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PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE E
BIOTECNOLOGIA-REDE BIONORTE**

**CONTRIBUIÇÕES À SISTEMÁTICA DO GÊNERO *Artibeus* Leach, 1821
(CHIROPTERA, PHYLLOSTOMIDAE) COM BASE EM DADOS MORFOLÓGICOS
E MOLECULARES: IMPLICAÇÕES PARA EVOLUÇÃO E CONSERVAÇÃO**

SAMIRA BRITO MENDES

Caxias - MA

2025

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Tese de doutorado apresentada ao Curso de doutorado do programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede BIONORTE, na Universidade Estadual do Maranhão, como requisito parcial para a obtenção do Título de Doutora em Biodiversidade e Biotecnologia.

Orientador (a): Profa. Dra. Maria Claudene Barros

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Banca examinadora

Documento assinado digitalmente
 **MARIA CLAUDENE BARROS**
Data: 23/07/2025 13:02:21-0300
Verifique em <https://validar.iti.gov.br>

Profa. Dra. Maria Claudene Barros (orientadora)
Universidade Estadual do Maranhão

Documento assinado digitalmente
 **ANDRE LUIZ SANTOS DE JESUS**
Data: 25/07/2025 12:55:09-0300
Verifique em <https://validar.iti.gov.br>

Prof. Dr. André Luiz Santos de Jesus
Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso

Documento assinado digitalmente
 **FABIANO CORREA**
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Prof. Dr. Fabiano Corrêa
Universidade Estadual do Maranhão

Documento assinado digitalmente
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Verifique em <https://validar.iti.gov.br>

Prof. Dr. Renato Gregorin
Universidade Federal de Lavras

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 **ALANA LISLEA DE SOUSA**
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Profa. Dra. Alana Lislea de Sousa
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RESUMO

O gênero *Artibeus* foi e tem sido alvo de grandes debates no que se refere a sua sistemática que se apresenta bastante confusa e controversa, principalmente por falta de informações suficientes e das enormes variações nos diferentes táxons do grupo. Este estudo integra duas abordagens para investigar a taxonomia, a sistemática e a dinâmica populacional do gênero *Artibeus*. A primeira parte da pesquisa foca em uma análise integrativa utilizando dados morfológicos, moleculares e morfogeométricos para elucidar as relações intra e interespecíficas das espécies do gênero. Neste estudo, geramos dois conjuntos de dados: um com dados de delimitação molecular com base na região do código de barras (COI) e o outro com dados de tamanho e forma cranianos usando métodos Morfometria Geométrica (MG). Os resultados da delimitação de espécies revelaram Unidades Taxonômicas Operacionais Moleculares (MOTUs) que apoiam a identificação morfológica das espécies. Análises usando técnicas MG demonstraram variações inter e intraespecíficas significativas no tamanho e forma cranianos entre espécies de *Artibeus*. Os resultados combinados sugerem a ausência de um sinal filogenético significativo influenciando a variação do crânio. Este cenário indica que potenciais fatores ecológicos históricos podem ter influenciado diretamente a morfologia craniana dessas espécies, atuando como forças de seleção significativas no espaço geográfico e gerando variações intraespecíficas em *Artibeus planirostris*. A segunda parte do estudo examinou a estrutura genética populacional de *A. planirostris* utilizando marcadores mitocondriais com uma amostragem extensiva. Nossas descobertas revelam considerável diferenciação genética entre populações com baixo fluxo gênico, especialmente em áreas periféricas quando comparadas com o norte da América do Sul. Isso sugere que adaptações locais, moldadas por pressões ambientais específicas e flutuações climáticas do Pleistoceno, podem ter influenciado as habilidades de dispersão e colonização de *A. planirostris* nessas regiões. Isso apoia a hipótese de que o isolamento por distância também pode contribuir para esse padrão de distribuição. A história demográfica de *A. planirostris* indica uma expansão populacional recente durante o Pleistoceno tardio, aproximadamente 50.000 a 60.000 anos atrás.

Palavras-chave: Morcegos; Morfologia; Delimitação molecular; Diversidade genética.

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ABSTRACT

The genus *Artibeus* has been the subject of extensive debate regarding its systematics, which remains complex and controversial, primarily due to insufficient information and significant variation across different taxa within the group. This study integrates two complementary approaches to investigate the taxonomy, systematics, and population dynamics of *Artibeus* in Brazil. The first part of the research employs an integrative analysis using morphological, molecular, and geometric morphometric data to elucidate intra- and interspecific relationships among species of the genus. In this study, we generated two datasets: one based on molecular species delimitation using the barcode region (COI) and another incorporating cranial size and shape data analyzed through Geometric Morphometrics (GM) methods. The species delimitation results identified molecular operational taxonomic units (MOTUs) that align with the morphological identification of the species. GM analyses revealed significant inter- and intraspecific variation in cranial size and shape among *Artibeus* species. The combined results suggest a lack of a strong phylogenetic signal driving skull variation. This finding indicates that historical ecological factors may have played a key role in shaping cranial morphology, acting as selective pressures across geographic space and contributing to intraspecific variation in *Artibeus planirostris*. The second part of the study investigated the population genetic structure of *A. planirostris* using mitochondrial markers with extensive sampling. Our findings reveal substantial genetic differentiation among populations, with limited gene flow, particularly in peripheral regions compared to northern South America. This suggests that local adaptations, shaped by specific environmental pressures and Pleistocene climatic fluctuations, may have influenced the dispersal and colonization capacity of *A. planirostris* in these areas. These findings support the hypothesis that isolation by distance may also contribute to the observed distribution pattern. The demographic history of *A. planirostris* indicates a recent population expansion during the Late Pleistocene, approximately 50,000 to 60,000 years ago.

Keywords: Bats; Morphology; Molecular delimitation; Genetic diversity.

APRESENTAÇÃO

A tese está estruturada em seis seções: introdução geral, referencial teórico, dois capítulos, o primeiro capítulo corresponde ao artigo publicado na *Biological Journal of the Linnean Society*, intitulado: **Morphological and molecular data combined reveal inter and intraspecific cranial shape variations in bats of *Artibeus* Leach, 1821 (Chiroptera: Phyllostomidae)**. O segundo capítulo corresponde ao artigo aceito para a publicação na *Mammalian Biology*, intitulado: **Population genetics and demographic history of flat-faced fruit-eating bat *Artibeus planirostris* (SPIX, 1823) (Chiroptera: Phyllostomidae)**. As seções finais da tese consistem em uma discussão integradora, na qual os principais achados dos capítulos são analisados em conjunto, e uma conclusão que sintetiza os resultados e suas implicações.

Esta tese foi desenvolvida no complexo GENBIMOL que compreende os laboratórios de Genética e de Biologia Molecular da Universidade Estadual do Maranhão, campus Caxias. A pesquisa com os morcegos *Artibeus* é resultante de projetos fomentados pela Fundação de Amparo à Pesquisa e o Desenvolvimento Científico e Tecnológico do Maranhão – FAPEMA Universal (#00797/13, #00715/15 e #00876/19), e pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES PROCAD Amazônia 2018 (#88887. 200514/2018-00).

O estudo recebeu aprovação do Comitê de Ética em Experimentação Animal (CEEa) da Universidade Estadual do Maranhão, sob o número de aprovação #06/2023-CEEa/CMV/UEMA. As coletas foram autorizadas pelo Sistema de Autorização e Informação em Biodiversidade e Instituto Chico Mendes de Conservação da Biodiversidade por meio das licenças SISBio/ICMBio nº 68047-4, 42670-3, 54384-2, e 74512-2. O manuseio e a manipulação dos morcegos foram conduzidos de acordo com as diretrizes estabelecidas pelo *Animal Care and Use Committee da American Society of Mammalogists*.

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1 INTRODUÇÃO

A morfologia do crânio em espécies de vertebrados reflete uma correlação com a evolução, uma vez que a dieta pode estar diretamente relacionada com a formação craniana. O crânio, os músculos cranianos e os dentes são exemplos de partes que desempenham funções específicas, a modificação de qualquer um desses componentes pode resultar em uma diversidade de partições morfológicas que podem dizer muito sobre a história evolutiva e dinâmica ecológica da espécie (MARCHÁN-RIVADENEIRA *et al.*, 2010; SANTANA *et al.*, 2012).

Dados morfológicos e de genes mitocondriais fornecem informações importantes na distinção entre espécies, principalmente em estudos de espécies crípticas, como ocorre em morcegos Neotropicais da família Phyllostomidae, que possuem uma alta diversidade morfológica (JONES *et al.* 2005; BAKER *et al.*, 2016). Ainda assim, estudos exclusivamente morfológicos e moleculares permanecem inconclusivos principalmente quando se refere as espécies do gênero *Artibeus* Leach, 1821 (REDONDO *et al.*, 2008; MARCHÁN-RIVADENEIRA *et al.*, 2010). Portanto, a integração desses estudos torna-se fundamental na elucidação das relações inter e intraespecíficas, fornecendo assim, subsídios para uma melhor compreensão acerca da taxonomia e sistemática do gênero *Artibeus*.

O gênero *Artibeus* é um dos mais diversos em termos de número de espécies, que são amplamente abundantes e distribuídas na região Neotropical, estendendo-se do México até a Argentina (SIMMONS, 2005; TAYLOR, 2019). As espécies possuem o hábito alimentar predominantemente frugívoro, mas ocasionalmente podem completar sua dieta com insetos, folha e néctar. Tem sido dividido em grandes e pequenos *Artibeus*, mas a classificação subgenérica permanece em debate. Os pequenos foram considerados como gênero *Dermanura* (Gervais, 1856) e os grandes como *Artibeus* (OWEN, 1991; LIM *et al.*, 2008; ROCHA *et al.*, 2018; SIMMONS e CIRRANELO, 2023; 2024), embora o gênero *Artibeus* no sentido amplo (*Artibeus* + *Dermanura*), seja considerado um grupo monofilético (SIMMONS, 2005; REDONDO *et al.*, 2008). O reconhecimento desses dois gêneros não é amplamente aceito com *Dermanura* sendo considerado por muitos autores como subgênero de *Artibeus* (OWEN, 1987; WETTERER *et al.*, 2000; SIMMONS, 2005; TAYLOR, 2019) ou classificado somente como *Artibeus* (GARBINO *et al.*, 2024).

Atualmente dez espécies de *Artibeus* são documentadas para o Brasil: *Artibeus amplus* Handley, 1987; *Artibeus concolor* Peters, 1865; *Artibeus fimbriatus* Gray, 1838; *Artibeus lituratus* (Olfers, 1818); *Artibeus obscurus* (Schinz, 1821); *Artibeus planirostris* (Spix, 1823); *Artibeus anderseni* Osgood, 1916; *Artibeus bogotensis* Andersen, 1906; *Artibeus cinereus*

(Gervais, 1856) e *Artibeus gnomus* Handley, 1987 (NOGUEIRA *et al.*, 2014; GARBINO *et al.*, 2024; ZORTÉA *et al.*, 2023). Aqui, seguimos a atualização de Garbino *et al.* (2024) com a classificação que considera todas as espécies como pertencentes ao gênero *Artibeus*.

Uma vez que as características morfológicas podem se sobrepor entre espécies dentro de um complexo, a integração de dados morfológicos e mitocondriais podem fornecer informações cruciais para distinguir espécies, como observado em morcegos do gênero *Artibeus*. Neste sentido a Morfometria Geométrica (MG) tem potencial para atuar como uma ferramenta bastante útil nas avaliações de diferenças morfológicas em espécies crípticas, uma vez que, fornece uma análise de forma refinada e informações mais robustas sobre variações inter e intraespecíficas (ZELDITCH *et al.*, 2012).

Além disso, análises baseadas em sequências de genes mitocondriais, fundamentadas em teorias evolutivas, permitem estabelecer limites para a identificação de espécies conhecidas e a delimitação de novas linhagens, por meio do conceito de *Molecular Operational Taxonomic Units* (MOTUs). No entanto, ainda não há estudos que integrem a análise conjunta de dados de forma e tamanho craniano, obtidos por MG, com a delimitação de MOTUs. A combinação dessas abordagens pode fornecer insights mais robustos sobre a diversidade do grupo, permitindo testar hipóteses sobre a existência de espécies crípticas e padrões de diversificação. Ainda, estudos populacionais que incorporem dados de genética mitocondrial podem esclarecer processos evolutivos subjacentes, como fluxo gênico, isolamento geográfico e eventos de especiação dentro do gênero, contribuindo para um entendimento mais abrangente da sua diversidade e estruturação populacional.

