ecosystem balance, helping to control insect populations, dispersing seeds, and pollinating flowers (Findley 1993; Muchhala 2013). Furthermore, they potentially transmit diseases such as white-nose syndrome, rabies, and histoplasmosis (Bredt and Silva 1998; Foley et al. 2011).

Artibeus (Leach 1821) is distributed in Neotropical region, and comprises more than 10 bat species (Guerrero et al. 2004; Simmons 2005; Larsen et al. 2007; Redondo et al. 2008). *Artibeus planirostris* (Spix 1823) is an ecologically versatile frugivorous species very common in South America being found in the most part of that continent and in Antilles. Their southern distribution limit encompasses Paraguay, southern Brazil and northwest Argentina. A wide and strong distribution gap of *A. planirostris* is observed in central Brazil, where xeromorphic environments like the Cerrado and the Caatinga biomes prevail (Hollis 2005; Gardner 2008; Reis et al. 2007; Garcia et al. 2014; Carvalho-Neto et al. 2016).

The present study investigated the phylogeography, the population genetic structure and molecular data for *A. planirostris.* Different and non-exclusive hypotheses that may explain how distribution of this species was affected by biogeographic events in space and time were evaluated. We considered two different mechanisms as competitive hypothesis: (I) Vicariance processes that promoted the Andean uplift in South America and rising of the Neotropical diagonal dry belt with associated bioclimatic events; or (II) Colonization processes caused by different subpopulations that altered genetic structure during recent bioclimatic cycles of the Earth's recent geological history.

MATERIAL AND METHODS

Sampling and DNA sequencing.— Sequences of *A. planirostris* were obtained from 41 locations across the entire distribution range of the species (Fig. 1). Samples were identified following the key proposed by Gardner [2008] and blood was collected from each individual and stored in tubes containing EDTA (5%) for subsequent analyses. This procedure was approved by *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBIO - license number 12264-1) and local ethics committee on animal use (license number UFRPE 067/2015). DNA extraction was performed according to a modified version of the salting out method described by Medrano et al. (1990). Mitochondrial cytochrome *b* gene (CytB) was partially amplified by polymerase chain reaction (PCR) using primers MVZ5 and MVZ16 (Smith and Patton 1993) in a final reaction volume of 25 µL containing 50-60 ng of DNA, 2 µm of each primer, 0.2 mM DNTP, 1.5 mM $MgCl_2$, 2.5 µL 1X reaction buffer, and 1.25 U Taq DNA polymerase (Invitrogen kit®). PCR procedure consisted of an initial denaturation step at 94 °C for 5 minutes; 35 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 1 minute, and extension at 72 °C for 2 minutes; and a final extension step at 72 °C for 5 minutes. PCR products were purified with a polyethylene glycol solution (20% PEG 8000, 2.5M NaCl) as described by Sambrook and Russel (2001), and DNA length was analyzed and quantified in agarose gel. DNA sequencing was accomplished in an Abi Prism 3500 Genetic Analyzer® (Applied Biosystems, Foster City). Generated dataset containing CytB was used to select additional sequences from GenBank, and all of them containing the region between locus +30 and locus +746 of the gene (sequence length 716 bp).

Phylogenetic analysis.— Sequences of CytB gene were aligned and edited using the program Molecular Evolutionary Genetics Analysis (MEGA) version 6 (Tamura et al. 2013). Parameters of nucleotide and haplotype diversity were calculated with DNAsp version 5.1 (Rozas et al. 2003). The most appropriate evolutionary model of nucleotide substitution for sequences was calculated by the Akaike Information Criterion (AIC) in jModelTest 2.1 (Darriba et al. 2012). The model TIM1 (Posada 2003), with proportion of invariant sites (*pinvar* = 0.665), was selected. This model was used in the Maximum Likelihood (ML) analysis. ML branch support was estimated using the approximate Likelihood-Ratio Test (aLRT) performed in PhyML 3.1 (Guindon and Gascuel 2003). Bayesian inference (BI) was performed using the program MrBayes 3.2.2. Four Markov Chain Monte Carlo (MCMC) simulations were run for $5x10^6$ generations with a sampling frequency of every hundred generations and burn-in of 25%. For the other variables, we used default parameters. Trees were sampled for every one hundred generations. Node support of BI was evaluated by posterior probability (Huelsenbeck and Ronquist 2001). Also, *A. inopinatus* (U66501), *A. hirsutus* (U66500), *A. fraterculus* (DQ869389), *A. fimbriatus* (KT149211, generated in this study), *A. amplus* (AY642924), and *A. jamaicensis* (DQ869447) were used as outgroups, based on the phylogenetic proximity defined by Redondo et al. (2008).

Molecular dating.— Divergence dates were estimated with the software BEAST version 1.5 using the Yule tree prior (Drummond and Rambaut 2007). The 11.7 Ma calibration obtained for the diversification of the *Artibeus* genus was used (Redondo et al. 2008).

Population structure and demographic history.— Analysis of Molecular Variance (AMOVA), Tajima's D and Fu's Fs neutrality tests (Tajima 1989; Fu and Li 1993; Fu 1997) were performed for the same groups defined in our study (*a prior*), and were carried out using the software Arlequin version 3.5.1.2, with number of permutation 99999 and significance level 0.05 (Excoffier and Lischer 2010). Both neutrality tests assume that groups are in mutation-drift and migration-drift equilibrium. Significant values for either may indicate that the populations are not evolving in a neutral selection or that they were previously subdivided and/or have practiced past population growth (Morando et al. 2004).

In order to understand better scenario from the origin of the actual geographic distribution of *A. planirostris*, we evaluated the posterior probability (PP) of the distinct historic scenarios (including different possibilities for vicariance and / or colonization). To do that, we employed Approximate Bayesian Computation approach (ABC) (Beaumont et al. 2002) as implemented in the software DIY ABC v. 1.0.4 (Cornuet et al. 2008). Phylogeographic tree topology was defined as baseline and reference for each population considered in the ABC analysis (details are showed in the Figure 1). Seven possible scenarios were tested (a-g), as follow: (a) vicariance 1+2+3; (b) vicariance 2, 3+1; (c) vicariance 3, 2+1; (d) vicariance 1, 2+3; (e) colonization from 1+3 to 2; (f) colonization from 1+2 to 3; and (g) colonization from 2+3 to 1.

RESULTS

We obtained a total of 110 individual sequences of CytB gene of 110 individuals. Haplotype numbers, location information, as well as GenBank vouchers and access numbers are shown in Supplementary Data S1. An overview of polymorphism data revealed 121 variable sites and 126 mutations. High haplotype (*hd* = 0.984) and low nucleotide ($\pi = 0.01536$) diversity values were observed.

Phylogeography of A. planirostris.— Phylogeographic trees produced using different methods (ML and BI) showed similar topology, with two main monophyletic clades whose approximate divergence time was 3.44 Ma (95% HPD: 2.08–4.89). One of the clades grouped individuals from the [1] Western Amazon Forest (WAF), while the other was composed by two subclades representatives from [2] Atlantic Forest (AF) plus Dry Forests (DF) and [3] Lesser Antilles (LA) plus Eastern Amazon Forest (EAF). The subdivision of this subclade was dated at 2.12 Ma (95% HPD: 1.27-2,96) (Fig.1 and 2).

Population structure and Demographic history.— Using the clusters observed in the phylogeographic tree, AMOVA analysis revealed the robust genetic structure of *A. planirostris* (Fst = 0.54, see supplementary data S3). The results of the Tajima's D and Fu's FS neutrality tests were negative and significant for the entire data set and for the clusters obtained in the present study (Table 1).

The ABC analysis strongly support the scenario "f" (colonization from 1+2 to 3) as most probably, demonstrating that the population of *A. planirostris* located in the east portion of the Amazon forest and Antilles (defined as "3") is a result of a colonization event. This population rise by an admixture between populations from western portion of Amazon forest (defined as "1") and Atlantic forests and Dry forests (defined as "2"). See details in the Table 2. The other scenarios tested and refused may be viewed in the Supplementary data S2.

DISCUSSION

Phylogeographic trees of CytB gene were constructed using samples representing the distribution of *A. planirostris* and indicate the existence of three groups geographically delimitated by different environments. Separation of the group that correspond to West portion of Amazon forest occurred approximately 3.44 Ma ago, which coincided with the early Pliocene period. The most conspicuous stage of the Andean uplift occurred exactly in this period, and leads to a strong modifications in the West and east portion of this biome (Hoorn et al. 2010). Geological evidence suggests that modifications in Central South America occurred progressively, one of the steps was alteration in drainage features of the Amazon River, from the western Andes to northeastern Amazonia during the Pleistocene (Frailey et al. 1988; Da Silva and Patton 1998). During and late the event of Andean uplift, the East portion of Andes increase significantly the precipitation and moisture index, these changes allowed a highly sediment deposition in this regions and important bioclimatic modifications (Hoorn et al. 2010; Poulsen et al. 2010). This gradative increase in the sediment deposition occurred from West to East Amazon, causing changes in river flows and leading to the biome conformations that we can find actually (Toivonen et al. 2007). This period was very intense for biodiversity radiation, such as plants (Hugues and Eastwood 2006) and even mammals (mustelíds, carnívors and perissodactils) (Cione et al. 2007). This scenario is very important considering that the dispersion of *A. planirostris* and the formation of the geographic structure encountered in our study for the species might be related with these biogeographic events.

Accordingly with our tree AF + DF, and LA+EAF are delimitated by the diagonal dry belt formed especially by xeric landscapes (Koopman 1976; Silva et al. 2001). Such geoclimatic event has affected the population structure of several bat species, including *Lonchorhina aurita* (Lopes and Ditchfield 2009), *Desmodus rotundus* (Martins et al. 2009), *Artibeus obscurus* (Ferreira et al. 2014) and *Carollia perspicillata* (Pavan et al. 2011), besides other animal groups, like reptiles (Pellegrino et al. 2005), amphibians (Elme et al. 2007) and birds (Bates et al. 1998).

Molecular dating that defined the separation of AF+DF from LA+EAF came close to the time recorded in previous phylogeographic studies about bats (Lopes and Ditchfield 2009; Martins et al. 2009). Similar dating values were obtained for rodents and marsupials, and coincided with the South American diagonal dry belt (Costa, 2003). Moreover, several investigations on birds have dated the separation of the Atlantic Forest from the Amazon Forest, which has been explained by the said geo-climatic event, at approximately 3.0 Ma (Ribas and Miyaki 2004; Cabanne et al. 2008; Weir and Price 2011; Batalha-Filho et al. 2013). Populations of *A. planirostris* from dry forests and coastal Atlantic Forest may be isolated from Amazon region since the Last Glacial Maximum and, posteriorly, spread out to occupy disjunct distributions as recorded in actual times. This biogeographic history is strongly supported by long distance migration events rather than vicariance (Mayle 2004), as showed by our ABC analysis. In this context, we observed a possible mixed zone (dotted line showed in the Figure 1) in ecoregions that present forested landscapes between the Amazon and Atlantic forest biomes (Olson et al. 2001), coinciding with this described geographic pattern of *A. planirostris*.

The results obtained in the present study did not allow to identify events that separated the Antilles from the South American continent. The samples of *A. planirostris* collected in these islands were genetically more related with representatives of the eastern portion of the Amazon Forest, indicating that the geographic isolation of the Antilles *per se* did not suffice to differentiate this species’ populations of the archipelago. Hunt et al. (2001) also observed low genetic divergence between populations of Antilles and continental ones in a study regarding birds (family Mimidae). However, other investigations have shown that this distinction is substantial in non-volant species (Irschick et al. 1997; Lovette et al. 1999; Camargo et al. 2009). This may be due to dispersion capacity of each organism, which is higher for birds and bats, increasing the probability of gene flow between populations from mainland and islands.

Demographic assays showed that the species and the three clusters underwent population expansion. This kind of event has been recorded for other organisms in Atlantic Forest (Mustrangi and Patton 1997; Birungi and Munstermann 2002; Brito et al. 2002; Costa 2003; Leite 2003; Pellegrino et al. 2005; Moraes-Barros et al. 2006), in the eastern portion of the Amazon Forest (Aleixo 2004; Mirabello and Conn 2006; Vasconcelos et al. 2006; Santos et al. 2007), and in the western portion of this biome (Aleixo 2004; Roberts et al. 2006; Ruiz-García et al. 2014). The fact that populations of different organisms are suffering expansions reinforces the hypothesis that the actual distribution of *A. planirostris* is a result of a colonization model (supported one more time by our ABC analysis). Analysis of the *A. planirostris* population structure confirmed the phylogeographic trees, highlighting the existence of three distinct groups.

Our results suggest phylogeographic pattern observed for *A. planirostris* originated at chronological moments represented by colonization events. Initially, the expansion of the Amazon Forest occurred and, in a second moment, the formation of the South American dry diagonal. Additionally, the intense and uninterrupted changes in the Neotropical region during the last 10 m.y. (Woodburne, 2010) support all scenarios described here. Molecular dating values obtained in the present study are in agreement with the two geo-climatic events. Finally, this is the first geographic structure mapping of a Neotropical bat species, very important for the maintenance of forested biomes, and can open up new avenues for investigations about interesting biodiversity and biogeographic patterns.