1.1 OBJETIVOS

1.1.1 OBJETIVO GERAL:

- Integrar dados morfológicos, moleculares e de morfometria geométrica para um melhor entendimento acerca da taxonomia e sistemática do gênero *Artibeus*.

1.1.2 OBJETIVOS ESPECÍFICOS:

- Identificar e diferenciar as espécies de *Artibeus* através do marcador mitocondrial Citocromo Oxidase c subunidade I (COI);
- Apontar quais caracteres diagnósticos são significativos para discriminar as espécies de *Artibeus* com base em dados de morfometria geométrica do crânio;
- Verificar presença/ausência de linhagens em espécies do gênero *Artibeus*, através dos métodos de delimitação molecular;

- Estimar a variabilidade e diferenciação genética de espécies de *Artibeus*, através dos marcadores mitocondriais Citocromo Oxidase c subunidade I (CO1) e Cytochrome b (Cyt b), a fim de fornecer informações acerca da conservação e aspectos evolutivos das populações.

2 REVISÃO BIBLIOGRÁFICA

2.1 ORDEM CHIROPTERA: DIVERSIDADE, ECOLOGIA E IMPORTÂNCIA DOS MORCEGOS

A ordem Chiroptera compreende os morcegos que representam a segunda maior ordem de mamíferos em diversidade, com cerca de 1.487 espécies descritas para o mundo (PAGLIA *et al.*, 2012; SIMMONS e CIRRANELLO, 2025). O Brasil representa cerca de 13% dessa diversidade, com 13 espécies endêmicas: *Dryadonycteris capixaba* Nogueira, Lima, Peracchi & Simmons, 2012, *Glyphonycteris behnii* (Peters, 1865), *Histiotus alienus* Thomas, 1916, *Lasiurus ebenus* Fazzolari-Corrêa, 1994, *Lonchophylla bokermanni* Vizotto e Taddei, 1978, *L. inexpectata* Moratelli e Dias, 2025, *L. mordax* Thomas 1903, *L. peracchii* (Días *et al.*, 2013), *Neoptesicus taddeii* Miranda, Bernardi e Passos, 2006, *Neonycteris pusilla* Sanborn, 1949, *Platyrrhinus recifinus* (Thomas, 1901), *Trachops ehrhardti* Felten, 1956 e *Xeronycteris vieirai* (Gregorin e Ditchfield, 2005) (GARBINO *et al.*, 2024).

A importância ecológica dos morcegos está associada à diversidade de hábitos alimentares, três quartos das espécies conhecidas são insetívoras, o que contribui para o controle das populações de insetos, cerca de um quarto são fitófagas, alimentando-se principalmente de frutos, podendo incluir na sua dieta pólen, néctar e folhas (HOLLIS, 2005). Aproximadamente 30% das espécies dependem das plantas como fontes de alimento e acabam dispersando sementes e pólen de um ambiente para outro. Um pequeno número é carnívora-piscívora ou hematófaga, outras onívoras (BREDDT *et al.*, 2012; TAYLOR, 2019). O Brasil é o quarto país com maior número de espécies de morcegos, atrás somente da Colômbia, Equador e Peru (DÍAZ *et al.*, 2021; PACHECO *et al.*, 2021; RAMÍREZ-CHAVES *et al.*, 2022). Atualmente o país registra 186 espécies e 68 gêneros distribuídos em nove famílias: Emballonuridae Gervais, 1856, Phyllostomidae Gray, 1825, Mormoopidae Saussure, 1860, Noctilionidae Gray, 1821, Furipteridae Gray, 1866, Thyropteridae Miller, 1907, Natalidae Gray, 1866, Molossidae Gervais, 1856 e Vespertilionidae Gray, 1821 (NOGUEIRA *et al.*, 2018; TAYLOR, 2019; GARBINO *et al.*, 2024).

A família Phyllostomidae é a mais representativa em número de espécies, com registro de 96 espécies para o Brasil, seguida pela família Molossidae com 34 espécies, Vespertilionidae

com 27 espécies, Emballonuridae com 17 espécies, Thyropteridae com cinco espécies, Mormoopidae com quatro espécies, Noctilionidae com duas espécies, Natalidae com uma espécie e Furipteridae também com uma espécie (GARBINO *et al.*, 2024). Essas 96 espécies de Phyllostomidae são classificadas em 10 subfamílias: Micronycterinae Van Den Bussche, 1992, Desmodontinae Wagner, 1840, Lonchorhininae Gray, 1866, Phyllostominae Gray, 1825, Glossophaginae Bonaparte, 1845, Lonchophyllinae Griffiths, 1982, Carollinae Miller, 1924, Glyphonycterinae Baker *et al.*, 2016, Rhinophyllinae Baker *et al.*, 2016, e Stenodermatinae Gervais, 1856 (NOGUEIRA *et al.*, 2018; GARBINO *et al.*, 2024).

Na subfamília Stenodermatinae encontra-se o gênero *Artibeus* Leach, 1821 que possui como característica a presença de listas faciais que podem ser visíveis ou fracamente perceptíveis, têm distribuição Neotropical e ocorrem desde o México até o norte da Argentina (GARDNER e CREIGHTON, 2008; REIS *et al.*, 2017). Os morcegos do gênero *Artibeus* são frugívoros e possuem uma alta plasticidade alimentar, são mais de 100 espécies de plantas utilizadas por estes morcegos. *Artibeus planirostris* por exemplo, faz uso de quase 30% dessas plantas, explorando seus frutos, flores e folhas, tanto em ambientes urbanos, periurbanos, rurais e naturais, é considerado o morcego fitófago mais estudado da América Latina, por seu grande papel ecológico na recuperação de áreas degradadas (BREDT *et al.*, 2012).

Os morcegos frugívoros são considerados os maiores reflorestadores naturais do planeta, isso porque ao comerem frutos sem destruir as sementes, acabam espalhando-as por toda a floresta através das fezes, que são então liberadas enquanto voam (NOVAES e LAURINDO, 2015). No entanto, existem vários fatores que ameaçam a sobrevivência e conservação destes morcegos, como a perda e alteração de habitat, urbanização, industrialização, agricultura e pecuária descontrolada, que contribuem para que venham a ser categorizadas, em um futuro próximo, nas listas de espécies ameaçadas de extinção (LAMIM-GUEDES e COSTA, 2018). Tendo em vista a importância destes morcegos para o equilíbrio dos ecossistemas, bem como para a biodiversidade de forma geral, é de fundamental importância estudos que visam a identificação e a diversidade dessas espécies.

Apesar da grande importância ecológica e da diversidade de morcegos no Brasil e no mundo, o conhecimento taxonômico de muitas espécies de quirópteros ainda permanecem bastantes insípidos (REDONDO *et al.*, 2008; SOLARI e MARTÍNEZ-ARIAS, 2014). O aumento na aplicação de métodos, como análises moleculares e morfométricas ajudam a esclarecer incertezas taxonômicas em várias espécies de morcegos. Como por exemplo, análises filogenéticas permitem-nos levantar hipóteses sobre relações evolutivas e distribuição de espécies (SOLARI e MARTÍNEZ-ARIAS, 2014). A filogeografia integra a filogenia e a

genética de populações com o propósito de analisar e entender as relações entre os processos micro e macro evolutivos, para compreender como os eventos históricos ajudam a formar a distribuição geográfica atual dos genes nas populações e nas espécies (AVISE 2000; PAPADOPOULOU e KNOWLES, 2016; THOME e CARSTENS, 2016; ZAMUDIO *et al.*, 2016). A MG por sua vez permite uma caracterização e discriminação morfológica refinada e acurada, principalmente em espécies crípticas (HEDRICK, 2021). Essa integração, destaca a importância de abordagens multidisciplinares para uma compreensão mais abrangente dessa biodiversidade.

O estudo da biodiversidade está diretamente alinhado com a Agenda 2030 para o Desenvolvimento Sustentável, lançada pela Organização das Nações Unidas (ONU) em 2015. Entre seus objetivos, destaca-se o 15º Objetivo de Desenvolvimento Sustentável (ODS 15), que propõe proteger, recuperar e promover o uso sustentável dos ecossistemas terrestres. Essa meta está intimamente relacionada à conservação da biodiversidade. Nesse contexto, os morcegos frugívoros desempenham um papel ecológico crucial na manutenção das florestas tropicais. No entanto, a perda de biodiversidade ameaça diretamente suas populações, comprometendo os serviços ecossistêmicos que esses animais prestam. Dessa forma, a proteção dos ambientes naturais que abrigam os morcegos é essencial para garantir a integridade dos ecossistemas e está em consonância com os princípios da ODS 15, ao promover uma gestão sustentável da terra e a conservação da diversidade biológica.

2.2 DESCRIÇÃO MORFOLÓGICA EM MORCEGOS *Artibeus*

Caracteres morfológicos desempenham um papel crucial na identificação de morcegos, oferecendo uma abordagem acessível e prática na diferenciação dessas espécies, entre estes pode ser citado: o formato do crânio, das asas, das orelhas, a coloração, o tamanho corporal, pois permitem classificar os morcegos com base em caracteres observáveis, complementando estudos genéticos e ecológicos. Isso evidencia que a morfologia ainda é uma ferramenta indispensável para a identificação e compreensão da biodiversidade (LIM *et al.*, 2008; REIS, *et al.*, 2013). Aqui, descrevo as características morfológicas para as espécies de *Artibeus* que ocorrem no Brasil. Tais características foram fundamentais para a identificação das espécies alvos registradas na presente pesquisa.

A espécie *A. cinereus* apresenta tamanho pequeno em relação as outras espécies do gênero, possui margem da folha nasal e orelhas com coloração variando entre pálida a amarela e listas faciais bastante evidentes (Fig. 1) (REIS *et al.*, 2017). Possui ocorrência na Venezuela, nas Guianas, no leste do Peru e no Brasil (MARQUES-AGUIAR, 2008). *Artibeus cinereus*

pode ser confundido com *A. anderseni*, sendo a concavidade na região frontal do crânio de *A. anderseni* um caráter diagnóstico bastante útil na separação dessas espécies (GONÇALVES e GREGORIN, 2004).

Figura 1-Indivíduo de *Artibeus cinereus* registrado na presente pesquisa, com características diagnósticas da espécie.



Fonte: Autor

A localidade tipo de *A. cinereus* é Belém no estado do Pará (PERACCHI *et al.*, 2006; ZORTÉA, 2007). Possui registro em quase todos os estados do Brasil, ocorre nos biomas Amazônia, Cerrado, Caatinga e Mata Atlântica (ZORTÉA, 2007; REIS *et al.*, 2013). É encontrado em florestas primárias, e também ocorre no domínio de Savanas (ZORTÉA, 2007; SCULTORI *et al.*, 2009). *Artibeus cinereus* usa tendas formadas por folhas (MACHADO *et al.*, 2008). No Brasil, há registros que indicam o consumo de espécies dos gêneros *Solanum*, *Ficus*, *Piper* e *Cecropia* por essa espécie, conforme relatado por Fabián *et al.* (2008) e Reis *et al.* (2013).

Artibeus gnomus apresenta coloração castanho claro, o trago a margem da folha nasal e das orelhas são de coloração frequentemente amarelados (REIS *et al.*, 2017). A espécie possui uma distribuição limitada à América do Sul, com registros em países como as Guianas, Venezuela, Equador, Bolívia, Peru e Brasil. No Brasil, foi registrada nos estados do Amazonas, Amapá, Bahia, Espírito Santo, Mato Grosso, Pará e Rondônia (PERACCHI *et al.*, 2011; REIS *et al.*, 2013). A localidade tipo dessa espécie é El Manaco, Bolívar, Venezuela (REIS *et al.*, 2007). Ocorre nos biomas Amazônia, Cerrado, Caatinga e Mata Atlântica (DO AMARAL *et al.*, 2023).

Artibeus anderseni, possui listas faciais brancas e visíveis, a borda das orelhas e folha nasal apresentam-se de cor clara, o uropatágio curto e cauda ausente. Pelagem de coloração

marrom-clara, na maior parte uniforme, com a coloração do ventre mais clara (REIS *et al.*, 2013). *Artibeus anderseni* é muitas vezes confundida com *A. cinereus*, mas possui tamanho menor. Gonçalves e Gregorin (2004), identificaram *A. anderseni* para o Cerrado, com base em dimensões cranianas e pela presença de apenas dois molares na maxila e mandíbula. Localidade tipo de *A. anderseni* é Rondônia, Porto Velho. Ocorre nos biomas Amazônia, Cerrado, Caatinga e Mata Atlântica (DO AMARAL *et al.*, 2023).

Artibeus bogotensis, além do Brasil possui registros para o Suriname, Guiana, Colômbia e nas ilhas de Trinidad e Tobago (REIS *et al.*, 2017). No Brasil possui ocorrência para o estado de Roraima. Localidade tipo é Bogotá, na Colômbia (MARQUES-AGUIAR, 2008). Existem poucos dados sobre essa espécie na literatura, já foi considerada como subespécie de *Artibeus glauca* (Thomas, 1893) por Marques-Aguiar (2008). *Artibeus bogotensis* apresenta listras faciais brancas bem definidas, uma membrana interfemoral menos peluda, uma região orbito rostral menos robusta e a ausência de um terceiro molar inferior pequeno (LIM *et al.*, 2008).

Artibeus concolor é endêmica da América do Sul, com ocorrência para o Peru, Guiana, Colômbia, Venezuela e Brasil (SIMMONS, 2005). Possui tamanho intermediário entre os pequenos e grandes *Artibeus*, as listas faciais são imperceptíveis ou ausentes, rostro largo e curto e sem margem anterior branca nas orelhas (ACOSTA e OWEN, 1993; REIS *et al.*, 2013). A pelagem de *A. concolor* é marrom-claro, com pelos dorsais apresentando três faixas de cores (MARQUES-AGUIAR, 2008). No Brasil, a espécie já foi registrada para os estados do Amapá, Amazonas, Ceará, Goiás, Pará, Piauí, Rondônia, Roraima e Tocantins (ZORTÉA *et al.*, 2006; TAVARES *et al.*, 2008; GREGORIN *et al.*, 2011). A localidade tipo de *A. concolor* é Paramaribo: Suriname (REIS *et al.*, 2007). Ocorre nos biomas Amazônia, Cerrado, Caatinga e Mata Atlântica (DO AMARAL *et al.*, 2023). Os dados sobre a história natural de *A. concolor* no Brasil são limitados, com registros indicando que sua dieta inclui *Vismia* spp., além de folhas, néctar e pólen (REIS e GUILLAUMET, 1983; BERNARD, 1997).

Em um estudo sobre diferenciação craniana em morcegos *Artibeus* a partir de dados morfológicos, Marchán-Rivadeneira *et al.* (2010), mostrou grandes diferenças na forma craniana de *A. concolor* em relação as outras espécies de *Artibeus*. *A. concolor* apresentou um morfotipo único com rostro mais largo, região escamosa alargada e basicrânio mais largo, sendo esses caracteres que mais contribuíram para diferencia-la das demais espécies do gênero.

As espécies de grandes *Artibeus*: *A. amplus*, *A. fimbriatus*, *A. lituratus*, *A. planirostris* e *A. obscurus* podem se sobrepor em algumas medidas corporais, devido as variações de peso, tamanho e coloração encontradas nessas espécies de acordo com a distribuição geográfica

(TADDEI *et al.*, 1998; MARCHÁN-RIVADENEIRA *et al.*, 2010), ressaltando a importância do uso de outras ferramentas para auxiliar na identificação taxonômica.

Artibeus amplus é uma das espécies menos conhecidas do gênero *Artibeus*. Sua distribuição abrange o norte da América do Sul, incluindo países como Colômbia, Venezuela, Guiana e Suriname. *A. amplus* possui semelhanças com *A. planirostris*, no entanto, *A. amplus* apresenta as pontas das asas de cor branca, rostro bem desenvolvido, crânio maior e mais estreito (LIM e WILSON, 1993). Zortéa *et al.* (2023) registraram pela primeira vez a ocorrência de *A. amplus* em uma área florestal do estado de Roraima no Brasil, onde analisaram dois exemplares de *A. amplus* de grande porte, e ressaltaram que há necessidade de revisar espécimes de museu na região norte do Brasil, o que pode revelar novas áreas de distribuição para a espécie. A localidade tipo de *A. amplus* é na Venezuela (HANDLEY, 1987).

Artibeus fimbriatus é endêmica da América do Sul está limitada às regiões nordeste, sudeste e sul do Brasil, além de ser encontrada no Paraguai e na Argentina (MARQUES-AGUIAR, 2008). *Artibeus fimbriatus* é um morcego de grande porte, apresentando tamanhos que ficam entre as espécies *A. planirostris* e *A. lituratus* (ZORTÉA, 2007). Possui coloração da pelagem de cor cinza, sendo a parte dorsal de coloração mais clara, listas faciais são fracamente perceptíveis, a folha nasal possui a borda inferior da ferradura soldada medianamente ao lábio e extremidades laterais livres, com bordas geralmente onduladas, bordas das orelhas e do trago de coloração marrom, uropatágio largo em forma de “V” (RUI *et al.*, 1999; REIS *et al.*, 2013). Alimenta-se de frutos, embora possa complementar sua dieta com insetos, e recursos florais, já foi visto se alimentando de frutos de Cecropiaceae, Moraceae, Solanaceae, Piperaceae e Curcubitaceae (PASSOS *et al.*, 2003). A localidade tipo de *A. fimbriatus* é Morretes na Serra do mar do estado do Paraná (REIS *et al.*, 2007). Ocorre nos biomas Cerrado, Caatinga e Mata Atlântica (DO AMARAL *et al.*, 2023).

Artibeus lituratus é a maior espécie dentro do gênero *Artibeus*, apresenta listas faciais evidentes, com coloração uniforme, variando de marron-escura e cinza, bordas das orelhas e do trago de coloração amarelada (Fig. 2) e uropatágio largo de em forma de “V” (RUI *et al.*, 1999; REIS *et al.*, 2013). Tem ampla distribuição na região Neotropical, com ocorrência no México, norte da Argentina, já foi registrado em todas as regiões do Brasil (ZORTÉA, 2007; MARQUES-AGUIAR, 2008). A sua dieta inclui vários frutos, dentre estes, estão os frutos de *Cecropia* spp., *Ficus* spp., *Solanum* spp., *Piper* spp. e ainda Anacardiaceae, Arecaceae, Cactaceae, Clusiaceae entre outros (REIS *et al.*, 2013). A localidade tipo de *A. lituratus* é Assunção: Paraguai (REIS *et al.*, 2007). Ocorre nos biomas Amazônia, Cerrado, Pampa,

Caatinga, Pantanal e Mata Atlântica (DO AMARAL *et al.*, 2023). Habita praticamente todos os ambientes da região neotropical, se adapta as áreas alteradas e áreas urbanas (REIS *et al.*, 2013).

Figura 2-Indivíduo de *Artibeus lituratus* registrado na presente pesquisa, com características diagnósticas da espécie.



Fonte: Autor

Marchán-Rivadeneira *et al.* (2012), com o intuito de avaliar a relação entre características morfológicas e condições ambientais específicas do habitat nas populações, encontrou uma alta correlação entre morfologia e ambiente, onde a sazonalidade foi correlacionada com o tamanho do crânio neste complexo. Isso evidencia que as pressões ambientais podem ser responsáveis pelas diferenças de tamanho de crânio em espécies de morcegos *Artibeus*.

Artibeus planirostris é de tamanho médio em relação aos grandes *Artibeus*, apresenta listas faciais em geral pouco evidentes em comparação com *A. lituratus*, folha nasal com bordas inferiores mediana livre e as bordas laterais onduladas, as orelhas apresenta-se pequenas de forma arredondadas e trago pequeno (Fig. 3) (HOLLIS, 2005; REIS *et al.*, 2013). Ocorre na Venezuela, nas Guianas, Brasil e leste dos Andes até o Norte da Argentina. Possui ocorrência em quase todos os estados do Brasil (TAVARES *et al.*, 2008). Sua principal fonte de alimentação constitui-se de frutos de *Cecropia* spp. e *Ficus* spp., pode consumir também frutos de Anacardiaceae, Arecaceae, Cactaceae entre outros (REIS *et al.*, 2013). Pode complementar sua dieta com folhas, néctar e pólen (HOLLIS, 2005). A localidade tipo de *A. planirostris* Bahia, Brasil (REIS *et al.*, 2007). Ocorre nos biomas Amazônia, Cerrado, Caatinga, Pantanal e Mata Atlântica (DO AMARAL *et al.*, 2023). Sendo muito abundante no Cerrado e Amazônia (DE SOUSA e DE CASSIA, 2023; MENDES *et al.*, 2024). Há diferenças significativas no tamanho

e na forma do crânio de indivíduos de *A. planirostris* nos biomas Cerrados, Amazônia e Caatinga em regiões do nordeste brasileiro (MENDES *et al.*, 2024).

Figura 3-Indivíduo de *Artibeus planirostris* registrado na presente pesquisa, com características diagnósticas da espécie.



Fonte: Autor

Artibeus obscurus tem pequeno porte em relação as demais espécies de grandes *Artibeus*, coloração mais enegrecida e listas faciais fracamente marcadas (HAYNES e LEE, 2004) (Fig. 4) uropatágio largo em forma com entalhe em forma de “V”, na sua dieta tem preferência por frutos de *Cecropia* spp. e *Ficus* spp. mas pode consumir frutos de *Philodendron* spp., Anacardiaceae e Aracaceae com pouca frequência (REIS *et al.*, 2013; REIS *et al.*, 2017). É endêmica da América do Sul, com ocorrência no Equador, Peru, Bolívia, Venezuela, Guiana e Brasil (ZORTÉA, 2007; MARQUES-AGUIAR, 2008). No Brasil apresenta registro em quase todas as regiões (TAVARES *et al.*, 2008). Localidade tipo de *A. obscurus* é Bahia, Brasil. Ocorre nos biomas Amazônia, Cerrado, Pampa, Caatinga, Pantanal e Mata Atlântica (DO AMARAL *et al.*, 2023). Costuma ocorrer em ambientes úmidos de floresta primária, mas também pode ser encontrada em ambientes xeromórficos e em áreas urbanas (REIS *et al.*, 2013).

Figura 4-Indivíduo de *Artibeus obscurus* registrado na presente pesquisa, com características diagnósticas da espécie.



Fonte: Autor

É importante ressaltar que as características morfológicas podem variar, dependendo do ambiente, sexo, localidade, entre outros fatores. Essas variações podem influenciar a identificação das espécies e dificultar a interpretação de dados taxonômicos. Portanto, ao realizar estudos sobre a diversidade, é fundamental considerar essas influências para garantir a precisão nas classificações e a compreensão das adaptações ecológicas de cada espécie. Além disso, uma análise abrangente que leve em conta essas variáveis pode contribuir para um melhor entendimento da biologia e da evolução dos organismos em questão.

2.3 DELIMITAÇÃO MOLECULAR (MOTUs): CONCEITO E APLICAÇÕES

Estudos com marcadores moleculares têm sido uma das abordagens mais utilizadas na distinção entre as espécies de *Artibeus* (LARSEN *et al.*, 2007; LIM *et al.*, 2008; REDONDO *et al.*, 2008). Estes se mostram uma ferramenta eficaz para estudos filogenéticos e caracterização genética de vários grupos de mamíferos, que são morfolologicamente similares, incluindo morcegos (MARCHESIN *et al.*, 2008). Apesar de representar apenas uma pequena fração do tamanho do genoma de um organismo, o DNA mitocondrial (mtDNA) tem sido de longe o marcador mais utilizado em estudos de diversidade molecular em animais nas últimas três décadas (GALTIER *et al.*, 2009).