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FIGURE AND TABLES

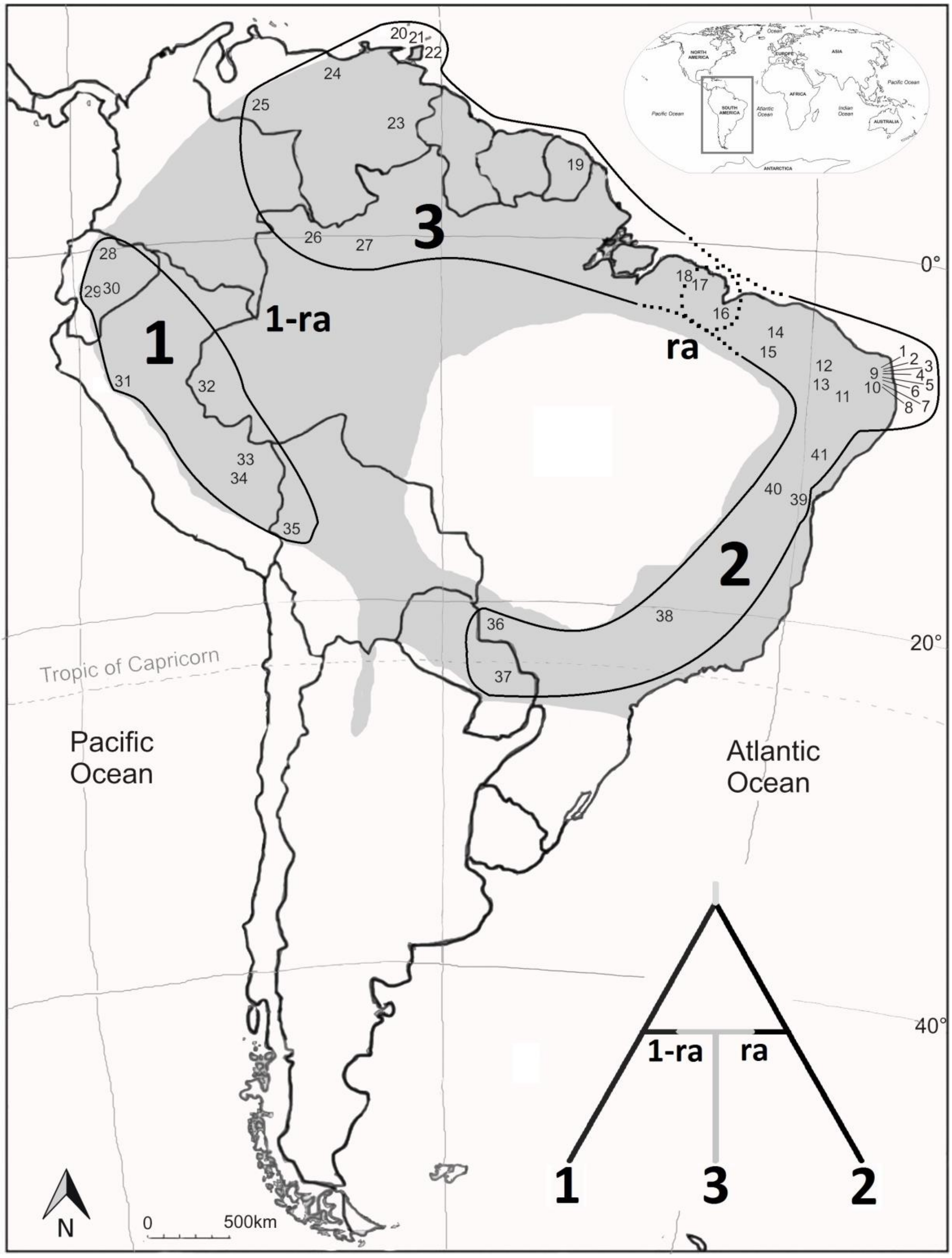


Fig. 1.—Section of the South America map showing the geographic distribution of *Artibeus planirostris* (grey shaded area, based on IUCN valid polygon). In the right down

corner is show the most probably scenario identified by ABC analysis. The bigger number labels indicate different populations identified (1. WAF = Western Amazon Forest; 2. AF = Atlantic Forest + DF = Dry Forest; 3. EAF = Eastern Amazon Forest + LA = Lesser Antilles) and small number labels indicate the sampling sites considered in our study. Brazil: Atlantic Forest: 1. Baía Formosa, 2. Mamanguape, 3. João Pessoa, 4. Goiana, 5. Igarassu, 6. Olinda, 7. São Lourenço da Mata, 8. Tamandaré, 38. Sales Oliveira, 39. Valença, 41. Entre Rios; Dry Forest: 9. Campina Grande, 10. Caruaru, 11. Buíque, 12. Triunfo, 13. Serra Talhada, 14. Palmeirais, 15. Uruçuí, 36. Aquidauana, 40. Rio de Contas; Amazon Forest: 16. São Luís, 17. Cândido Mendes, 18. Godofredo Viana, 26. Santa Isabel do Rio Negro, 27. Barcelos, 32. Rodrigo Alves. French Guiana: Atlantic Forest: 19. Remire. Granada: Tropical Forest: 20. Carriacou Island, 21. St. Georges. Trinidad and Tobago: Amazon Forest: 22. Trinidad. Venezuela: Amazon Forest: 23. Bolivar, 24. Guarico, 25. Barinas. Ecuador: Amazon Forest: 28. Sucumbios, 29. Pastaza, 30. Puyo. Peru: Amazon Forest: Chachapoyas, 33. Madre de Dios, 34. Cuzco. Bolívia: Amazon Forest: 35. La Paz. Paraguay: Dry Forest: 37. San Pedro.

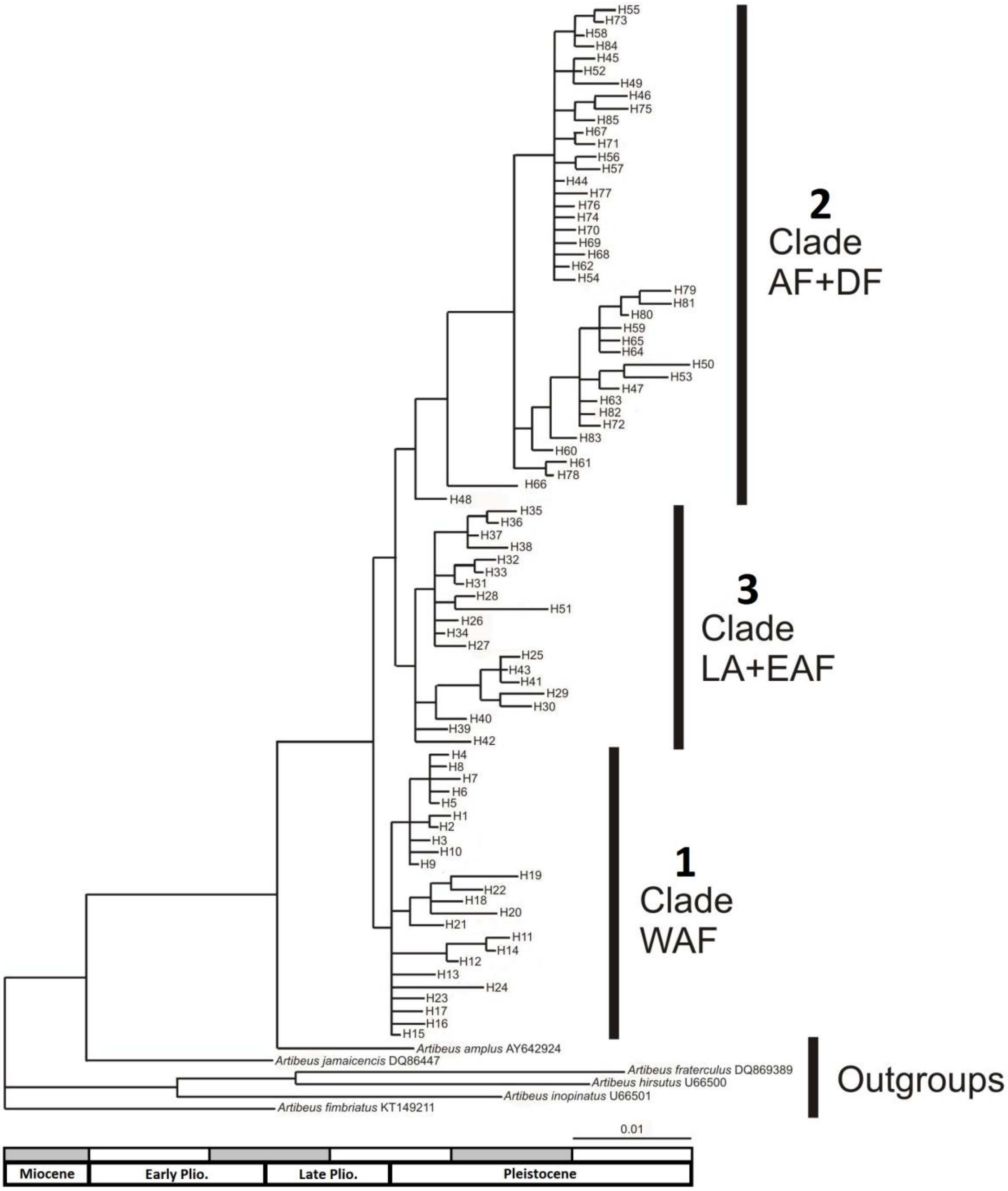


Fig. 2. —Phylogeographic reconstruction based on the Bayesian inference tree of the CytB gene haplotypes of *Artibeus planirostris*. The statistical supports for the clades are presented in the following order: Bayesian inference posterior probability and approximate likelihood ratio test. Information about locations and accession number of each haplotype (H) from GenBank is given in Supplementary data S1. Clade1 - WAF = Western Amazon Forest; Clade 2 - AF = Atlantic Forest + DF = Dry Forest; Clade 3 - EAF = Eastern

Amazon Forest + LA = Lesser Antilles.

Table 1.—Tajima's D and Fu's FS neutrality tests and p value for each cluster and for the data set generated using the CytB gene of *Artibeus planirostris*. WAF = Western Amazon Forest, EAF = Eastern Amazon Forest, LA = Lesser Antilles, AF = Atlantic Forest, and DF = Dry Forest.

	Statistics	WAF	EAF + LA	AF + DF	Mean
Tajima's D test	Tajima's D	-1.79100	-1.54402	-1.79232	-1.70911
	Tajima's D p-value	0.01970	0.03930	0.01400	0.02433
Fu's FS test	FS	-22.39267	-7.69645	-25.05525	-18.38146
	FS p-value	0.00000	0.00330	0.00000	0.00110

Table 2.—Scenarios evaluated for *Artibeus planirostris* (a-g) with the respective values of posterior probability (PP) and Bayes Factor (BF), followed by ABC analysis and according Jeffreys (1961). Numbers in the description of each scenario follow the same geographic pattern showed in the Figure 1.

Scenarios	PP	BF
(a) Vicariance 1+2+3	0.0042	Negative
(b) Vicariance 2, 3+1	0.0000	Negative
(c) Vicariance 3, 2+1	0.0000	Negative
(d) Vicariance 1, 2+3	0.0010	Negative
(e) Colonization from 1+3 to 2	0.1069	Substancial evidence
(f) Colonization from 1+2 to 3	**0.8803**	**Very Strong**
(g) Colonization from 2+3 to 1	0.0075	Negative

SUPPLEMENTARY DATA

Supplementary Data S1.—Haplotype codes, geographic location, and GenBank voucher and accession number of CytB gene sequences used in the phylogeographic analyses of *Artibeus planirostris*. Haplotype codes are the same as used in Figure 2.

Haplotype	**Locality**	**Voucher number**	**Accession number**
H1	Pastaza, Ecuador	TK104419; TTU85191	DQ869418
H2	Pastaza, Ecuador	TK104413; TTU85185	DQ869417
H3	Sucumbios, Ecuador	TK125306; QCAZ6913	DQ869412
H4	Sucumbios, Ecuador	TK125307; QCAZ6862	DQ869411
H5	Pastaza, Ecuador	TK104404; TTU85176	DQ869409
H6	Pastaza, Ecuador	TK104017; TTU84789	DQ869408
H7	Puyo, Ecuador	TK 104404	AY684714
H8	Cuzco, Peru	MVZ 166539	AY684725
H9	Puyo, Ecuador	TK 104403	AY684713
H10	Cuzco, Peru	MVZ 166536	AY684726
H11	Puyo, Ecuador	TK 104333	AY684721
H12	Chachapoyas, Peru	TK 22634	AY684722
H13	Pastaza, Ecuador	TK104016; TTU84788	DQ869407
H14	Pastaza, Ecuador	TK104410; TTU85182	DQ869410
H15	Madre de Dios, Peru	TK16634	DQ869397
H16	La Paz, Bolivia	TK 14535	AY684718

H17	La Paz, Bolivia	TK 14536	AY684723
H18	Puyo, Ecuador	TK 104345	AY684717
H19	Chachapoyas, Peru	TK 22630	AY684754
H20	Chachapoyas, Peru	TK 22626	AY684724
H21	Chachapoyas, Peru	TK 22629	AY684716
H22	Puyo, Ecuador	TK 104419	AY684753
H23	Puyo, Ecuador	TK 104008	AY684719
H24	Rodrigues Alves, Brazil	CA010	EU160909
H25	Guarico, Venezuela	TK15020; TTU33340	DQ869425
H26	Barinas, Venezuela	TK 19025	GQ861700
H26	Bolivar, Venezuela	TK19025	DQ869426
H27	Barinas, Venezuela	TK 19026	GQ861701
H28	Barinas, Venezuela	TK 19023	GQ861699
H28	Remire, French Guiana	TK143051; CM83901	DQ869398
H29	Guarico, Venezuela	TK15011; TTU33331	DQ869423
H30	Guarico, Venezuela	TK15013; TTU33333	DQ869424
H31	Carriacou Island, Grenada	TK 128674	GQ861671
H31	Carriacou Island, Grenada	TK 18520	GQ861610
H31	Carriacou Island, Grenada	TK 18527	GQ861611
H31	Carriacou Island, Grenada	TK 18530	GQ861612
H31	Saint George, Grenada	TK18522; CM63324	DQ869437
H31	Saint George, Grenada	TK18527; CM63329	DQ869438
H31	Saint George, Grenada	TK18530; CM63332	DQ869439
H31	Trinidad, Trinidad and Tobago	TK25185; TTU44046	DQ869427

H31	Trinidad, Trinidad and Tobago	TK25187; TTU44048	DQ869431
H31	Trinidad, Trinidad and Tobago	TK25188; TTU44049	DQ869428
H32	Saint George, Grenada	TK18528; CM63330	DQ869436
H32	Trinidad, Trinidad and Tobago	TK25186; TTU44047	DQ869430
H33	Trinidad, Trinidad and Tobago	TK25085; TTU44061	DQ869433
H34	Trinidad, Trinidad and Tobago	TK25026; TTU44060	DQ869432
H35	São Luis, Brazil	VCT324	EU160933
H36	São Luis, Brazil	VCT305	EU160903
H37	Godofredo Viana, Brazil	LK01	EU160941
H38	São Luis, Brazil	VCT318	EU160931
H39	Santa Izabel do Rio Negro, Brazil	AD876	EU160896
H40	Santa Izabel do Rio Negro, Brazil	AD884	EU160897
H41	Santa Izabel do Rio Negro, Brazil	AD824	EU160891
H42	Santa Izabel do Rio Negro, Brazil	AD855	EU160893
H43	Santa Izabel do Rio Negro, Brazil	AD856	EU160894
H44	Aquidauana, Brazil	4521	EU160866
H44	Mamanguape, Brazil	AD072	EU160866
H44	Entre Rios, Brazil	AD184	EU160888
H44	Tamandaré, Brazil	M1538	KT149197 #

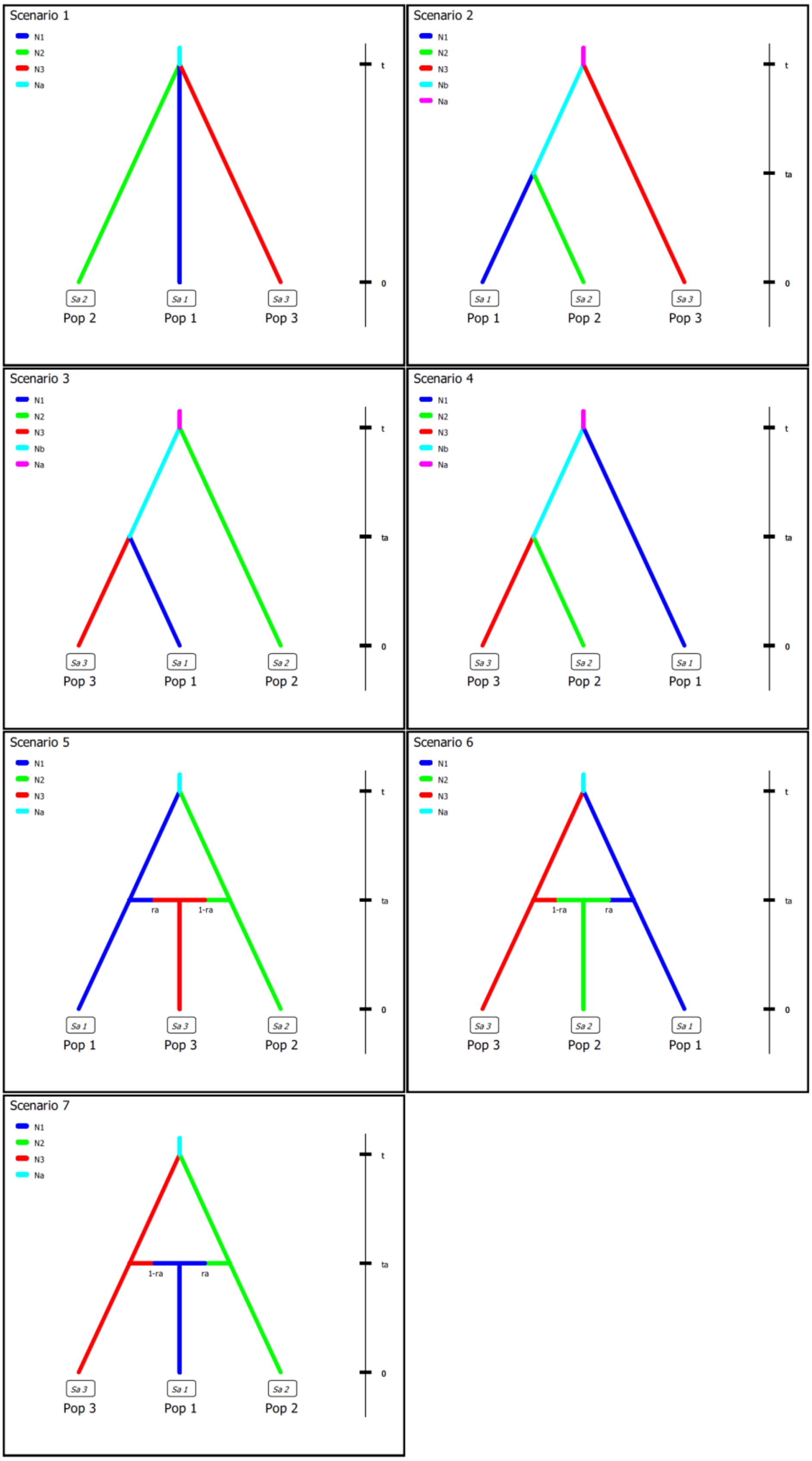
H44	Caruaru, Brazil	M1774	KT149203 #
H44	Mamanguape, Brazil	M2115	KT149191 #
H44	Rio de Contas, Brazil	UFPB5125	EU160866
H44	Palmeirais, Brazil	UFPB5129	EU160866
H45	São Luis, Brazil	VCT326	EU160934
H46	São Luis, Brazil	VCT345	EU160936
H47	São Luis, Brazil	VCT359	EU160937
H48	São Luis, Brazil	VCT372	EU160938
H49	São Luis, Brazil	VCT376	EU160939
H50	São Luis, Brazil	VCT377	EU160940
H51	Barcelos, Brazil	RZ122	EU160926
H52	Cândido Mendes, Brazil	LK09	EU160942
H53	São Luis, Brazil	VCT321	EU160932
H54	San Pedro, Paraguay	TK56656; TTU96811	DQ869394
H55	Mamanguape, Brazil	AD083	EU160875
H55	Goiana, Brazil	M1471	KT149202 #
H55	Igarassu, Brazil	M1583	KT149204 #
H55	Goiana, Brazil	M1994	KT149205 #
H55	Mamanguape, Brazil	M2122	KT149189 #

H56	Serra Talhada, Brazil	M1277	KT149190 #
H57	Serra Talhada, Brazil	M1334	KT149192 #
H58	Mamanguape, Brazil	M2119	KT149193 #
H59	Buíque, Brazil	M1443	KT149194 #
H59	Triunfo, Brazil	M2075	KT149195 #
H60	Caruaru, Brazil	M1767	KT149196 #
H61	São Lourenço da Mata, Brazil	M1710	KT149198 #
H62	Triunfo, Brazil	M2068	KT149199 #
H63	Triunfo, Brazil	M2080	KT149200 #
H64	Campina Grande, Brazil	AD034	EU160868
H64	São Lourenço da Mata, Brazil	M1756	KT149207 #
H65	Tamandaré, Brazil	M1555	KT149208 #
H66	Serra Talhada, Brazil	M1345	KT149209 #

H67	Goiana, PE, Brazil	M1990	KT149210 \#
H68	Entre Rios, Brazil	AD176	EU160887
H69	Valença, Brazil	AD192	EU160889
H70	Mamanguape, Brazil	AD077	EU160874
H71	Mamanguape, Brazil	AD091	EU160877
H72	Olinda, Brazil	AD158	EU160886
H73	Tamandaré, Brazil	AD145	EU160883
H74	Tamandaré, Brazil	AD144	EU160882
H75	Joao Pessoa, Brazil	AD038	EU160869
H76	Joao Pessoa, Brazil	AD040	EU160871
H77	Joao Pessoa, Brazil	UFPB5120	EU160930
H78	Mamanguape, Brazil	AD076	EU160873
H79	Olinda, Brazil	AD156	EU160884
H80	Tamandaré, Brazil	AD134	EU160879
H81	Tamandaré, Brazil	AD136	EU160880
H82	Palmeirais, Brazil	UFPB5111	EU160928
H83	Uruçui, Brazil	UFPB5106	EU160927
H84	Baia Formosa, Brazil	AD107	EU160878
H85	Sales Oliveira, Brazil	AD753	EU160890

Generated in this study.

Supplementary Data S2.— Proposed scenarios for the demographic history of Artibeus planisrostris obtained by ABC approach.



Supplementary Data S3.— Analysis of molecular variance (AMOVA) of Cytochrome b gene; results based on 41 localities for *A.* planirostris based across three main geographical clades.

Source of variation	% Total variance	Sum of Squares	Variance components
Among groups	54.01	263.77	3.71
Within populations	45.99	344.99	3.16

Fixation index Fst = 0.540
Populations were subdivided into 3 groups according to the phylogeographic tree (see Results section).

5 CAPÍTULO II

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Original Article

Runnig title: **Genetic differentiation: A study in two bat species**

Genetic differentiation patterns between the Atlantic and dry forests: A study of population structure in two phyllostomid bat species

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Abstract

Biomes Seasonally Dry Tropical Forest (SDTF) and Atlantic Forest exhibit different abiotic characteristics that reflect the adaptation of living organisms. There are a great variety of bats in these natural environments, though few are known regarding their genetic diversity. This study aimed to evaluate the genetic structure of two phyllostomid bat species, *Artibeus planirostris* (n=91) and *Carollia perspicillata* (n = 60), from seven locations (SDTF: Serra Talhada and Triunfo; Buíque and Caruaru, inserted in the sub region of the Atlantic Forest, Brejo; Tamandaré, São Lourenço da Mata and Igarassu, inserted in the subregion of the Atlantic Forest, Pernambuco). Genetic diversity was assessed by inter-simple sequence repeat (ISSR) technique based on the application of four primers: (AC) 6AT, (CA) 6GC, (CA) 6AG, and (AGC) 4T. It was possible to identify 259 ISSR markers, all polymorphic. Analysis of molecular variance indicated a strong genetic structure population of both species, with F_{st}=0.30 for *A. planirostris* and F_{st}=0.24 for *C. perspicillata*; however, the last one also showed genetic difference between the environments (F_{sc}=0.09). These data evince the different foraging behavior between the two *taxa*, with *C. perspicillata* being a more specialist specie and moving less when searching for resources. SDTF populations showed greater genetic differentiation, reflecting the recent developments of this biome. Neutrality test indicated that expanding populations (*A. planirostris*: -65.387, p <0.01; *C. perspicillata*: -39.186, p <0.01) may be common in this region. Positive and moderate correlation were verified when comparing the genetic and geographical distances, indicating that isolation by distance also causes genetic differentiation. This study reveals that different variables (displacement capacity, environmental specialization, geographic distance) influence the genetic structure of bat populations.

Introduction

Two important biomes of Neotropical region are considered hot spots because of the high rate of endemism, diversity and species at risk: the Atlantic Forest and the Seasonally Dry Tropical Forest (SDTF) (Trejo & Dirzo 2000; Galindo-Leal & Gusmão-Câmara 2005). The Brazilian Atlantic Forest, extending along the coast from 7°S to 23°S, is one of the most widespread environments in Brazil; it is considered a set of subregions rather than a single type of vegetation (Rizzini 1997; Rodal *et al.* 2008). In this group can be found *Brejos* which are "islands" of the Atlantic Forest inserted into SDTFs (Pôrto *et al.* 2004). SDTFs are considered to be critically endangered (Trejo & Dirzo 2000), even more than tropical rainforests (Gentry 1995). In Brazil, SDTF consists of a mosaic of scrub vegetation and patches of dry forest representing approximately 10% of the national landmass (Santos *et al.* 2011). According to the ecological vicariance model, these environments can act on the genetic differentiation of natural populations through evolutionary forces such as drift and divergent selection (Moritz *et al.* 2000).