Os genes mitocondriais COI e Cyt b são marcadores comumente utilizados em estudos filogenéticos, de diversidade genética e de estudos de delimitação molecular (CARABALLO *et al.*, 2020; PUILLANDRE *et al.*, 2020; KOWALCZYK *et al.*, 2021). O gene COI, é amplamente usado como um código de barras molecular, especialmente para identificação de espécies em estudos de taxonomia molecular por sua alta taxa de evolução em comparação com

outros marcadores mitocondriais (HEBERT *et al.*, 2003; RATNASINGHAM e HEBERT, 2007). O gene Cyt b, é empregado principalmente em estudos com morcegos, por ser um gene bastante variável, e apresenta uma taxa de substituição relativamente alta, o que facilita a detecção de diferenças genéticas mesmo entre populações ou espécies que divergiram há relativamente pouco tempo (PAVAN *et al.*, 2011; KARTAVTSEV, 2011; CARABALLO *et al.*, 2020).

Atualmente com base em sequências de genes mitocondriais, estão disponíveis as Unidades Taxonômicas Operacionais Moleculares (do inglês *Molecular Operational Taxonomic Unit* – MOTUs) (FLOYD *et al.*, 2002; VOGLER e MONAGHAN, 2007; GOLDSTEIN e DESALLE, 2011). As MOTUS têm sido utilizadas para designar um conjunto de sequências representantes de genomas dos quais são derivados com o intuito de delimitar espécies em um contexto taxonômico molecular (FLOYD *et al.*, 2002; JONES *et al.*, 2011). Dentre os modelos de delimitação molecular mais usados estão os modelos GMYC (*Generalized Model Yule Coalescent*) (PONS *et al.*, 2006), ABGD (*Automatic Barcode Gap Discovery*) (PUILLANDRE *et al.*, 2012) e ASAP (*Assemble Species by Automatic Partitioning*) (PUILLANDRE *et al.*, 2020).

O GMYC usa uma árvore filogenética como arquivo de entrada estimando taxas de eventos de ramificação para inferir modelos de especiação e coalescência. O ABGD e ASAP usam distâncias genéticas avaliando as distâncias intra e interespecíficas entre os táxons. O ABGD sugere várias partições que correspondem diferentes valores de P , ou seja, identifica uma partição de espécie para cada valor de P definido a priori, essa divisão acontece quando se detecta lacunas internas no código de barras. O ASAP também fornece partições, no entanto, são mais refinadas indicando a partição com melhor pontuação (PUILLANDRE *et al.*, 2020).

Muitos estudos já testaram o uso dos modelos de delimitação molecular em várias espécies de animais, incluindo morcegos (GALIMBERTI *et al.*, 2012; MENDES *et al.*, 2024) todos os métodos geralmente apresentam ótimos resultados. No entanto, podem apresentar desempenho ruim se em um conjunto de dados o número de indivíduos por espécie for pequeno (AHRENS *et al.*, 2016), mas geralmente todos os métodos apresentam ótimos resultados sendo congruentes com outros dados, como dados morfológicos, biogeográficos, ecológicos e outros (PUILLANDRE *et al.*, 2020), fornecendo assim, um conjunto de dados mais robusto para uma melhor compreensão acerca de estudos sistemáticos e taxonômicos de várias espécies.

2.4 MORFOMETRIA GEOMÉTRICA COMO UMA FERRAMENTA REFINADA PARA QUANTIFICAR VARIAÇÃO MORFOLÓGICA

Na década de 1990 foi desenvolvida a técnica de MG, que se constitui de ferramentas matemáticas que possibilitam a quantificação da variação de forma de vários organismos (ASTÚA *et al.*, 2015). Diferente da morfometria tradicional (linear), a MG, descreve a forma de maneira detalhada, bem como, localizam as áreas de mudança de forma e representam graficamente essas mudanças (ROHLF e MARCUS, 1993; ZELDITCH *et al.*, 2012). A MG utiliza informações completas sobre uma configuração de pontos de referência, não apenas um conjunto selecionado de distâncias, mas todos os aspectos do arranjo dos pontos de referência e todas as inter-relações entre eles. Portanto, considera todos os aspectos da forma, incluindo proporções, ângulos e o arranjo relativo das partes (KLINGENBERG, 2016).

A MG tem sido aplicada em estudos de sistemática, evolução (ADAMS *et al.*, 2004; SEBASTIÃO e MARROIG, 2013) e morfologia (MARCHÁN-RIVADENEIRA *et al.*, 2010) possibilitando uma melhor caracterização morfológica em espécies crípticas, como em espécies de *Artibeus* (HEDRICK, 2021). Dentre as ferramentas utilizadas na MG encontra-se a alometria, que se refere as mudanças de características morfológicas relacionadas ao tamanho (centroide), sendo essencial para os estudos taxonômicos, sistemáticos e evolutivos (KLINGENBERG, 2016). Portanto, a utilização da MG tem sido essencial para entender a variação na morfologia craniana de *Artibeus*, especialmente quando se considera a adaptação dessas características a diferentes biomas e ambientes. Estudos que se basearam em técnicas de morfometria lineares (LIM, 1997; GUERRERO *et al.*, 2003; MARCHÁN-RIVADENIERA, 2006, 2008; LIM *et al.*, 2008; LARSEN *et al.*, 2010) demonstraram que, embora as variações na forma craniana possam ser evidentes, essas abordagens muitas vezes não levam em conta o efeito da alometria residual, ou seja, a variação morfológica que é explicada apenas pelo tamanho do organismo. Essa limitação pode obscurecer a real influência de outros fatores ecológicos e evolutivos sobre a forma. A abordagem integrativa proposta, que combina dados moleculares com morfometria geométrica MG e considera o efeito da alometria residual (KLINGENBERG, 2016), proporciona uma visão mais precisa das diferenças na forma do crânio de *Artibeus*.

A evolução pode alterar o tamanho dos organismos e produzir alterações na morfologia devido a alometria evolutiva (CARDINI e POLLY, 2013; KLINGENBERG e MARUGÁN-LOBÓN, 2013; SHERRATT *et al.*, 2014). Avaliar essas variações morfológicas torna-se cada vez mais importantes em estudos taxonômicos, pois estas podem estar associadas aos fatores genéticos e ambientais que podem fornecer informações de como esses fatores estão influenciando no tamanho e na forma dos organismos, ajudando a entender como a evolução das espécies está relacionada a seus hábitos alimentares e ao ambiente em que vivem. Além

disso, esses estudos podem ser usados para inferir comportamentos e interações ecológicas (KLINGENBERG, 2016).

A MG tem sido amplamente aplicada em estudos sobre morcegos, oferecendo insights sobre a diversidade morfológica, adaptações ecológicas e evolução desses animais. Ruelas e López (2018) por meio da MG analisaram a variação na forma craniana das espécies morfológicamente similares de *Carollia* Gray, 1838 do Peru, e apesar das sobreposições, as diferenças morfológicas foram estatisticamente suportadas, principalmente a vista ventral, que foi fundamental pra discriminar as espécies. Estudos da forma craniana podem fornecer informações valiosas sobre aspectos alimentares, incluindo características como o tamanho das mandíbulas, a forma dos dentes e a configuração das fossas cranianas podem indicar adaptações a diferentes tipos de dieta (HEDRICK e DUMONT, 2018).

Hedrick (2021) empregou a MG no complexo de espécies *Artibeus*, utilizou dados de tamanho e forma craniana (no entanto não utilizou dados de alometria residual) em pequenos e grandes *Artibeus*. O estudo revelou uma variação craniana significativa entre as espécies, onde a maioria das espécies apresentaram características únicas de tamanho e forma, evidenciando que cada espécie pode estar explorando recursos ecológicos distintos. Além disso, os dados intraespecíficos mostraram dimorfismo sexual de tamanho e forma para a espécie de *A. lituratus*, onde as fêmeas apresentaram um perfil de crânio mais baixo em vista lateral, e tamanho maiores do que os machos, levantando a hipótese de que diferentes estratégias de forrageamento em machos e fêmeas refletem na forma e tamanho de crânios de *A. lituratus* indicando que as espécies de *Artibeus* apresentam adaptações morfológicas específicas que refletem suas ecologias e nichos.

A MG é uma técnica que juntamente com outras abordagens, como a delimitação molecular, estudos de diversidade genética, permite que as características morfológicas sejam melhores compreendidas evidenciando a importância dessa integração. Além do que essas metodologias integradas podem ser cruciais para subsidiar estratégias de conservação e para monitorar alterações morfológicas e genéticas em resposta a pressões ambientais.

2.5 ESTUDOS POPULACIONAIS EM MORCEGOS: ABORDAGEM E IMPORTÂNCIA PARA A CONSERVAÇÃO

A filogeografia preocupa-se com os princípios e processos que regem as distribuições geográficas das linhagens genealógicas, especialmente aquelas no nível intraespecífico (AVISE, 2000). Um dos principais objetivos da filogeografia é integrar a filogenia e a genética de populações com o propósito de analisar e entender as relações entre os processos micro e

macro evolutivos, para compreender como os eventos históricos ajudam a formar a distribuição geográfica atual dos genes nas populações e nas espécies (AVISE, 2000; PAPADOPOULOU e KNOWLES 2016; THOME e CARSTENS 2016; ZAMUDIO *et al.*, 2016).

Um dos grandes desafios da biologia é estabelecer ligações entre ecologia e evolução das espécies que pode ser fornecida pela relação entre a capacidade de dispersão, magnitude e escala espacial, sobre a qual as populações diferem geneticamente. Estudos de diferenciação genética populacional são particularmente fundamentais nestes aspectos, pois permitem inferências sobre como as forças microevolutivas interagem ao longo da história de uma espécie, bem como, podem indicar o potencial para a adaptação local ou especiação no futuro (BOHANAK, 1999; ANDERSON *et al.*, 2018; GALETTI, 2023).

Compreender como uma espécie está distribuída geograficamente no espaço é uma das fontes mais comuns de evidências em estudos de dinâmica populacional de vários grupos de animais. A exemplo dos morcegos, sabe-se que espécies com alta capacidade de dispersão tendem a apresentar menos estruturação genética em grandes escalas geográficas e que existem vários fatores que podem interferir nestes processos, como o acasalamento fora das áreas de reprodução e os movimentos de longa distância (MOUSSY *et al.*, 2012). Apesar desses fatores, uma série de eventos históricos como barreiras ecológicas e biogeográficas também podem influenciar na estruturação genética em morcegos (GUEVARA-CHUMACERO *et al.*, 2010; LOUREIRO *et al.*, 2020), bem como, em vários outros organismos (JOLY e BRUNEAU 2006; MOUSSALLI *et al.*, 2009; BELL *et al.*, 2012; CARNAVAL *et al.*, 2014; ZAMUDIO *et al.*, 2016; STEFANELLO *et al.*, 2020).

Os morcegos estão entre os mamíferos com alta capacidade de dispersão o que lhes permite colonizar até mesmo ilhas isoladas (SPEER *et al.*, 2017). A família Phyllostomidae é uma das famílias mais diversificada da região Neotropical (SIMMONS, 2005), onde encontra-se o gênero *Artibeus* Leach, 1821, um dos maiores e mais diversos gêneros dentro de Phyllostomidae, com registro de 23 espécies para o mundo (TAYLOR, 2019). O gênero se destaca por incluir espécies frugívoras com ampla distribuição geográfica e versatilidade ambiental.

Várias espécies do gênero *Artibeus* ainda são consideradas um complexo (DAVIS, 1984; LIM *et al.*, 2004; LARSEN *et al.*, 2007; REDONDO *et al.*, 2008; MARCHAN-RIVADENEIRA *et al.*, 2012; MENDES *et al.*, 2024). Como por exemplo, *Artibeus planirostris* (Spix, 1823) que já foi considerada como subespécie de *Artibeus jamaicensis* (Leach, 1821). Entretanto, vários estudos com base em dados morfológicos, moleculares ou ambos (PHILLIPS *et al.*, 1989; LIM, 1997; GUERRERO *et al.*, 2004; LIM *et al.*, 2004) identificaram *A.*

jamaicensis e *A. planirostris* como duas espécies distintas. Larsen *et al.* (2007) em um estudo filogeográfico representando todas as subespécies do complexo *A. jamaicensis* utilizando sequências do gene mitocondrial Cyt b também reconheceu *A. planirostris* como espécie distinta de *A. jamaicensis*. Estes estudos fornecem informações sobre a diversidade genética e as relações entre *A. planirostris* e *A. jamaicensis* e suas subespécies. No entanto, para uma compreensão mais abrangente da genética populacional de *A. planirostris*, são necessárias mais pesquisas e análises genéticas de diferentes populações e regiões ao longo da sua distribuição.