Advances in the field of molecular biology provides tools to further investigation on geographical variation; this information enables to understand diversification processes and assessment of the biogeographic history (Patton *et al.* 1994). For the last few years, the inter-simple sequence repeat (ISSR) technique has been widely applied in ecological and evolutionary studies to estimate the genetic diversity of different animal and plant species, to determine population structures, and to evaluate hybridization and genetic conservation (Lu *et al.* 2006; Vijayan *et al.* 2006; Antunes *et al.* 2010; Lin *et al.* 2012; Manrique-Poyato *et al.* 2013; Quezada *et al.* 2014; Rodríguez-Rodríguez *et al.* 2015). The application of this technique may enable assessing if the differences in environments may cause significant differentiation between organisms.

Due to its ability to fly, bats can disperse over long distances, a fact that has generated interest among evolutionary biologists. These animals present low divergence of genetic structure when compared to other mammals, being up to ten times lower than the values found for rodents and marsupials, for distances that are ten times greater. They can potentially display phylogeographic patterns different from other non-volant organisms (Ditchfield 2000).

Given the lack of knowledge regarding the effect of natural bat populations on the environment, using ISSR markers, this study aimed to: 1) assess the genetic diversity and population structure of two phyllostomid bat species (*Carollia perspicillata* Linnaeus and *Artibeus planirostris* Spix); 2) analyze environmental effects of SDTF and two subregions of the Atlantic forest, Pernambuco and Brejo; and 3) verify if the geographical distance is related to genetic differentiation.

Materials and methods

Samples and molecular methods

C. perspicillata and *A. planirostris* specimens were collected in natural forests of the Brazilian northeastern region, at seven different sites in the state of Pernambuco, comprising SDTF (Triunfo and Serra Talhada), PAF (Tamandaré, Igarassu and São Lourenço da Mata) and BAF (Caruaru and Buíque, Fig. 1) environments.

The bats were captured by mist nets. The identification of the species was accomplished based on Gardner (2007). Blood was collected from each individual and stored in tubes containing EDTA (5%) for subsequent analysis in the laboratory. This procedure was approved and allowed by the responsible organization (ICMBIO - License Number 12264-1).

Genomic DNA extraction was carried out by salting-out method, according to the protocol proposed by Medrano *et al.* (1990). Polymerase chain reactions (PCR) were performed in a total volume of 25µL containing: 50-60 ng of DNA, 4µm of primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 2.5 µL 1X reaction buffer, and 1.25 U Taq DNA polymerase (Invitrogen Kit®). The PCR procedure consisted in initial denaturation at 94°C for two minutes, followed by 35 denaturation cycles at 94°C for 30 seconds; annealing between 42°C and 51°C for 30 seconds; extension at 72°C for two minutes; and final extension at 72°C for 5 minutes.

Four out of 20 ISSR primers were selected [$(CA)_6AT$; $(CA)_6GC$; $(CA)_6AG$; $(AGC)_4T$], as they showed satisfactory results based on the number of clean, bright, and easily interpretable and reproducible polymorphic bands produced. The ISSR amplification products were separated by electrophoresis in 3% agarose gels, and the length of each band was determined using 100-bp DNA Ladder (Invitrogen®). Each sample was amplified and run in duplicates; a third replicate was made as guarantee**.** Presence or absence of each fragment was coded in a data matrix as 1 or 0, respectively.

Data analysis

Descriptive analysis of ISSR markers was performed using the program FAMD version 1.3 (Schlüter & Harris 2006), which evaluated the number of polymorphic *loci* and private bands.

An AMOVA (Analysis of Molecular Variance) algorithm was used for the hierarchical analysis of genetic diversity of the population and groups. Beyond that, matrices of genetic distance between populations and groups were generated with the software Arlequin version 3.5 (Excoffier & Lischer 2010). Twenty independent simulations were run for each value of K from 1 to 10, with 10^5 permutations and an initial burn-in of 10^4 generations with the Structure 2.3 software (Pritchard *et al.* 2000). Best-fit K value was estimated by Evanno's test (Evanno *et al.* 2005).

Fu's neutrality test (Fu 1997) as well as the observed and expected distribution of pairwise differences (mismatch distribution) were evaluated with the DnaSP software version 5.1 (Rozas *et al.* 2003).

Mantel test was performed using the software Alleles in Space, version 1.0 (Miller 2005), to determine the level of association between genetic and geographical distance in the data set. The analysis used a Dice distance matrix of individuals and geographical distances, which were calculated from the Universal Transverse Mercator (*UTM*) coordinates of the sample locations, then log-transformed. Significance was assessed by 5,000 replications.

Results

The genetic analysis of 151 bat samples, using concatenated data obtained from four primers, identified 259 ISSRs markers, where 100% of which were polymorphic (Table 1). AMOVA results revealed strong population structure in *A. planirostris* ($F_{st} = 0.30$) and *C. perspicillata* ($F_{st}=0.24$). In addition, *C. perspicillata* was also found to be structured at regional level, which was not observed in *A. planirostris* (Table 2).

The population structure observed in *A. planirostris* and *C. perspicillata* is depicted in a bar plot graphic constructed with the software Structure, with K=2, $\Delta K = 22.59$ (Fig. 2) and $\Delta K=88.24$ (Fig. 3), respectively. Comparing the fixation index among regions, the

SDTF has always been more genetically distant in relation to the other regions, a result observed in both species (Table 3).

Analysis of pairwise differences (mismatch distribution) for populations of both species indicates expansion (Fig. 4), which is supported by neutrality tests. F_s test showed negative values and significance for *A. planirostris* (-65.387, p <0.01) and *C. perspicillata* (-39.186, p <0.01).

The correlation between genetic and geographical distance of the samples was positive/moderate for *A. planirostris* (r = 0.32, p<0.01) and positive/weak (r = 0.25, p<0.1) for *C. perspicillata*.

Discussion

This study revealed an important population structure in two bat species with different ecological features, found in different environments. The results showed high polymorphism rate (100%) for 259 identified ISSR markers (143 and 116 *loci* for *A. planirostris* and *C. perspicillata,* respectively).

AMOVA showed strong genetic structure of the two species. However, 40.20% of the variation in *C. perspicillata* was due to regional differences, whereas *A. planirostris* presented no regional, but only interpopulation variation. This demonstrates that the applied grouping procedures were justified, given that each region is not totally heterogeneous. This result was also observed in a study conducted in the same environment with *Drosophila* populations using mitochondrial DNA (Brito *et al.* 2002).

The fact that *C. perspicillata* presents regional structure can be explained by the species' low-mobility foraging tactics, varying between 0.5 to 2.5 km, as shown in studies with telemetry data (Heithaus & Fleming 1978; Bernard & Fenton 2003). Such low mobility stimulates regional differentiation, as observed in the present study. Other research has detected regional morphological variations of this species that corroborate this idea (Cloutier & Thomas 1992; Nowak 1994). *C. perspicillata* has a specialized diet, targeting Piperaceae species (Peracchi et al. 2006), and specialist species are known to present greater genetic structure (Kitahara *et al.* 2000; Carnaval 2002). The genus *Artibeus* has greater foraging movement, reaching 25km in the rainforest (Esberard 2003), which promotes connectivity between regions. This observation supports the explanation for the absence of morphological variation in this specie, shown by Taddei (1998) in analysis of individuals from the northern, Brazil's northeast and southeast.

Similarly, no geographic pattern was found in a study conducted by Carnaval (2002) in *Hylabranneri*, a generalist specie of frog just as *A. planirostris*. It is known that the exploratory behavior of bats is the product of different ecological and evolutionary pressures (Bianconi *et al.* 2006). Therefore, the observed genetic differentiation between populations probably occurs due to the founder effect, i.e., populations originated by a group of individuals share similar characteristics, but which differ from other groups.

The SDTF region was more differentiated in relation to other subregions of Atlantic Forests when comparing the F_{st} values for both species. There are two possible explanations for this fact. First, species move more within dry environments, as they require more foraging area to obtain sufficient food and because of the scarcity of resources. This constant movement results in more homogeneous populations (Fleming 1988; Bernard & Fenton 2003). In this same context, it has been observed that the living area of the opossum (*Didelphis albiventris*) is also influenced by the availability of fruits in SDTF, with more frequent movements to find food during the resource-scarce season; the resulting larger living area increases the flow between close populations (Cerqueira 1985). As a second explanation, the two subregions of Atlantic forest (Pernambuco and Brejo) would have the same evolutionary origin, sharing many characteristics and resulting in greater differentiation in relation to SDTF (Pôrto *et al.* 2004). In addition, other aspects, such as breeding system, might affect the levels of genetic diversity within a specie or population (Levsen & Mort 2008). Regional variations indicate that the patterns observed today are a reflection of many events varying in time and scale, as suggested by Costa (2003).

Intrapopulation analysis revealed significant evidence of population expansion in both species, with multimodal mismatch distribution, as observed in populations of *Artibeus obscurus* of the Atlantic and Amazon forest (Redondo *et al.* 2008). In addition, F_s test is one of the most successful for this type of analysis, providing sufficient evidence for population expansion.

Evidence for a moderate/weak positive correlation between the pairwise genetic and the geographical distance shows that, besides the environmental variations in the spatial gradient, the isolation by distance, which in this study reached 380km, influences the structure of the species. ISSR markers were effective in this study to evaluate structure and genetic diversity, constituting a fast and inexpensive technique. Recent studies with a similar approach have shown satisfactory results in animal research (Manrique-Poyato *et al.* 2013; Rodriguéz-Rodriguéz *et al.* 2015).

This study suggests that different variables (displacement capacity, environmental specialization, and geographical distance) can influence the genetic structure of bat populations. Moreover, ISSR markers are suitable tools to *evaluate* regional genetic variations in the studied environments.

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Figures and tables

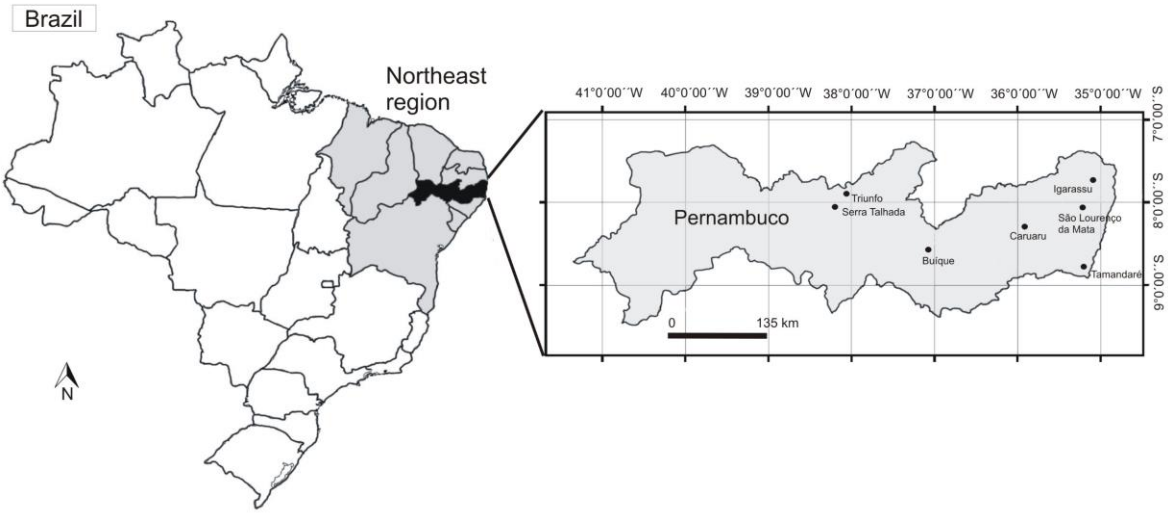

Fig. 1 Map showing the location of where the samples were collected from the seven analyzed populations of *A. planirostris* and *C. perspicillata*.

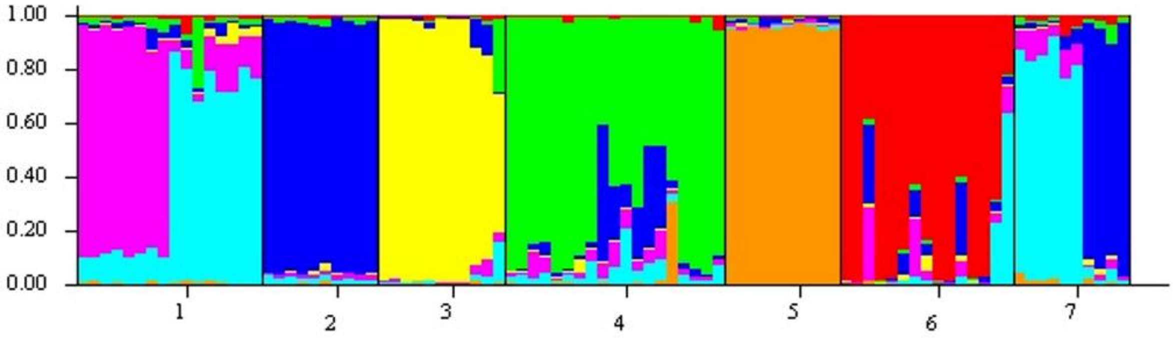

Fig. 2 Graphical output of Bayesian clustering analysis of 91 *A. planirostris* samples constructed with the software Structure for the model $K = 7$. Each vertical bar corresponds to a single individual; the length of each color is proportional to the estimated membership coefficient. Sampling sites: 1 – Tamandaré, 2 –Igarassu, 3 – São Lourenço da Mata, 4 – Serra Talhada, 5 –Triunfo, 6 – Caruaru and 7 –Buíque.

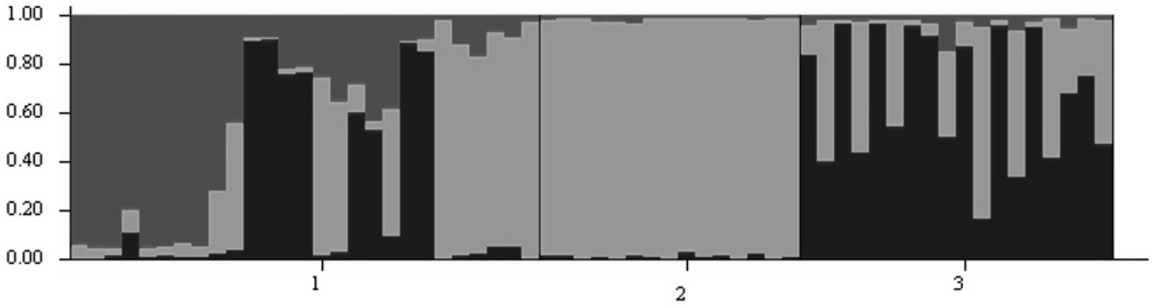

Fig. 3 Graphical output of Bayesian clustering analysis of 60 *C. perspicillata* samples created with the software Structure for the model *K* = 3. Each vertical bar corresponds to a single individual; the length of each color is proportional to the estimated membership coefficient. Sampling regions: 1 – Pernambuco, subregion of the Atlantic forest 2 – Seasonnaly dry tropical forest, 3 – Brejo, subregion of the Atlantic forest.

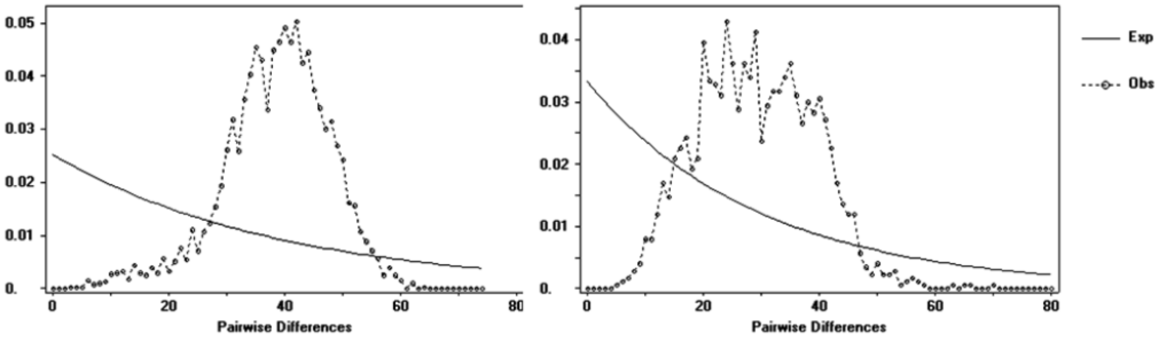

Fig. 4 Expected (solid line) and observed (dashed line) mismatch distributions among individual of *Artibeus planirostris* (left) and *Carollia perspicillata* (right) in a population growth model.

Table 1 Number of bat specimens (n), polymorphic *loci* and private bands for regions and populations.

Species	Region	Population	n	Polymorphic	Private

		Loci	Bands
Artibeus planirostris	91	143	
Pernambuco subregion Atlantic forest	37	111	18
Tamandaré	16	89	6
Igarassu	10	30	1
São Lourenço da Mata	11	66	8
SDTF	29	115	16
Serra Talhada	19	105	5
Triunfo	10	39	1
Brejo subregion Atlantic forest	25	83	13
Caruaru	15	67	1
Buíque	10	66	0
Carollia perspicillata	60	116	
Pernambuco subregion Atlantic forest	27	99	27
Tamandaré	10	39	0
Igarassu	11	92	19
São Lourenço da Mata	6	29	0
SDTF	15	43	4
Serra Talhada	8	33	1
Triunfo	7	36	2

Brejo subregion	Atlantic forest	18	75	12
	Caruaru	9	71	4
	Buíque	9	61	1

Table 2 Results of a three-level analysis of molecular variance.

Species	**Source of variation**	**df**	**Summ of square**	**Variance components**	**Percentage of variation**
A. planirtostris					
	Among Regions	2	194.075	-0.9282	-0.46
	Among population within regions	4	358.671	6.14328	30.51
	Within population	84	1183.364	14.08766	69.96
C. perspicillata					
	Among Regions	2	121.574	1.52455	9.93
	Among population within regions	4	124.178	2.29874	14.97
	Within population	53	611.348	11.53487	75.11

Table 3 F_{st} values between regions. The lower diagonal corresponds to *A. planirostris* and the upper diagonal to *C. perspicillata*.

	Pernambuco subregion		Brejo subregion
	Atlantic forest	SDTF	Atlantic forest

Pernambuco subregion			
Atlantic forest	--	0.16502	0.13767
SDTF	0.12181	--	0.19784
Brejo subregion Atlantic forest	0.11205	0.16699	--

6 DISCUSSÃO GERAL

Diante das questões levantadas no presente trabalho foi possível identificar um padrão filogeográfico na espécie *A. planirostris*. Assim como, compreender melhor a estrutura genética de *A. planirostris* e *C. perpscillata* ao longo de diferentes populações e ambientes. É fato que o entendimento da diversidade genética é uma das principais linhas de estudo em biologia da conservação, pois fornece informações relevantes sobre o pontecial adaptativo das espécies (Moritz, 1994). Não é por menos que nos últimos anos tenha se tornado primordial para ações de cunho conservacionista (Farjado *et al.,* 2016).

Para avaliação da filogeografia de *A. planirostris* foi fundamental o uso de amostras que compreendessem toda a distribuição geográfica da espécie. Desta forma, foi possível avaliar a espécie em todo o gradiente espacial em que ela se encontra, tornando mais efetivo e robusto o estudo. As árvores filogeográficas, construídas através de diferentes algoritmos, convergiram para a mesma topologia. Esta topologia indicou, geograficamente, a influência da vicariância causada pela elevação dos Andes, fato este documentado em estudos com morcegos (Hoffman e Baker, 2003; Redondo *et al.*, 2008) além de outros organismos (Cortés-Ortiz *et al.,* 2003; Dick *et al.*, 2003; Zeh *et al.*, 2003; Cheviron *et al.*, 2005; Eberhard e Bermingham, 2005; Gutiérrez *et al.*, 2014b).

Para aumentar a robustez destas conclusões, datações, com abordagem molecular, vem sendo empregadas (Eizirik *et al.,* 2001). Elas auxiliam no esclarecimento de controvérsias geradas por estudos paleontológicos clássicos e modernos (Bromham *et al.,* 1999; Foote *et al.*, 1999). Como visto no presente estudo, a datação foi essencial para associar a principal divisão de clados da árvore com o evento de elevação dos Andes. A datação que estimou em aproximadamente 3,44 milhões de anos a separação deste ramo, coincide com um dos momentos de elevação mais acentuada dos andes ocorrido no Plioceno (Hoorn *et al*., 2010).

Outros eventos de vicariância sucedidos no neotrópico puderam ser testados no estudo com *A. planirostris.* Como a separação entre as Antilhas e o continente sul-americano. Aonde mostrou-se similar com a porção leste da Floresta Amazônica, indicando o possível fluxo gênico entre continente e ilha e/ou isolamento recente. Este fato podemos atribuir a alta vagilidade dos morcegos devido ao voo. Sendo esta conclusão corroborada por outro estudo realizado por Hunt *et al.* (2001) com aves (vertebrado de vagilidade similar) que obteve resultado similar. A biologia dos quirópteros permite que explorem paisagens fragmentadas em busca de alimento e abrigo por longas distâncias (Bernard e Fenton, 2003). Portanto os diferentes ambientes visitados pelos morcegos são relevantes na estruturação das populações (Fogaça e Reis, 2008).

Assim como a formação dos Andes, outros eventos geo-climáticos influenciaram diretamente a dispersão da biota neotropical. Este é o caso do cinturão seco formado pela Caatinga, Cerrado e Chaco (Koopman, 1976; Silva *et al*. 2001). Esta formação xérica, pode ter funcionado como uma barreira ambiental separando a Floresta Atlântica da Amazônia. Em nosso estudo as amostras de florestas secas agruparam-se com as de Floresta Atlântica, provavelmente estas amostras ficaram isoladas nos períodos inter-glaciais ocorridos no Pleistoceno, devido a transgressões e retrações marítimas. Estando este momento alinhado com nossa datação (~2,12 milhões de anos). A influência deste cinturão seco também foi verificada em outros morcegos (Lopes e Ditchfield, 2009; Martins *et al.,* 2009; Pavan *et al.*, 2011; Ferreira *et al*., 2014). Além de outros grupos animais como répteis (Pellegrino *et al.*, 2005), anfíbios (Elme *et al.*, 2007), e aves (Bates *et al.*, 1998). Estes eventos acarretam um afunilamento das populações e posterior expansão, fato observado no nosso estudo através de testes de neutralidade.

Diante do exposto, é evidente que os eventos geoclimáticos durante o plioceno e pleistoceno atuaram diretamente na estrutura genética dos vertebrados neotropicais. Não obstante a isto, nosso estudo sobre a estruturação genética de duas espécies de morcegos no nordeste brasileiro, evidenciou a diferenciação genética de populações, mesmo diante de curtas distâncias entre populações (até ~400km).

A partir da análise molecular de variância, a espécie *C. perspicillata* mostrou-se sensível ao ambiente de origem. Este resultado pode estar associado com a baixa mobilidade da espécie para forrageio que não ultrapassa 2,5 km (Heithaus & Fleming, 1978; Bernard & Fenton, 2003). Isto diminui a capacidade de fluxo gênico da espécie e pode causar diferenciação regional. Podendo ter relação com estudos que detectaram variação morfológica a nível regional (Cloutier e Thomas, 1992; Nowak, 1994). Outro fato que contribui para isto é a dieta especializada da espécie que consiste de espécies da Família Pipperaceae (Peracchi *et al.*, 2006). Pois dietas especializadas estão associadas a forte estruturação populacional (Kitahara *et al.*, 2000; Carnaval, 2002).

Em contrapartida, espécies do gênero *Artibeus* podem deslocar-se por até 25 km em busca de recurso (Esberard, 2003). Isto acarreta maior fluxo interpopulacional, fato reforçado pela ausência de variação morfológica (Taddei,1998). Segundo Carnaval (2002) esta espécie possui grande plasticidade em ocupar ambientes, espécies com esta característica costumam não possuir um padrão de estrutura genética definido.

Ao avaliar especificamente o ambiente, pode-se perceber que os espécimes de florestas secas sazonais são os mais distintos geneticamente em ambas as espécies, existindopossíveis explicações para isto. Primeiramente, o fato de que neste ambiente, devido à escassez de recursos, os animais necessitam se deslocar mais, acarretando em maior homogeneidade dentro da população (Cerqueira, 1985; Fleming, 1988; Bernard e Fenton, 2003). Outro motivo é que as sub-regiões de Floresta Atlântica (Pernambuco e Brejo) possuem uma história evolutiva em comum, compartilham características que as fazem se diferenciarem da floresta seca (Pôrto *et al.,* 2004). Além disso, a diferenciação destas populações foi também influenciada pelo isolamento por distancia que se mostrou moderado, mas significativo. Análises intrapopulacionais indicaram expansão populacional em ambas espécies o que é comum em organismos da região (Carnaval e Moritz, 2008, Redondo *et al.,* 2008).

Haja vista o exposto, os padrões genéticos (filogeografia, estruturação populacional e história demográfica) observados hoje são um reflexo da biologia do animal e eventos que variam em tempo, escala e intensidade.

7 CONCLUSÕES

1. A filogeografia da espécie *A. planirostris* mostrou uma divisão em subgrupos delimitados geograficamente pelos Andes e pela diagonal seca. O que está em acordo com os eventos de vicariância da Região Neotropical;
2. As datações propostas neste estudo coincidem com os eventos geo-climáticos que causaram diferenciação genética dentro da espécie *A. planirostris*;
3. A filogeografia de *A. planirostris* também indicou que a espécie passa por um processo de expansão, também observado em outros vertebrados da Região Neotropical;
4. As populações de *A. planirostris* e *C. perspicillata* estudadas no nordeste brasileiro apresentaram forte estruturação ao nível de população. Isto indica que mesmo com a grande vagilidade dos morcegos em relação a outros mamíferos, eles conseguem manter características genéticas distintas em populações próximas;
5. A espécie *C. perspicillata* apresentou também diferenciação genética ao nível de ambiente fitofisionômico. Em estudo prévio havia sido relatada diferenciação morfológica relacionado a variação ambiental. Esta espécie possui deslocamento de forrageio limitado o que favoreceria a especialização por ambiente;
6. A maior semelhança genética entre os indivíduos de *A. planirostris* e *C. perspicillata* das populações da floresta seca sugere maior fluxo genético dentro deste ambiente. Podendo ser explicado pela maior escassez de recursos que gera necessidade um maior deslocamento dos morcegos.