Artibeus planirostris é um dos morcegos frugívoros neotropicais mais conhecido e sua distribuição se estende por grande parte da América do Sul, abrange países como o Brasil, Bolívia, Colômbia, Guiana, Guiana Francesa, Paraguai, Peru, Suriname, Venezuela (LIM, 1997; LIM e ENGSTROM, 2001; LARSEN *et al.*, 2013; TAYLOR, 2019). Os indivíduos dessa espécie possuem alta plasticidade alimentar e grande importância ecológica (HOLLIS, 2005). Esta espécie tem sido um organismo modelo em estudos moleculares (LIM *et al.*, 2004; LARSEN *et al.*, 2007; LINO *et al.*, 2021; MENDES *et al.*, 2024). Estudos de importância médica com hepatovírus (DE MORAES PIRES *et al.*, 2024) e Leishmaniose (DE CASTRO FERREIRA *et al.*, 2017). Apesar da existência de estudos morfológicos baseados em morfometria linear (DE MEDEIROS FILHO *et al.*, 2018) e morfometria geométrica (MENDES *et al.*, 2024), ainda não há um estudo abrangente sobre a dinâmica populacional de *Artibeus planirostris*. Essa lacuna no conhecimento torna a espécie um modelo ideal para investigações de genética populacional em larga escala geográfica, permitindo uma compreensão mais aprofundada de seus padrões de variação genética, fluxo gênico e estrutura populacional.

3 RESULTADOS

3.1 CAPÍTULO 1

Artigo publicado na revista *Biological Journal of the Linnean Society*, com JCR (2.0) intitulado: **Morphological and molecular data combined reveal inter- and intraspecific cranial shape variations in bats of *Artibeus* Leach, 1821 (Chiroptera: Phyllostomidae).**

Original Article

Morphological and molecular data combined reveal inter- and intraspecific cranial shape variations in bats of *Artibeus* Leach, 1821 (Chiroptera: Phyllostomidae)

Samira Brito Mendes^{1,*}, Fabiano Stefanello², Cleison Luís da Silva Costa¹,
Amanda Cristiny da Silva Lima³, Ana Priscila Medeiros Olímpio³,
Walna Micaelle de Moraes Pires³, Elmary da Costa Fraga^{4,5} and Maria Claudene Barros^{4,5}

¹Programa de Pós-graduação em Biodiversidade e Biotecnologia da Rede Bionorte-PPGBIO-NORTE, Universidade Estadual do Maranhão, Cidade Universitária Paulo VI, Avenida Lourenço Vieira da Silva, no. 1000, Jardim São Cristóvão, 65055-310, São Luís, Maranhão, Brazil

²Laboratório de Entomologia Aquática, Universidade Estadual do Maranhão (UEMA), Campus Caxias, Praça Duque de Caxias S/N, Morro do Alecrim, 65604-380, Caxias, Maranhão, Brazil

³Programa de Pós-graduação em Genética e Biologia Molecular – PPGBM, Universidade Federal do Pará (UFPA), R. Augusto Corrêa, 01-Guamá, 66075-110, Belém, PA, Brazil

⁴Programa de Pós-graduação em Biodiversidade, Ambiente e Saúde – PPGBAS, Universidade Estadual do Maranhão (UEMA), Campus Caxias, Praça Duque de Caxias S/N, Bairro morro do Alecrim, 65604-380, Caxias, Maranhão, Brazil

⁵Laboratório de Genética e Laboratório de Biologia Molecular, Universidade Estadual do Maranhão (UEMA), Campus Caxias, Praça Duque de Caxias S/N, Morro do Alecrim, 65604-380, Caxias, MA, Brazil

*Corresponding author. Programa de Pós-graduação em Biodiversidade e Biotecnologia da Rede Bionorte-PPGBIO-NORTE, Universidade Estadual do Maranhão, Cidade Universitária Paulo VI, Avenida Lourenço Vieira da Silva, no. 1000, Jardim São Cristóvão, 65055-310, São Luís, Maranhão, Brazil. E-mail: britosamira503@gmail.com

ABSTRACT

Since morphological traits may overlap among species within a complex, the integration of morphological and mitochondrial data could provide crucial insights for distinguishing species, as observed in fruit-eating bats of the genus *Artibeus*. Therefore, the application of geometric morphometric (GM) techniques could yield more refined and robust analyses of inter- and intraspecific variations. In this study, we generated two datasets: one with molecular delimitation data based on the barcode region (*COI*) and the other with cranial size and shape data using GM methods. Our aims were to investigate variations between large and small species within the genus *Artibeus*, as well as to explore potential factors influencing such variations. The results from species delimitation revealed molecular operational taxonomic units (MOTUs) supporting the morphological identification. Analyses using GM techniques demonstrated significant inter- and intraspecific variations in cranial size and shape among *Artibeus* species. The combined outcomes suggest the absence of a significant phylogenetic signal influencing skull variation. This scenario indicates that potential historical ecological factors may have directly influenced the cranial morphology of these species, acting as significant selection forces in geographical space and generating intraspecific variations in *Artibeus planirostris*.

Keywords: biomes; *COI* gene; geometric morphometrics; fruit-eating bats; molecular delimitation

INTRODUCTION

Cranial morphology can be influenced by adaptive evolution for optimized foraging performance. Key components such as the skull, cranial muscles, and teeth serve specific dietary functions, and modifications to any of these elements can lead to various morphological variations. These variations provide valuable insights into the evolutionary history and ecological dynamics of species (Cheverud 1982, Marchán-Rivadeneira *et al.* 2010, Santana *et al.* 2012, Bardua *et al.* 2020, Bon *et al.* 2020).

The combination of morphological and molecular data is crucial for distinguishing between species, especially in studies of cryptic species such as Neotropical fruit-eating bats (Phyllostomidae), which exhibit high morphological diversity (Simmons and Conway 2003, Jones *et al.* 2005, Baker *et al.* 2016). However, morphological and molecular studies, particularly those related to species of the genus *Artibeus* Leach, 1821, have remained inconclusive. Therefore, additional studies are essential to elucidate inter- and intraspecific relationships,

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contributing to a better understanding of the taxonomy and systematics of *Artibeus*.

Fruit-eating bats of the genus *Artibeus* are widespread and abundant in the Neotropical Region from Mexico to Argentina (Simmons 2005, Taylor 2019). They are primarily frugivorous but may occasionally supplement their diet with insects, leaves, and nectar. The species have been categorized into large and small *Artibeus*, but the subgeneric classification remains controversial. The smaller species were considered as *Dermanura* Gervais, 1856, and the larger species as *Artibeus* (Owen 1991, Lim et al. 2008, Rocha et al. 2018). However, the genus *Artibeus* in the broader sense (*Artibeus* + *Dermanura*) has been recovered as monophyletic (Simmons 2005, Redondo et al. 2008). The recognition of these two genera is not universally accepted, with *Dermanura* considered by many authors as a subgenus of *Artibeus* (Owen 1987, Wetterer et al. 2000, Simmons 2005, Taylor 2019) or simply as *Artibeus* (Garbino et al. 2020).

Currently, 10 species of *Artibeus* are documented in Brazil: *Artibeus amplus* Handley, 1987; *A. concolor* Peters, 1865; *A. fimbriatus* Gray, 1838; *A. lituratus* (Olfers, 1818); *A. obscurus* (Schinz, 1821); *A. planirostris* (Spix, 1823); *A. anderseni* Osgood, 1916; *A. bogotensis* Andersen, 1906; *A. cinereus* (Gervais, 1856); and *A. gnomus* Handley, 1987 (Nogueira et al. 2014, Garbino et al. 2020, Zortéa et al. 2023). In this study, we adhere to Garbino's updated classification, considering all large and small species as *Artibeus*. The large and small species constitute two complexes that show many overlapping traits in body and skull morphology, but it remains essential to discriminate between them. Therefore, geometric morphometric (GM) techniques could serve as a valuable tool for assessing morphological differences among *Artibeus* species, offering more refined analyses and robust information about inter- and intraspecific variations (Zelditch et al. 2012).

While some analyses based on mitochondrial genes use the phylogenetic tree of the species and estimate limits for recognition or the identification of new species (molecular operational taxonomic units, MOTUs) (Puillandre et al. 2020), there is a gap in research integrating MOTU delimitation and cranial shape and size data through GM techniques. Such an integrated approach could help verify the genetic support for taxa recognized based on morphology, as is the case with *Artibeus* bats, or still confirm the identity of taxa providing valuable background information.

Hence, our proposed objectives are: (i) to delimit the species of fruit-eating bats of *Artibeus* in northern and northeastern Brazil using molecular data (MOTUs) and (ii) to analyse inter- and intraspecific variations in the shape and size of the skull among these species using GM techniques. Consequently, we aim to test several hypotheses regarding cranial size and shape variations in *Artibeus* species, addressing the following questions: (i) Do molecular delimitation models recover *Artibeus* MOTUs congruent with morphology? Our hypothesis is that the MOTUs corroborate the taxa defined based on morphology. (ii) Do the larger *Artibeus* species exhibit distinct skull shapes and sizes? Building upon prior GM studies on *Artibeus* species (Hedrick 2021), we hypothesize that the larger *Artibeus* species will be distinguishable based on both skull size and shape, whereas the discrimination between larger and smaller species will be primarily size-dependent and not shape-dependent. (iii)

Is there a phylogenetic signal in cranial size and shape of these species? Our hypothesis posits the absence of a phylogenetic signal affecting size and shape variations, suggesting that external ecological factors are driving these variations rather than phylogenetic relationships. (iv) Do individuals of *A. planirostris* demonstrate intraspecific differences in cranial size and shape across their geographical distribution? We anticipate significant variations in size and shape, indicating that environmental factors directly impact this structure. Variations in skull shape and size, as well as the emergence of new lineages, are probably influenced by local environmental conditions (Gaston, et al. 2008, Luxbacher and Knouft 2009).

MATERIAL AND METHODS

Sampling

Bats were collected from three biomes and different states and municipalities in northern and northeastern Brazil: Caatinga [Piauí (PI)], Cerrado [Maranhão (MA) and Tocantins (TO)], and Amazon [Maranhão and Pará (PA)]. The municipalities of Picos/PI and Milton Brandão/PI comprise the Caatinga biome; Caxias/MA, Codó/MA, Chapadinha/MA, Timon/MA, Carolina/MA, São João do Soter/MA, Lajeado/TO, and Palmas/TO comprise the Cerrado; and the municipalities of Imperatriz/MA, Cândido Mendes/MA, Carutapera/MA, Turiaçu/MA, Centro Novo do Maranhão/MA, Godofredo Viana/MA, Augusto Corrêa/PA, and Viseu/PA comprise the Amazon biome (Fig. 1). The collections were licensed by ICMBIO/SISBIO under licences #42670-1, #54384-1, #68047-1, and #74512-1, and the study was approved by the Animal Ethics and Experimentation Committee (CEEA—Universidade Estadual do Maranhão) no. 06/2023-CEEA/CMV/UEMA.

The collections were conducted during various field expeditions using mist-nets placed near bat shelters. Captured bats were transported to an improvised laboratory near the collection sites. There, they were killed, weighed, photographed, and measured using a manual caliper (300 mm). Subsequently, they were tagged, and muscle tissue was removed and stored in microtubes with 70% alcohol for molecular studies. The samples were then transported to the GENBIMOL facilities, housing the Genetics and Molecular Biology Laboratories of the Universidade Estadual do Maranhão. Species identification was performed using taxonomic classification keys (Reis et al. 2013, 2017, Díaz et al. 2021) and subsequently confirmed by specialists.

The skull of the specimens was extracted through the mouth opening while carefully removing and folding the skin. Subsequently, the skulls underwent a cleaning process with the assistance of larvae and adults of dermestids (Coleoptera, Dermestidae) in a dedicated breeding facility at GENBIMOL. Following the cleaning process, the skulls were subjected to bleaching with 10% hydrogen peroxide and then placed in an oven at a 30°C for ~6 h to facilitate drying. Once all treatments were completed, the skulls were coded and individually packaged in clean bottles. Only adult specimens were included, determined based on epiphyseal–diaphyseal fusion and reproductive condition (Anthony 1988). Vouchers are deposited in the Coleção de História Natural da Universidade Federal do Piauí (CHNUFPI), Campus Amílcar Ferreira Sobral (CAFS), in Floriano, Piauí, Brazil.