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ANEXO A - INSTRUÇÕES PARA AUTORES (JOURNAL OF MAMMALOGY)

Instructions to authors

The *Journal of Mammalogy* is an international, peer-reviewed publication of the American Society of Mammalogists (ASM). We publish manuscripts presenting original research and scholarship on mammals, including topics in mammalian evolution, ecology, behavior, systematics, management, and conservation. Articles should be of interest to a broad scientific readership and may include theoretical or empirical studies that advance our understanding of any aspect of mammalian biology. Book reviews generally may be solicited or readers may request to review a book of general interest to mammalogy (contact the Editor for Reviews).

All submissions are subject to review. Initial review is done by our Editor-in-Chief and by 1 of the Associate Editors, who evaluate whether the manuscript is of sufficient quality and general interest for outside review. Manuscripts that pass this initial evaluation will be sent to 1 or more outside reviewers. The assigned Associate Editor then evaluates the reviews and the manuscript and makes a recommendation to the Editor-in-Chief, who makes the final decision regarding suitability for publication.

Submitted manuscripts should be free of jargon. The editors reserve the right to edit all manuscripts for style and clarity. Contributions are accepted for review and publication on the condition that they are submitted solely to *Journal of Mammalogy* and will not be reprinted or translated without the publisher’s permission, although authors retain copyrights. ASM recommends that original data reported be deposited in a suitable public database. Send inquiries to the editorial office.

Manuscript Preparation

Submission

Submit all manuscripts through ScholarOne. Once you have prepared your manuscript according to the instructions below, please visit the online submission web site. All articles must include a statement in the Materials and Methods section confirming that the study conforms to published ASM guidelines for the use of wild mammals in research (see below) as well as to any relevant institutional requirements (e.g., in the United States, IACUC approval must be confirmed). Submissions from authors whose research involved the use of “human subjects” (as defined in federal law) must include evidence of approval from an institutional review board.

Document Format

Use double-spacing and 12-point Times New Roman font throughout all text, tables, references, and figure captions. Number all pages and insert line numbers continuously throughout your manuscript. Avoid the use of appendices and footnotes in the text. Put tables and figure captions at the end of the document. The title page should contain authors’ names, titles, affiliations, and postal and e-mail addresses.

Style.—Follow *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers* (currently in its 8th ed.), for conventions in biology. For general style and spelling, consult the *Chicago Manual of Style* (currently in its 16th ed.), *The Elements of Style* (currently in its 4th ed.), and a dictionary such as *Merriam-Webster’s Collegiate Dictionary*. The *Journal of Mammalogy* uses idiomatic American English. *Mammal Species of the World*, 3rd ed., or *Handbook of the Mammals of the World* (5 of 9 volumes published as of March 2016) are our baselines for mammal taxonomy. Newer names accepted; older names need justification. Serial commas should be employed (“a, b, and c” rather than “a, b and c”). Avoid modifier strings, sequential parentheses, and lost subjects. Distinguish between hyphens (“wild-caught”), en-dashes (“4–5 dolphins”), and em-dashes (used in many JM header styles). En-dashes and em-dashes may be inserted as Special Characters, although most word processors will convert a dash to an en-dash if bracketed by spaces; em-dashes may also be indicated with 3 sequential dashes (this ensures that type setters do not inadvertently confuse this for a different symbol).

Symbols, Abbreviations, Acronyms, and Units of Measure.—Define all nonstandard symbols, and spell out all acronyms. Avoid abbreviations and acronyms unless a term is used numerous times (minimum 3–5). Use the metric system, SI units (Système international d’unités), to express weights and measures. For details on technical style follow the most recent edition of the CSE Manual (currently 8th ed.). “Holocene” not “Recent”; “K/Pg” not “K/T”; use BP for years before present, My to indicate 10^6 years, and Ma to indicate million years ago (equivalent to Ma BP for million years before present); e.g., “The Cretaceous Periods lasted 80 My, from 144 Ma to 65 Ma” (from CSE 8th ed.).

All details of statistical outcomes reported should be provided, and degrees of freedom should be reported as subscripts of test statistics (e.g., $t_2 = 3.76$, $P < 0.04$; $F_{6,198} = 0.253$, $P = 0.618$).

Other statistical standards:

SD, SE, d.f.
All tests in italics: *F, G, H, P, R, r, R2, t, U, V, W, z,* etc.
Spell out mean in text, but use $\bar{X}$ with values; e.g., mean ± *SD*, but $\bar{X} \pm SD$.
Spell out Chi-square test but use X^2 with values; e.g., “We used a chi-square test . . .”, but $X^2 = 234.55$.
Define analysis of variance and other statistical acronyms (e.g., coefficient of variation, confidence interval, etc.) at 1st use; thereafter use ANOVA, *CV*, *CI*, etc.

Always a space on either side of =, <, >, ≤, ≥.

General.—Your manuscript should include: title page, text of manuscript, Acknowledgments, Supporting Information (if needed), Literature Cited, Figure Legends, Appendix(es) as needed, Tables, Figures.

1. Title page should include:

a. Contact information for corresponding author, including email address (single spaced).

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f. Abstract. Summarizes key findings. NO heading. ≤ 5% of the length of the text (Introduction through Discussion).

g. All manuscripts reporting on research from Latin America MUST include a summary in either Spanish or Portuguese, usually a translation of the Abstract. For work based in other non-English speaking countries, a foreign-language abstract is encouraged and welcome. No heading is required for the translation.

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2. Text of manuscript.

a. The *Journal of Mammalogy* employs up to 4 levels of headings, although most articles skip #2 headings. These are as follows:

#1	**Cap and Small Cap Bold Centered**
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#3	*Italic, paragraph indent, initial cap only: lowercase after colon.—Text follows em-dash (either use 3 dashes or insert em-dash as a Special Character).*
#4	Roman, Paragraph Indent, Cap and Lowercase Followed by Period: lowercase after colon (text follows a standard en-dash, or 2 spaces).

b. Additionally, taxonomic synonymic headings may be used. These differ from headings listed above, and include full attribution of the author of the name followed by a comma and the year of publication. Common names of new species, if provided, are centered on the next line. NOTE that attribution is distinct from citation, and this formatting style formally distinguishes nomenclatural attribution from literature citation (which lack the comma), although authors should include these nomenclatural references in the Literature Cited. This formatting keeps the *Journal of Mammalogy* consistent with attribution given in synonymies for *Mammalian Species*; for further guidance see Gardner and Hayssen (2004) 'A guide to constructing and understanding synonymies for Mammalian Species' (*Mammalian Species* 739:1–17):

Family Molossidae

(genus and species indeterminate)

Rattus detentus, new species Timm, Weijola, Aplin, Flannery, and Pine

Admiralties Rat

Sorex merriami Dobson, 1890

Crocidura rapax G. Allen, 1923

c. Left-justify text following a #1 header unless this includes a lower header; indent subsequent paragraphs.

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The following examples are typical of references in *Journal of Mammalogy* and *Mammalian Species*; refer to recent issues for additional formatting guidance.

Journal Articles

Dumbacher, J. P., G. B. Rathbun, T. O. Osborne, M. Griffin, and S. J. Eiseb. 2014. A new species of round-eared sengi (genus *Macroscelides*) from Namibia. Journal of Mammalogy 95:443–454.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy in press.

Smith, J. B., T. W. Grovenburg, K. L. Monteith, and J. A. Jenks. 2015. Survival of female bighorn sheep (*Ovis canadensis*) in the Black Hills, South Dakota. American Midland Naturalist 174:290–301.

Stapp, P., and G. A. Polis. 2003. Influence of pulsed resources and marine subsidies on insular rodent populations. Oikos 102:111–123.

Vieira, M. V. 2003. Seasonal niche dynamics in coexisting rodents of the Brazilian cerrado. Studies on Neotropical Fauna and Environment 38:7–15.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Studies 46 (Supplement):120–139.

Books

Gardner, A. L. (ed.). 2007 [2008]. Mammals of South America. 1. Marsupials, xenarthrans, shrews, and bats. University of Chicago Press. Chicago, Illinois. (See Emmons [2008] below as example of reference for a treatment within this book).

Groves, C., and P. Grubb. 2011. Ungulate taxonomy. Johns Hopkins University Press. Baltimore, Maryland.

Hall, E. R. 1981. The mammals of North America. 2nd ed. John Wiley & Sons, Inc. New York 1:1–600 + 90. [OR . . . 2:601–1181 + 90.]

Neal, E. G., and D. C. Cheeseman. 1996. Badgers. Poyser Natural History. London, United Kingdom.

Peterson, A. T., et al. 2011. Ecological niches and geographic distributions. Princeton University Press. Princeton, New Jersey. (Note that this reference has 7 authors).

Wilson, D. E., and D. M. Reeder. 2005. Mammal species of the world: a taxonomic and geographic reference. 3rd ed. Johns Hopkins University Press. Baltimore, Maryland. (See Musser and Carleton (2005) below for how to cite sections within this book).

Chapter in Edited Books

Emmons, L. H. 2007 [2008]. Genus *Caluromysiops*. Pp. 11–12 in Mammals of South America. 1. Marsupials, xenarthrans, shrews, and bats (A. L. Gardner, ed.). University of Chicago Press. Chicago, Illinois. (See Gardner [2008] above for citing the entire volume).

Fahr, J. 2013. *Rhinolophus ziama*. Ziama horseshoe bat. Pp. 355–356 in Mammals of Africa. 4. Hedgehogs, shrews, and bats (M. Happold and D. C. D. Happold, eds.). Bloomsbury Publishing. London, United Kingdom.

Goin, F. J., J. N. Gelfo, L. Chornogubsky, M. O. Woodburne, and T. Martin. 2012. Origins, radiations, and distribution of South American mammals: from greenhouse to icehouse worlds. Pp. 20–50 in Bones, clones, and biomes: the history and geography of Recent Neotropical mammals (B. D. Patterson and L. P. Costa, eds.). University of Chicago Press. Chicago, Illinois.

Kley, N. J., and M. Kearney. 2007. Adaptations for digging and burrowing. Pp. 284–309 in Fins into limbs (B. Hall, ed.). University of Chicago Press. Chicago, Illinois.

Musser, G. G., and M. D. Carleton. 2005. Superfamily Muroidea. Pp. 894–1531 in Mammal species of the world: a taxonomic and geographic reference. 3rd ed. (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press. Baltimore, Maryland. (See Wilson and Reeder (2005) above for citing the full book).

Technical Reports

Carey, A. B., B. L. Biswell, and J. W. Witt. 1991. Methods for measuring populations of arboreal rodents. U.S. Forest Service, General Technical Report PNW-GTR-273:1–24.

Griggs, F. T., and River Partners. 2009. California riparian habitat restoration handbook. 2nd. ed. July 2009. California Riparian Habitat Joint Venture. Sacramento, California.

Zielinski, W. J. 1995. Track plates. Pp. 67–89 in American marten, fisher, lynx, and wolverine: survey methods for their detection (W. J. Zielinski and T. E. Kucera, eds.). U.S. Forest Service, General Technical Report PSW-GTR-157:1–163.

Proceedings

Armitage, K. B., and D. T. Blumstein. 2002. Body-mass diversity in marmots. Pp 22–40 in Holarctic marmots as a factor of biodiversity. Proceedings of the 3rd International Conference on Marmots, Cheboksary, Russia, 25–30 August 1997 (K. B. Armitage and V. Y. Rumiantsev, eds.). ABF Publishing House. Moscow, Russia.

Theses or Dissertations

Quaife, L. R. 1978. The form and function of the North American badger (*Taxidea taxus*) and its fossorial way of life. M.S. thesis, University of Calgary. Calgary, Alberta, Canada.

Steward, P. D. 1997. The social behaviour of the European badger, *Meles meles*. Ph.D. dissertation, University of Oxford. Oxford, United Kingdom.

Web sites (Do not include web addresses in manuscript text, cite them as “(Author Year)” and include web address in Literature Cited).

CDFW [California Department of Fish and Wildlife]. 2008. State & federally listed endangered & threatened animals of California. January 2013. www.dfg.ca.gov/biogeodata/cnddb/pdfs/TEAnimals.pdf. Accessed 15 July 2013.

IUCN. 2015. The IUCN Red List of threatened species. Ver. 2015.3. www.iucnredlist.org. Accessed 6 November 2015.

To cite the IUCN assessment of a given species, use the following template

Author(s). Year. *Species name*. In: IUCN 2015. The IUCN Red List of Threatened Species. Version 2015.3. www.iucnredlist.org. Accessed 6 November 2015.

Software (Cite software as "(Author Year)" in text and include citation in literature cited.)

ESRI. 2002. ArcView GIS. Ver. 3.3. Environmental System Research Institute, Inc. Redlands, California.

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Jenness Enterprises. 2002. Kit fox telemetry. A custom-written extension for ArcView 3.3. Jenness Enterprises. Flagstaff, Arizona.

Kenward, R. E., N.M. Casey, S. S. Walls, and A. B. South. 2014. Ranges9: for the analysis of tracking and location data. Online manual. Anatrack Ltd. Wareham, United Kingdom.

McCune, B., and M. J. Mefford. 2011. PC_ORD. Multivariate analysis of ecological data. Ver, x. MjM Software. Gleneden Beach, Oregon.

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. www.R-project.org/.

Rodgers, A. R., and A. P. Carr. 1998. HRE: the Home Range Extension for ArcView. Centre for Northern Forest Ecosystem Research, Ontario Ministry of Natural Resources. Thunder Bay, Ontario, Canada.

SAS Institute Inc. 2008. SAS/STAT user's guide. Release 9.2. SAS Institute, Inc. Cary, North Carolina.

Statsoft Inc. 2002. Statistica. Release 6. Statsoft, Inc. Tulsa, Oklahoma.

Swofford, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods). Ver. 4. Sinauer Associates, Inc., Publishers. Sunderland, Massachusetts.

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ANEXO B - INSTRUÇÕES PARA AUTORES (ZOOLOGICA SCRIPTA)

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For the journals Aims and Scope please click on this link

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Molecular sequences should be deposited in GenBank, and authors are encouraged to deposit anatomical images in MorphoBank database. Authors are strongly encouraged to deposit voucher specimens for DNA, and to desposit alignments in e.g. the EMBL database.

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Names of species and genera should be italicized or underlined. Spell out the entire scientific name the first time a species is mentioned; thereafter, abbreviate the generic part of the name (first letter and period) except at the beginning of a paragraph or sentence. New taxa should be indicated by the Latin abbreviations 'sp. n.', 'gen. n.' etc, and should be mentioned in the abstract. Use SI units and appropriate standard abbreviations.

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Description of taxa

Descriptions of new taxa should be arranged as in the following example:

Genus *Pachygnatha* Sundevall, 1823

Pachygnatha atromarginata sp. n. (Figs 100-111)

Holotype. m, CAMEROON, Mount Koupé, 1600 m, rain forest, 8 February 1983, Bosmans & Bosselaers (sweep net), (MRAC).

Paratypes. Same data as for holotype, 4 mm 8ff W (MRAC).

Other material. Mount Koupé, 1300 m, rain forest, 1f, 31 January 1983 (pitfall trap); 1f, 2 February 1983 (sweep net) (MRAC Mount Koupé, 900 m, rain forest, 1 m (subadult) 2ff, 31 January 1983 (pitfall trap), Bosmans & Bosselaers (all MRAC).

Etymology. The name refers to the dark, reticulated marginal zone of the carapace.

Diagnosis. This species can be distinguished by the shape of the paracymbium (Fig. 103) and the vulvar morphology (Fig. 111) in the female.

Description

Male (holotype). Total body length 2.38mm, 1 car 1.15mm, 1 stern 0.66 mm, 1 abd 1.45 mm, w car 0.93 mm ...

Female (paratype). Total body length 3.03mm ...

The complete data of the holotype and paratypes, and the institutions in which they are deposited, must be recorded in the original description. All material examined should be listed in similar format: localities should be cited in order of increasing precision as in the examples; names of countries should be in capitals. Sex symbols will be used in the journal; abbreviations used to indicate sex must be clearly stated in the manuscript.

Redescriptions of taxa should be arranged as in the following example:

Family PHYLLODOCIDAE

Genus *Phyllodoce* Lamarck, 1818

Phyllodoce citrina Malmgren, 1865: Fig. 2.

Phyllodore citrina Malmgren, 1865: 95-96, pl. XIII, fig. 24.

Phyllodoce badia Malmgren, 1867: 22, pl. II, fig. 6.

Anaitides citrina Bergström 1914: 140-141, fig. 41; Eliason 1962a: 18; Hartmann-Schröder 1971: 105-107, fig. 33D-F; Uschakov 1972: 136-137, pl. V, figs 5, 6.

Material examined. Phyllodoce citrina: 3 syntypes from Spitsbergen (SMNH type collection 2419 and 2420); 1 syntype from Spitsbergen (BMNH 1865.9.23.3); about 30 specimens from Wales, Shetland, western Norway, Spitsbergen, and Greenland (SMNH, MZB); about 10 specimens from the Arctic, Bering Sea and the Sea of Okhotsk (ZIL). *Phyllodoce badia*: several syntypes (at least 4 specimens and some fragments) from Bohuslän, Sweden (SMNH type collection 2423).

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Computer programs

Swofford, D. L. 1993. PAUP - *Phylogenetic Analysis Using Parsimony. Ver. 3. 1*. [Computer software and manual]. Champaign, Illinois: Illinois Natural History Survey.

On-line source

Eriksson, T. (1996, June). AutoDecay version 2.9.2. Available via http:/www.botan.su.se/systematik/Folk/Torsten.html

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ANEXO C - CURRÍCULO LATTES

Francisco Geraldo de Carvalho Neto

Curriculum Vitae

Dados pessoais

Nome	Francisco Geraldo de Carvalho Neto
Filiação	CLÁUDIO GERALDO RODRIGUES DE CARVALHO e MARIA LEONICE BEZERRA DE BRITO CARVALHO
Nascimento	19/06/1988 - SERRA TALHADA/PE - Brasil
Carteira de Identidade	7199962 SDS - PE - 04/12/2002
CPF	075.906.684-19

Formação acadêmica/titulação

2013 - 2017

Doutorado em Genética.
Universidade Federal de Pernambuco, UFPE, Recife, Brasil
Título: Uma abordagem molecular da filogeografia e estruturação populacional de morcegos filostomídeos neotropicais, Ano de obtenção: 2017
Orientador: Neide Santos
Co-orientador: Martín Alejandro Montes
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2011 - 2013

Mestrado em Saúde Humana e Meio Ambiente.
Universidade Federal de Pernambuco, UFPE, Recife, Brasil
Título: Ecologia, assimetria flutuante e dimorfismo sexual de pequenos mamíferos em três remanescentes de Floresta Atlântica na região Nordeste do Brasil, Ano de obtenção: 2013
Orientador: Ana Cristina Lauer Garcia
Co-orientador: Martín Alejandro montes
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2006 - 2010

Graduação em Bacharelado em Ciências Biológicas.
Universidade Federal Rural de Pernambuco-Unidade Acadêmica de Serra Talhada, UFRPE-UAST, Brasil
Título: COMUNIDADES DE MORCEGOS EM DUAS ÁREAS DE CAATINGA NO SERTÃO DE PERNAMBUCO
Orientador: Ednilza Maranhão dos Santos
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico

Formação complementar

2010 - 2010

Curso de curta duração em Morfologia Craniana de Mamíferos. (Carga horária: 12h).
Universidade Federal do Vale do São Francisco, UNIVASF, Petrolina, Brasil

2008 - 2008

Curso de curta duração em Métodos Cromatográficos. (Carga horária: 5h).
Unidade Acadêmica de Serra Talhada, UAST-UFRPE, Brasil

2007 - 2007

Curso de curta duração em Ecologia e comportamento de Aranhas.

Unidade Acadêmica de Serra Talhada, UAST-UFRPE, Brasil

Atuação profissional

1. Universidade Federal de Pernambuco - UFPE

Vínculo institucional

2013 - 2017	Vínculo: Bolsista , Enquadramento funcional: Doutorando, Regime: Dedicação exclusiva
2011 - 2013	Vínculo: Outro (especifique) , Enquadramento funcional: Estudante de mestrado, Regime: Dedicação exclusiva

2. Secretaria de Educação do Estado de Pernambuco - SE-PE

Vínculo institucional

2010 - 2010	Vínculo: Professor Contratado , Enquadramento funcional: Professor Substituto , Carga horária: 25, Regime: Parcial Outras informações:

Professor substituto das disciplinas de Biologia e matemática para turmas do ensino médio e fundamental.

3. Universidade Federal Rural de Pernambuco - UFRPE

Vínculo institucional

2009 - 2009	Vínculo: - , Enquadramento funcional: Estagiário , Carga horária: 20, Regime: Parcial Outras informações:

Estágio em zoologia e genética de Morcegos com o Prof. Dr. Martín Alejandro Montes

2007 - 2008	Vínculo: livre , Enquadramento funcional: Estagiário, Regime: Parcial

Atividades

05/2007 - 06/2008	Estágio, Unidade Acadêmica de Serra Talhada *Estágio:* *Estagiário do laboratório de Informática e membro do NTI(núcleo de tecnologia de informação) da UFRPE-UAST, desenvolvendo soluções em tecnologia de informação.*

4. Prefeitura da Cidade do Recife - PCR

Vínculo institucional

2017 - Atual	Vínculo: Servidor público , Enquadramento funcional: Coordenador

de Gabinete , Carga horária: 40, Regime: Integral

Outras informações:

Coordenador de Articulação no Gabinete de Representação em Brasília e Relações Internacionais.

Projetos

Projetos de pesquisaProjetos de pesquisa**2013 - Atual** DIVERSIDADE GENÉTICA E ECOLÓGICA DE PEQUENOS MAMÍFEROS EM DIFERENTES BIOMAS NO ESTADO DE PERNAMBUCO, BRASIL

Descrição: O projeto pretende mensurar variabilidade genética inter e intrapopulacional de mamíferos em diferentes fitofisionomias; Realizar uma análise ecológica das comunidades de pequenos mamíferos avaliando a variação temporal e espacial das espécies em diferentes ambientes; Correlacionar o tamanho e o nível de conservação do ambiente com sua variabilidade genética e ecológica; Auxiliar, com as informações genéticas e ecológicas geradas, no manejo das unidades de conservação estudadas.
Situação: Em andamento Natureza: Projetos de pesquisa
Alunos envolvidos: Doutorado (1);
Integrantes: Francisco Geraldo de Carvalho Neto; martin alejandro montes; Neide Santos (Responsável); Valdir de Queiroz Balbino

2011 - 2012 Diversidade ecológica de pequenos mamíferos em três fragmentos de Floresta Atlântica na região Nordeste do Brasil

Descrição: Descrever as espécies de pequenos mamíferos presentes em três fragmentos de Floresta Atlântica no estado de Pernambuco, são eles:Estação Ecológica do Tapacurá (município de São Lourenço da Mata), Reserva Ecológica de Dois Irmãos (município de Recife) e Refúgio Ecológico Charles Darwin (município de Igarassu). Além disso, analisar a estrutura das populações, verificar o efeito da ação antrópica e estudar a variação estacional nesses fragmentos.
Situação: Em andamento Natureza: Projetos de pesquisa
Alunos envolvidos: Mestrado acadêmico (1);
Integrantes: Francisco Geraldo de Carvalho Neto; martin alejandro montes; Ana Cristina Lauer Garcia (Responsável)

2009 - 2010 Comunidades de Morcegos do Sertão do Pajeú

Descrição: Levantar a fauna de quirópteros de Serra Talhada, identificando as espécies da região, de forma a conhecer melhor aspectos como habitats, biologia reprodutiva e alimentação de cada uma delas. Este é um subprojeto do projeto: MASTOFAUNA DO SERTÃO DO PAJEÚ/PERNAMBUCO.Possuido uma bolsa de iniciação científica PIBIC/FACEPE/CNPq
Situação: Concluído Natureza: Projetos de pesquisa
Alunos envolvidos: Graduação (1);
Integrantes: Francisco Geraldo de Carvalho Neto; Ednilzão Maranhão dos Santos; martin alejandro montes (Responsável)

2008 - 2010 Mastofauna do Sertão do Pajeú

Situação: Concluído Natureza: Projetos de pesquisa
Alunos envolvidos: Graduação (1);
Integrantes: Francisco Geraldo de Carvalho Neto; Ednilzão Maranhão dos Santos (Responsável); martin alejandro montes

2008 - 2009 RÉPTEIS DA FAZENDA SACO, SERRA TALHADA/PE - INDICADORES DE CONSERVAÇÃO

Situação: Concluído Natureza: Projetos de pesquisa
Integrantes: Francisco Geraldo de Carvalho Neto; Ednilzão Maranhão dos Santos (Responsável)
Financiador(es): Universidade Federal Rural de Pernambuco-UFRPE

Revisor de periódico

1. BIOLOGICAL JOURNAL OF THE LINNEAN SOCIETY

Vínculo

2017 - Atual Regime: Parcial

Prêmios e títulos

2014 Menção Honrosa na área de Genética, Evolução e Melhoramento Animal, nível Doutorado., XX Encontro de Genética do Nordeste

Producão

Produção bibliográfica

Artigos completos publicados em periódicos

1. COUTINHO-SILVA, R.D.; MONTES, M.A.; Oliveira, G.F.; **DE CARVALHO-NETO, F.G.**; ROHDE, C.; GARCIA, A.C.L.
Effects of seasonality on drosophilids (Insecta, Diptera) in the northern part of the Atlantic Forest, Brazil. Bulletin of Entomological Research. , v.1, p.1 - 11, 2017.