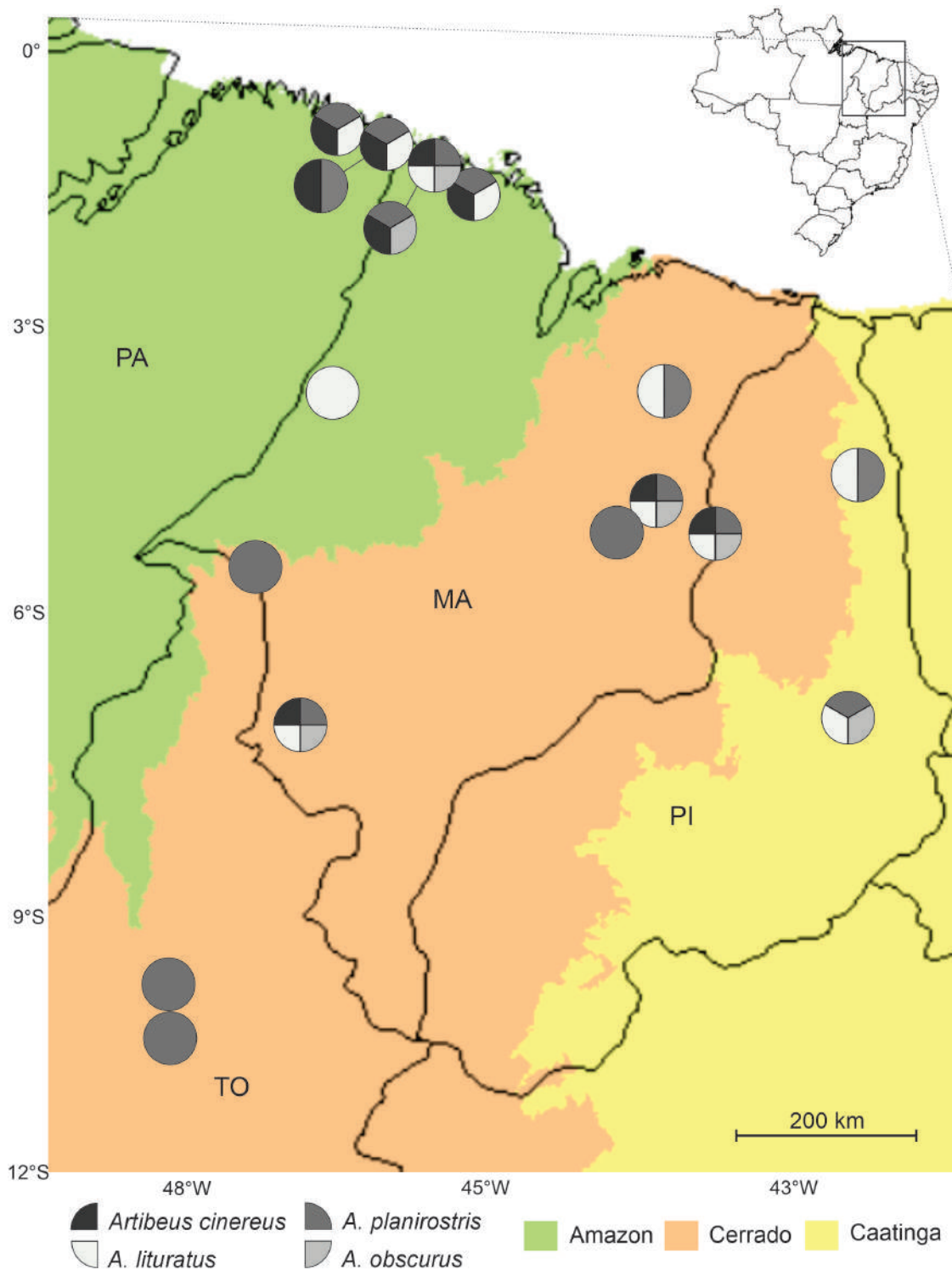


Figure 1. Study area showing collection sites and the species of *Artibeus* collected at each site. The biomes are indicated by different colours on the map. The species collected at each site are indicated by grey scale in the circles. Abbreviations for Brazilian states: MA, Maranhão; PA, Pará; PI, Piauí; TO, Tocantins.

Molecular data collection and analyses

Genomic DNA was extracted from muscle tissue using the Wizard Genomic[®] DNA Purification kit from Promega, following the manufacturer's instructions. The mitochondrial COI gene was then amplified through polymerase chain reaction (PCR) using the universal primers described by Folmer *et al.* (1994).

Sequencing was carried out using the dideoxyterminal method (Sanger *et al.* 1977) on the ABI Prism[™] 3500 automatic DNA sequencer (Applied Biosystems, USA). In total, 91 sequences, each 600 bp long, were obtained, corresponding to the following species: *A. cinereus* ($N = 11$), *A. lituratus* ($N = 25$), *A. obscurus* ($N = 07$), and *A. planirostris* ($N = 48$).

The generated COI gene sequences have been deposited in the GenBank database (see [Supporting Information, Table S1](#)). For species identification and determination of levels of similarity, sequences were compared using the BOLD Systems v.3 (The Barcode of Life Data Systems, www.barcodinglife.org) and BLAST (Basic Local Alignment Search Tool, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) tools. Sequence editing and alignment were performed using ClustalW (Thompson *et al.* 1994) within the Bioedit 7.2.5 program (Hall 1999).

The suitable substitution model for COI was determined using jModelTest v.2.1.10 (Darriba *et al.* 2012) with the Akaike information criterion (AIC; Posada and Buckley 2004). The best-fitted model was the Hasegawa–Kishino–Yano model (HKY+G+I). A Bayesian inference (BI) tree was generated in the BEAST v.1.10.4 software (Drummond *et al.* 2012, Suchard *et al.* 2018), employing a strict clock and the Yule speciation process as priors. The analysis was performed based on 80 million Markov chain Monte Carlo (MCMC) generations with 10% burn-in, and the parameter convergence were checked in Tracer v.1.6 (Rambaut *et al.* 2014), with the results accepted when the effective sample size (ESS) exceeded 200. The tree obtained was summarized in Tree Annotator v.1.0.4 (Suchard *et al.* 2018) to obtain the consensus tree, which was then visualized and edited in FigTree v.1.4.2 (Rambaut 2014). The species *Sturnira lilium* (Geoffroy, 1810) was used as an outgroup to root the tree.

The MOTU delimitation analyses employed three methods: GMYC (Generalized Model Yule Coalescent) (Pons *et al.* 2006), ABGD (Automatic Barcode Gap Discovery) (Puillandre *et al.* 2012), and ASAP (Assemble Species by Automatic Partitioning) (Puillandre *et al.* 2020). The GMYC model was implemented using the consensus ultrametric tree built in the BEAST software. The lineages were estimated in the packages ape v.5.7-1 (Paradis and Schliep 2019), splits v.1.0-20 (Ezard *et al.* 2009), paran v.1.5.2 (Dinno 2009), and MASS v.7.3-60 (Venables and Ripley 2002) available in the R software 4.2.3 (R Core Development Team 2023). The ABGD test was conducted in the web graphical version (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>) with the aligned sequence database as the input. The distance model was Kimura's two-parameter (K2P) and other values were set as default (Pmin: 0.001, Pmax: 0.1, Steps: 10, NBins: 20, relative range: 1.5). The ASAP test was performed using the web graphical interface (<https://bioinfo.mnhn.fr/abi/public/asap/>), using the aligned sequence bank as input and the K2P distance model. After delimitation analyses, FigTree v.1.4.4 and the Inkscape v.1.1 (<https://inkscape.org/pt-br/>) were used to edit the consensus tree. Average genetic distances between MOTUs were calculated in MEGA X (Kumar *et al.* 2018) using the K2P model.

Cranial shape data acquisition

Following dissection, the skulls were photographed in dorsal and ventral views using a Canon T7i camera equipped with an EFS 18–55-mm lens, mounted on a photographic stand to ensure consistent angles in all images. A total of 148 *Artibeus* specimens were analysed in dorsal view, including males and females of *A. cinereus* ($N = 37$), *A. lituratus* ($N = 30$), *A. obscurus* ($N = 12$), and *A. planirostris* ($N = 69$). For the ventral view, 138 specimens were included comprising males and females of *A. cinereus* ($N = 32$), *A. lituratus* ($N = 30$), *A. obscurus* ($N = 11$), and

A. planirostris ($N = 65$). The variation in the number of individuals sampled in each view was due to the structural condition of the skulls. Our preliminary test of shape variation between the sexes indicated an absence of sexual dimorphism; hence, subsequent analyses were not performed for each sex separately.

Eleven landmarks were plotted in the dorsal view and 17 in the ventral view (Fig. 2), following the protocols of Marchán-Rivadeneira *et al.* (2010), Ruelas and Lopéz (2018), and Hedrick (2021). These landmarks were strategically selected to capture the greatest variation in both ventral and dorsal views of the skull. A TPS format file was generated using the tpsUtil 1.83 software (Rohlf 2015), and bidimensional coordinates of landmarks were obtained using the tpsDig 2.32 software (Rohlf 2015). Two separate datasets were analysed—one to explore interspecific cranial shape variation, encompassing all species, and another to investigate intraspecific cranial shape variation in *A. planirostris*, given its larger sampling and geographical range.

Interspecific analyses of shape data

The bidimensional coordinates of both the dorsal and ventral sets of the bat skull were superimposed using the 'procSym' function of the Morpho v.2.12 package (Schlager 2017) in R. This function performs a Generalized Procrustes Analysis (GPA) which removes nonshape effects of position, size, and rotation by minimizing Procrustes distances between landmarks (Bookstein 1991). The number of principal shape components (PCs) to be analysed was selected by measuring the correlation between the matrix of Procrustes shape distances in the full shape space and pairwise Euclidean distances in the reduced shape space, according to Cardini *et al.* (2007). Ten principal components analysis (PCA) axes representing 91.6% of the variation in dorsal shape and 92.1% of that in ventral shape were then used to test cranial shape variation (both dorsal and ventral) among species.

For this purpose, an analysis of variance (ANOVA) was conducted, with species as the categorical independent variable. Additionally, a boxplot of centroid-size of dorsal view landmark configurations was generated for each species. In this case, the dorsal view provides a more accurate estimate of the species' skull size. This procedure enables the assessment of centroid-size variability for each species and allows testing for residual allometry (the effect of size on the variation in the shape data) (Klingenberg 2016). Thus, centroid-size was used as a predictor in a linear regression analysis to test whether there is an effect of this variable on shape. In instances where a significant effect was observed, the residuals from this regression were used to eliminate the residual allometry.

A canonical variate analysis (CVA) with cross-validation and 999 permutations was implemented based on the PCA axes to test the cranial shape variation (dorsal and ventral) among species of *Artibeus*. Since the effect of centroid-size on the shape of the structures was significant, the CVA was conducted with the PC scores (shape data) and with the regression scores (i.e. residuals). These analyses were performed using the 'CVA' function of the Morpho v.2.12 package (Schlager 2017). Additionally, a hierarchical cluster of Mahalanobis distances was generated to illustrate cranial shape similarity between *Artibeus* species.

A two-block partial least squares (2-block PLS) analysis was performed to evaluate the degree of integration between blocks of Procrustes shape variables, in this case the dorsal and

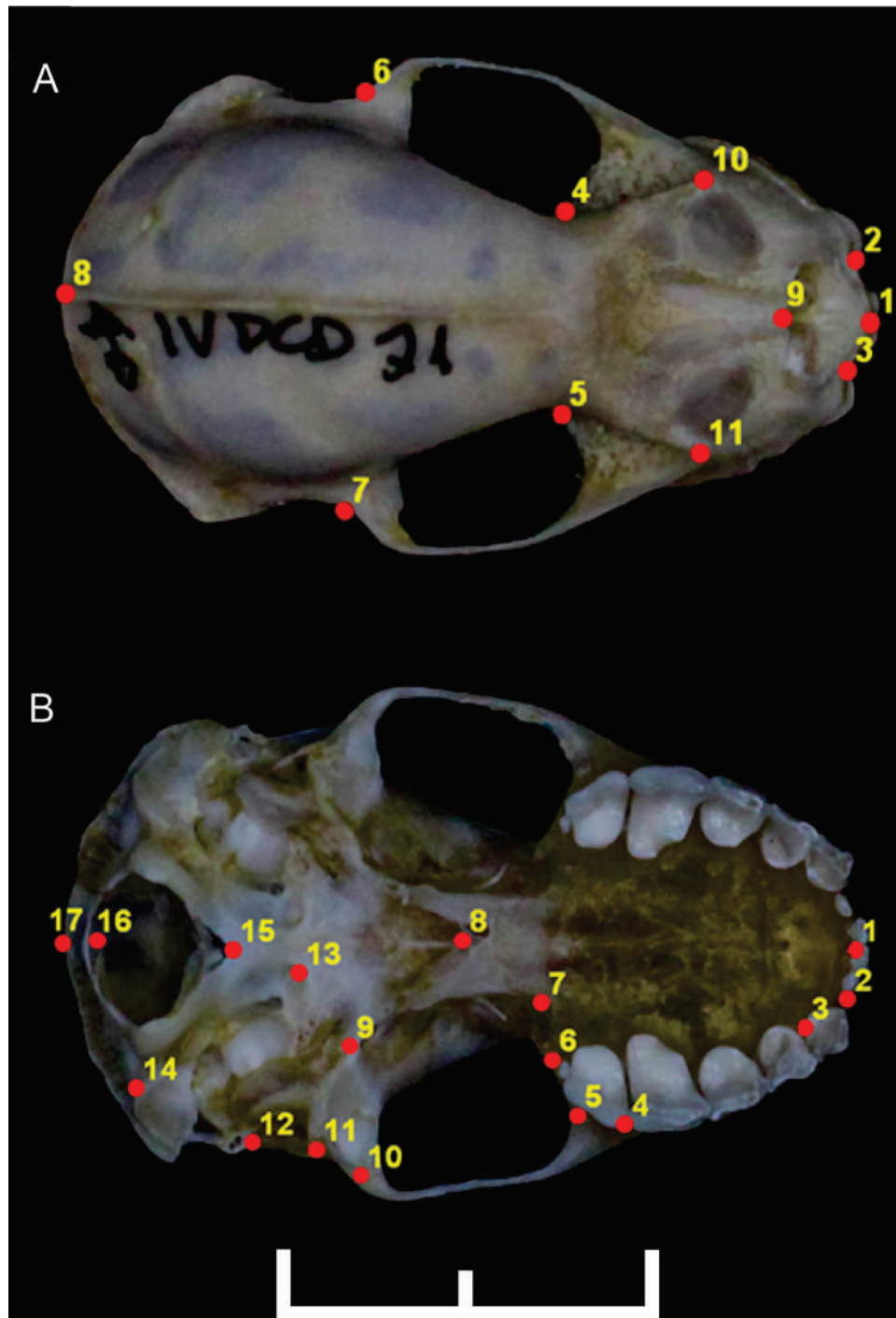


Figure 2. Dorsal (A) and ventral (B) views of the *Artibeus planirostris* skull with landmark locations. Scale bar = 1.0 cm.

ventral cranial shapes. This procedure was implemented with the ‘two.b.pls’ function from the package geomorph v.4.0.6 (Adams and Collyer 2022). This test enables us to determine whether the parts of the bat’s skull are integrated, i.e. varying in the same way, or whether there are two separate blocks of structures varying independently, possibly with selection forces acting differently on each (Adams and Collyer 2019). If they are integrated, only the dorsal view was considered in most analyses.

Furthermore, to evaluate whether there is an influence of phylogenetic relationships on cranial shape variation,

we randomly selected individuals from different biomes, which were also included in the molecular delimitation analysis encompassing the four species (i.e. 10 terminals). This association allowed for testing the phylogenetic signal and the configuration of the tree in shape space, referred to as phylomorphospace. These analyses were conducted in R, using the ‘physignal’ and ‘gm.prcomp’ functions from the geomorph package (Blomberg et al. 2003, Collyer et al. 2022). The phylogenetic signal test enables the investigation of whether shape variation is driven by the phylogenetic

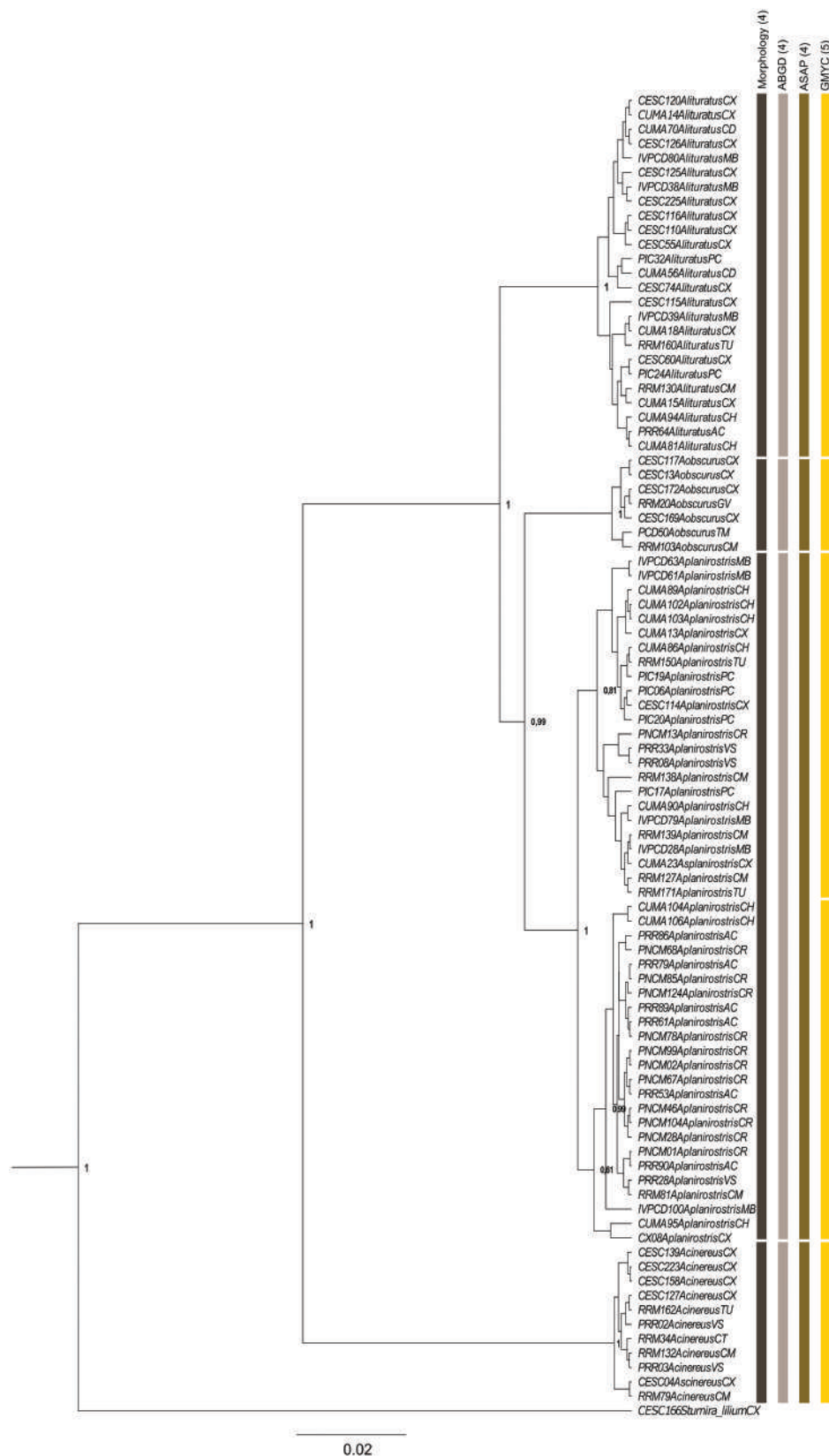


Figure 3. Bayesian inference phylogenetic tree for *Artibeus* species. Bars to the side show results of species delimitation (ABGD, ASAP, and GMYC) based on the cytochrome oxidase subunit I gene. Collection sites: CX = Caxias, CD = Codó, CHA = Chapadina, TM = Timon, and CR = Carolina (comprising the Cerrado biome); CM = Cândido Mendes, CR = Carutapera, TU = Turiaçu, AC = Augusto Corrêa, and VS = Viseu (comprising the Amazon biome); and MB = Milton Brandão and PC = Picos (comprising the Caatinga biome).

history of the species or by other ecological selection forces, for instance.

Intraspecific analyses of shape data

The specimens of *A. planirostris* examined were initially categorized into three groups according to the biome where they were collected (Amazon, Caatinga, and Cerrado). The bidimensional coordinates of the dorsal view of the skull were superimposed following the same procedures described in the interspecific analyses. A boxplot of centroid-size (which best represents the overall skull size of the specimens) was generated for each group representing the biomes. This procedure enables verification of how variable centroid-size is for specimens occurring in different biomes and also allows testing for residual allometry in the species (Klingenberg 2016).

First, we employed an ANOVA with groups as the categorical independent variable to assess whether the cranial shape of *A. planirostris* varies across different biomes. Second, a CVA with cross-validation and 999 permutations was conducted using the PCA axes of the dorsal cranial shape of *A. planirostris* to visualize the variation in cranial shape among biomes. Due to the significant effect of centroid-size on shape, the CVA was performed with the shape data incorporating size effects, as well as with the regression scores (allometry-free data). Additionally, a hierarchical cluster of Mahalanobis distances was generated to illustrate the similarity in dorsal cranial shape of *A. planirostris* among biomes.

RESULTS

Molecular delimitation

Our Bayesian tree recovered the monophyly of the species of *Artibeus* with high support, revealing two major clades. One clade comprised large species of *Artibeus* (*A. lituratus*, *A. planirostris*, and *A. obscurus*) while the other included the small species (*A. cinereus*) (Fig. 3) with robust clade support. *Artibeus obscurus* was recovered as sister to *A. planirostris* with also high support. The ABGD and ASAP distance methods yielded consistent results, delimiting four MOTUs (*A. lituratus*, *A. planirostris*, *A. obscurus*, and *A. cinereus*) in the dataset. The GMYC model identified five MOTUs, indicating the presence of two MOTUs in *A. planirostris*. However, there is no indication that the MOTUs of *A. planirostris* were formed based on the geographical distribution of the species across biomes, as both MOTUs included specimens from all biomes (Fig. 3).

The genetic distance matrix derived from the molecular taxonomic units supported interspecific limits, with mean intra-MOTU values ranging from 0.2% for *A. cinereus* to 1.0% for *A. planirostris*. The average inter-MOTU distances ranged from 3.4% between *A. planirostris* and *A. lituratus* to 12.6% between *A. obscurus* and *A. cinereus* (Table 1).

It is noteworthy that the highest average inter-MOTU genetic distances were observed between the species of large *Artibeus* (*A. planirostris*, *A. lituratus*, and *A. obscurus*) and the species of small *Artibeus* (*A. cinereus*) while the lowest averages were observed among large species of *Artibeus* (Table 1).

Interspecific cranial shape variation

ANOVA indicated a significant variation in cranial shape in dorsal and ventral views among *Artibeus* species ($P < .001$ for

Table 1. Matrix with average genetic distances with the Kimura two-parameter algorithm generated by MEGA X for MOTUs of the genus *Artibeus*, defined by delimitation methods (ABGD, ASAP and GMYC): interspecific mean (below the diagonal) and intraspecific mean (diagonal in bold).

MOTU	Mean genetic distance			
	1	2	3	4
1. <i>Artibeus lituratus</i>	0.4			
2. <i>Artibeus cinereus</i>	10.9	0.2		
3. <i>Artibeus obscurus</i>	5.2	12.6	0.3	
4. <i>Artibeus planirostris</i>	3.4	11.8	4.2	1.0

both tests). Moreover, centroid-size also exhibited a significant effect on cranial shape in dorsal and ventral views ($P < .001$ for both tests).

The centroid-size of the dorsal view was significantly different ($P < .001$), indicating that size of the skull varies among species of *Artibeus*, with higher values in *A. planirostris*, *A. lituratus*, and *A. obscurus*, and the lowest in *A. cinereus* (Fig. 4). The large species displayed a shorter and wider braincase region and a more protruding rostrum compared to the small species (Fig. 5).