2. **CARVALHO-NETO, F. G.**; SILVA, J. R.; SANTOS, N.; RHODE, C.; GARCIA, A. C. L.; MONTES, M. A.
The heterogeneity of Caatinga biome: an overview of the bat fauna. mammalia. , v.., p.. - , 2016.

3. GARCIA, ANA CRISTINA L.; ROHDE, CLAUDIA; **CARVALHO-NETO, FRANCISCO G.**; MONTES, MARTÍN A.; LEAL, EDSON S.B.
The bats of northeastern Brazil: a panorama. Animal Biology (Print). , v.64, p.141 - 150, 2014.

Capítulos de livros publicados

1. SANTOS, E. M.; MORAES, B. L. C.; ALMEIDA, G. V. L.; **CARVALHO-NETO, F. G.**
Vertebrados tetrápodes In: PARQUE ESTADUAL MATA DA PIMENTEIRA RIQUEZA NATURAL E CONSERVAÇÃO DA CAATINGA.1 ed.Recife-PE : Editora Universitária da UFRPE, 2013, v.1, p. 175-206.

Trabalhos publicados em anais de eventos (resumo)

1. VIEIRA, A. V. B.; SILVA, I. I. F. G.; **CARVALHO-NETO, F. G.**; LIMA, G. D. C.; COSTA, V. V.; RUSHANSKY, E.; MARIANO, M. H.; SOUZA, P. R. E.; MONTES, M. A.; MAIA, M. M. D.

ESTUDO PILOTO DO PERFIL DE METILAÇÃO DOS DINUCLEOTÍDEOS CPG -628, -610, -574 E -491 DO GENE IL-6 EM PACIENTES COM ARTRITE REUMATOIDE In: XXI Encontro de Genética do Nordeste, 2016, Recife, PE.
Anais do XXI Engene. , 2016.

2. SILVA, I. I. F. G.; **CARVALHO-NETO, F. G.**; ANGELO, H. D.; RUSHANSKY, E.; MARIANO, M. H.; SOUZA, P. R. E.; MONTES, M. A.; MAIA, M. M. D.
ESTUDO PRELIMINAR DO SNP -174 G/C E PERFIL DE METILAÇÃO DO GENE INTERLEUCINA-6 EM PACIENTES COM ARTRITE REUMATOIDE In: XXI Encontro de Genética do Nordeste, 2016, Recife, PE.
Anais do XXI Engene. , 2016.

3. SILVA, I. I. F. G.; **CARVALHO-NETO, F. G.**; ANGELO, H. D.; RUSHANSKY, E.; MARIANO, M. H.; SOUZA, P. R. E.; MONTES, M. A.; MAIA, M. M. D.
ESTUDO PRELIMINAR DO SNP -174 G/C E PERFIL DE METILAÇÃO DO GENE INTERLEUCINA-6 EM PACIENTES COM ARTRITE REUMATOIDE In: XXI Encontro de Genética do Nordeste, 2016, Recife, PE.
Anais do XXI Engene. , 2016.

4. **CARVALHO-NETO, F. G.**; MAIA, M. M. D.; GARCIA, A. C. L.; BALBINO, V. Q.; MONTES, M. A.; SANTOS, N.
FILOGEOGRAFIA DE ARTIBEUS PLANIROSTRIS (CHIROPTERA, PHYLLOSTOMIDAE): INFLUÊNCIA DOS ANDES E DA DIAGONAL SECA NEOTROPICAL In: XXI Encontro de Genética do Nordeste, 2016, Recife, PE.
Anais do XXI Engene. , 2016.

5. **CARVALHO-NETO, F. G.**; SANTOS, T. C.; BALBINO, V. Q.; SANTOS, N.; MONTES, MARTÍN A.
DIVERSIDADE E ESTRUTURA GENÉTICA POPULACIONAL DE ARTIBEUS PLANIROSTRIS E CAROLLIA PERSPICILLATA (CHIROPTERA; PHYLLOSTOMIDAE) NO NORDESTE DO BRASIL In: V Jornada da Pós-graduação em Genética - UFPE, 2015, Recife - PE.
Resumos da V Jornada da Pós-graduação em Genética - UFPE. , 2015.

6. Andrade, I. S.; **CARVALHO-NETO, F. G.**; GARCIA, A. C. L.; MONTES, M. A.; SANTOS, N.
Diversidade genética de Lonchirhina aurita (Mammalia), uma espécie de morcego vulnerável a extinção In: I SIMBRAFAUNA, 2015, Ipojuca - PE.
RESUMOS DO I SIMBRAFAUNA. , 2015.

7. SANTOS, T. C.; **CARVALHO-NETO, F. G.**; SANTOS, N.; MONTES, M. A.
DIVERSIDADE GENÉTICA NO GENE CITOCROMO DE CAROLLIA PERSPICILLATA NO ESTADO DE PERNAMBUCO In: XV JEPEX-UFRPE, 2015, RECIFE.
RESUMOS DO XV JEPEX. , 2015.

8. **CARVALHO-NETO, F. G.**; GARCIA, A. C. L.; BALBINO, V. Q.; MONTES, M. A.; SANTOS, N.
FILOGEOGRAFIA E ESTRUTURAÇÃO GENÉTICA DE ARTIBEUS PLANIROSTRIS, SPIX 1823 (CHIROPTERA; PHYLLOSTOMIDAE) In: V jornada da Pós-graduação em Genética - UFPE, 2015, Recife - PE.
Resumos da V Jornada da Pós-graduação em Genética - UFPE. , 2015.

9. da Silva, R. D. C.; Oliveira, G.F.; **CARVALHO-NETO, FRANCISCO G.**; MONTES, M. A.; RHODE, C.; GARCIA, A. C. L.
Seasonal variations in abundance and richness of drosophilids (Insecta, Diptera) in Atlantic Forest environments in Northeastern Brazil In: IX SIMPÓSIO DE ECOLOGIA,GENÉTICA E EVOLUÇÃO DE DROSOPHILA, 2015, Brasília - DF.
RESUMOS DO IX SIMPÓSIO DE ECOLOGIA,GENÉTICA E EVOLUÇÃO DE DROSOPHILA. , 2015.

10. FREITAS, L. C.; **CARVALHO-NETO, F. G.**; MONTES, M. A.; GARCIA, A. C. L.
VARIABILIDADE HAPLOTÍDICA NO GENE CITOCROMO b DE Artibeus planirostris NO ESTADO DE PERNAMBUCO In: XV JEPEX-UFRPE, 2015, RECIFE.

RESUMOS DO XV JEPEX. , 2015.

11. **CARVALHO-NETO, F. G.**; PESSOA, L. A.; GARCIA, A. C. L.; MONTES, M. A.; SANTOS, N. Diversidade genética de Artibeus Planirostris, Spix 1823 (Chiroptera; Phyllostomidae) em diferentes fitofisionomias no Nordeste do Brasil In: XX Encontro de Genética do Nordeste, 2014, Campina Grande - PB.
Anais do XX Encontro de Genética do Nordeste. , 2014.

12. SANTOS, T. C.; **CARVALHO-NETO, F. G.**; SANTOS, N.; MONTES, M. A.; GARCIA, A. C. L. DNA barcoding revela novo registro de roedor Echimyidae para a Floresta Atlântica do Nordeste In: XX Encontro de Genética do Nordeste, 2014, Campina Grande - PB.
Anais do XX Encontro de Genética do Nordeste. , 2014.

Apresentação de trabalho e palestra

1. **CARVALHO-NETO, FRANCISCO G.**
Cromossomos Metafásicos de mamíferos, 2015. (Conferência ou palestra,Apresentação de Trabalho)

2. **CARVALHO-NETO, FRANCISCO G.**
Técnicas de citogenética para observação de cromossomos de mamíferos, 2015. (Conferência ou palestra,Apresentação de Trabalho)

3. **CARVALHO-NETO, FRANCISCO G.**
Importância da Citogenética para identificação taxonômica: métodos e abordagens, 2014. (Conferência ou palestra,Apresentação de Trabalho)

4. **CARVALHO-NETO, FRANCISCO G.**
Técnicas modernas para análise de cariótipos, 2014. (Conferência ou palestra,Apresentação de Trabalho)

5. **CARVALHO-NETO, FRANCISCO G.**
Montagem e interpretação de cariótipos de cromossomos metafásicos de morcegos, 2013. (Conferência ou palestra,Apresentação de Trabalho)

Produção técnica

Demais produções técnicas

1. MONTES, M. A.; **CARVALHO-NETO, F. G.**
Genética da Conservação, 2013. (Outro, Curso de curta duração ministrado)

Orientações e Supervisões

Orientações e supervisões

Orientações e supervisões concluídas

Trabalhos de conclusão de curso de graduação

1. Ana Claúdia Caetano Pinheiro Leite. **Avaliação do conhecimento e impacto de palestras expositivas/demonstrativas sobre morcegos nos alunos de ensino fundamental e médio da região metropolitana do Recife.**. 2014. Curso (Ciências Biológicas) - Universidade Federal Rural

de Pernambuco

2. Jackson Roberto da Silva. **AVALIAÇÃO DA QUIROPTEROFAUNA DAS ECORREGIÕES DA CAATINGA**. 2013. Curso (Ciências Biológicas) - Universidade Federal Rural de Pernambuco

Eventos

Eventos

Participação em eventos

1. **V jornada da Pós-graduação em Genética**, 2015. (Outra)
Phylogeography and population structure of the Artibeus planirostris, Spix 1823 (Chiroptera; Phyllostomidae).

2. Simposista no(a) **I Simpósio de Genética e Biotecnologia**, 2014. (Simpósio)
Estudo de genes mitocondriais em estudos ecológicos.

3. Avaliador no(a) **VIII SIMPÓSIO INTEGRADO DE CIÊNCIAS DA SAÚDE E BIOLÓGICAS**, 2014. (Simpósio)
..

4. **XX Encontro de Genética do Nordeste**, 2014. (Encontro)
Diversidade genética de Artibeus Planirostris, Spix 1823 (Chiroptera; Phyllostomidae) em diferentes fitofisionomias no Nordeste do Brasil.

Organização de evento

1. **CARVALHO-NETO, F. G.**
XXI Encontro de Genética do Nordeste, 2016. (Congresso, Organização de evento)

2. **CARVALHO-NETO, F. G.**
V Jornada da Pós-graduação em Genética UFPE, 2015. (Outro, Organização de evento)

Bancas

Bancas

Participação em banca de trabalhos de conclusão

Curso de aperfeiçoamento/especialização

1. MONTES, M. A.; **CARVALHO-NETO, F. G.**; GUENTHER, M.
Participação em banca de Bruna Gonçalves Miller. **As Unidades de Conservação da Região Nordeste do Brasil**, 2013
(Perícia, auditoria e gestão ambiental) Universidade de Pernambuco

Graduação

1. MAIA, M. M. D.; CAVALCANTI, Y. V. N.; **CARVALHO-NETO, FRANCISCO G.**
Participação em banca de Amanda Virginia Batista Vieira. **Estudo do polimorfismo -174 G>C do gene da IL-6 (rs1800795) em pacientes com artrite reumatoide do Estado de Pernambuco**, 2016

(Ciências Biológicas) Universidade Federal Rural de Pernambuco

2. **CARVALHO-NETO, F. G.**; SANTOS, E. M.; MONTES, M. A.
Participação em banca de Anna Claudia Aca Ferreira. **Análises das dimensões básicas do nicho ecológico(espaço e alimento) de Lontra longicaudis (Olfers,1818) (Carnivora, Mustelidae) em um fragmento florestal no município de Igarassu - PE.**, 2014
(Ciências Biológicas) Universidade Federal Rural de Pernambuco

3. **CARVALHO-NETO, F. G.**; SANTOS, E. M.
Participação em banca de Luis Eduardo Alves Bezerra do Nascimento. **Modelagem de Distribuição Especial de Lontra longicaudis (Olfers, 1818) no Centro de Endemismo Pernambuco.**, 2013
(Ciências Biológicas) Universidade Federal Rural de Pernambuco

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Totais de produção

Produção bibliográfica

Artigos completos publicados em periódico	4
Capítulos de livros publicados	1
Trabalhos publicados em anais de eventos	30
Apresentações de trabalhos (Conferência ou palestra)	9

Produção técnica

Curso de curta duração ministrado (outro)	2

Orientações

Orientação concluída (trabalho de conclusão de curso de graduação)	2
Orientação em andamento (trabalho de conclusão de curso de graduação)	1

Eventos

Participações em eventos (congresso)	3
Participações em eventos (seminário)	2
Participações em eventos (simpósio)	3
Participações em eventos (oficina)	1
Participações em eventos (encontro)	5
Participações em eventos (outra)	4
Organização de evento (congresso)	1
Organização de evento (outro)	2
Participação em banca de trabalhos de conclusão (curso de aperfeiçoamento/especialização).	1
Participação em banca de trabalhos de conclusão (graduação)	4