CVAs using shape data of both the dorsal and ventral views of the skull successfully discriminated among the large *Artibeus* (*A. planirostris*, *A. lituratus*, and *A. obscurus*), but with some overlap, and between large species and the small *Artibeus* (*A. cinereus*), achieving a high correct classification of 84.45% for the dorsal view (Fig. 6A) and 77.53% for the ventral view (Fig. 7A) after cross-validation. However, CVAs with residuals of cranial shape (regression scores) showed no discrimination between *A. cinereus* and other large species of the genus, displaying high similarity with *A. lituratus*, with a correct classification of 48.64% for the dorsal view (Fig. 6B) and 48.55% for the ventral view (Fig. 7B) after cross-validation.

The results from the Mahalanobis distance of dorsal and ventral views considering shape data indicated that *A. cinereus* is morphologically distant from the other large species of the genus (Fig. 8A, C). Nevertheless, considering the regression scores, in both views, *A. obscurus* was morphologically more distant from the other species, with *A. cinereus* and *A. lituratus* being morphologically similar in cranial shape (Fig. 8B, D).

The integration test indicated that the shapes of the dorsal and ventral views of the skull of species of *Artibeus* are closely associated ($r\text{-PLS} = 0.86$, $P = .001$). There is no significant phylogenetic signal acting on the variation in the dorsal shape of the skull ($k = 0.15$ and $P = .138$). The PC1 axis of phylomorphospace, explaining 89.7% of the total variation, is represented by species with a skull featuring a shorter rostrum and shorter skull on the negative axis, while species with a more elongated rostrum and skull are positioned on the positive axis (Fig. 9).

Intraspecific cranial shape variation

Centroid-size of the dorsal view in *A. planirostris* varied significantly between biomes ($P = .01$). Specimens from the Cerrado exhibited a highly variable centroid-size, whereas specimens from the Amazon had the highest centroid-size value, and those from the Caatinga showed the lowest values (Fig. 10).

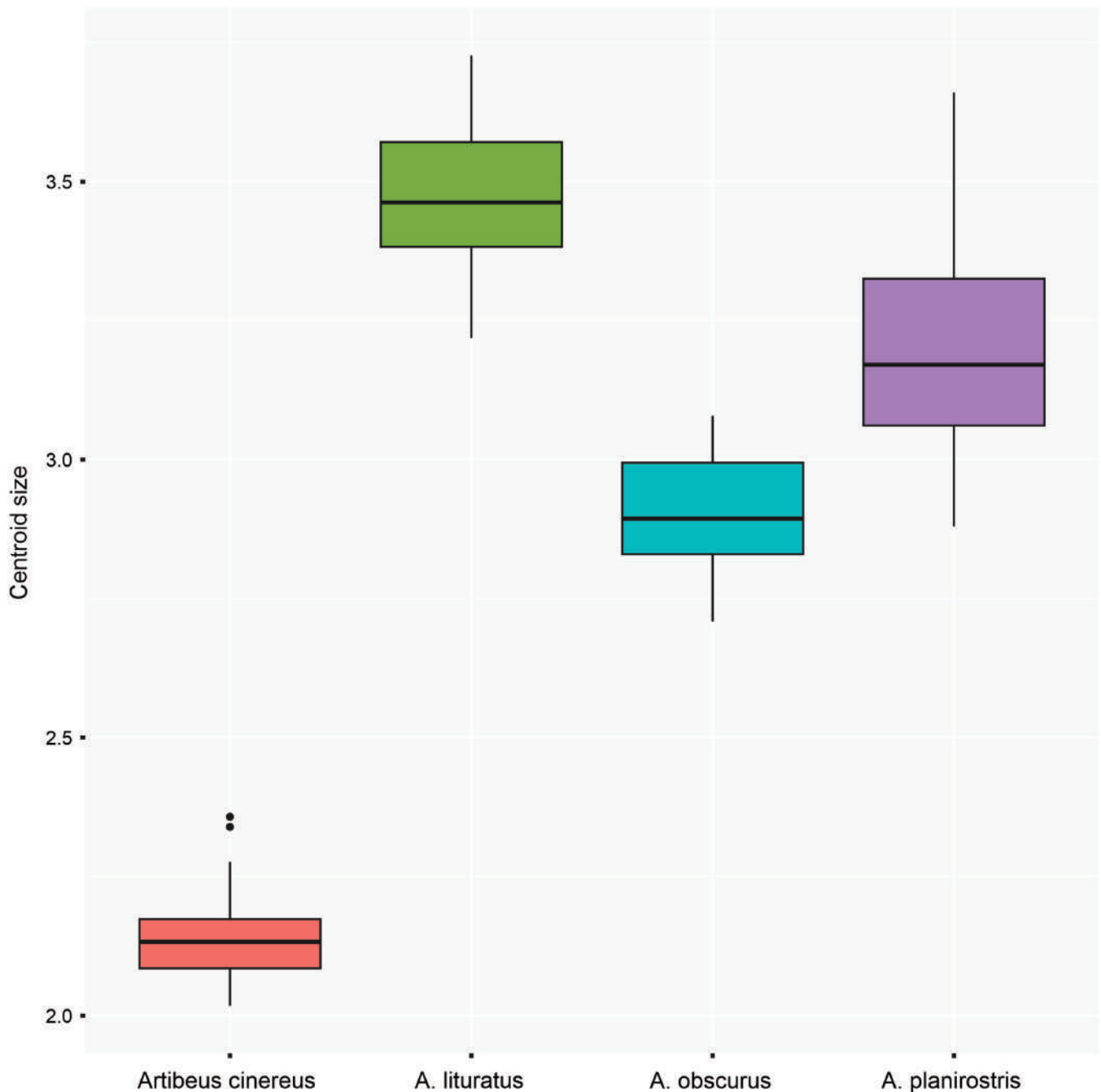


Figure 4. Boxplot of dorsal view centroid-size, indicating skull size variation among evaluated *Artibeus* species.

Furthermore, centroid-size also had a significant effect on the cranial shape of this species ($P < .001$) (Fig. 11).

The cranial shape of *A. planirostris* varied significantly between biomes based on ANOVA ($P < .001$). CVA using shape data of the dorsal view demonstrated a high discriminatory power among specimens from each biome, with a correct classification of 69.56% (Fig. 12A) and using regression scores a correct classification of 59.42% after cross-validation (Fig. 12B). Specimens from the Amazon were morphologically more similar to those from the Cerrado.

Concerning the lineages proposed by the GMYC model for *A. planirostris*, it was verified, using the ANOVA test, that there is no morphological support for the two lineages ($P = .18$),

indicating that the shape of the skull does not vary between the specimens representing MOTUs.

DISCUSSION

Some species of fruit-eating bats of the genus *Artibeus* have been considered as a species complex, due primarily to inconsistencies in morphological and molecular data (Davis 1984, Larsen *et al.* 2007, Marchán-Rivadeneira *et al.* 2012, Ferreira *et al.* 2014, Hedrick 2021). The taxonomic status of the species within the genus is not widely accepted, given the lack of information and the significant morphological variations among different taxa in the group (Taddei *et al.* 1998, Simmons 2005, Lim *et al.* 2008,

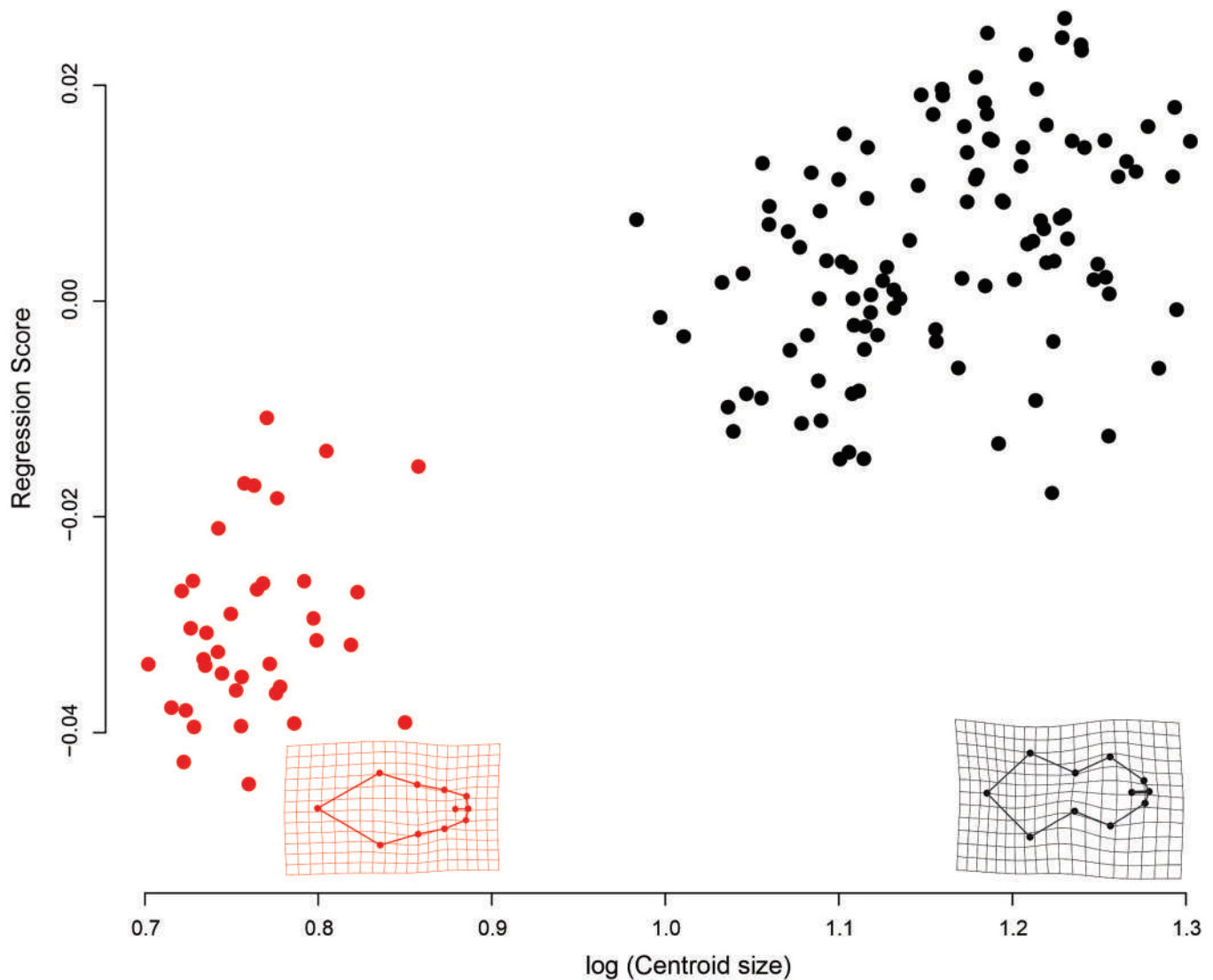


Figure 5. Regression analysis of cranial shape scores and log centroid-size of *Artibeus* species. Deformation grids depict shape variation associated with centroid-size. Red dots represent *Artibeus cinereus*, while black dots represent large *Artibeus* species sampled.

Redondo *et al.* 2008, Baker *et al.* 2016, Cirranello *et al.* 2016). Therefore, this group provides an excellent opportunity for a study that integrates molecular and morphological data to discriminate species, as well as to investigate whether the variations in skull morphology are related to the evolutionary history of the group, or whether they may be driven by external environmental conditions based on geographical distribution.

We advocated for an integrative approach, proposing a molecular delimitation analysis utilizing MOTUs combined with cranial size and shape data for fruit-eating *Artibeus* bats. This combined methodology has the potential to significantly contribute to the differentiation among *Artibeus* species as our findings clearly demonstrated. Given the often-challenging task of distinguishing species within this group through a single approach, our results underscore the importance of integrating data. In this study, two datasets were generated—one based on *COI* sequences of *Artibeus* bats and the other based on cranial shape data using GM techniques. Also, the integration of these datasets allowed us to examine, for instance,

the influence of phylogenetic signals on cranial shape variation in *Artibeus* species.

Previous studies on *Artibeus* species have mainly utilized linear morphometric techniques (Lim 1997, Guerrero *et al.* 2003, 2004, Marchán-Rivadeneira, 2006, 2008, Lim *et al.* 2008, Larsen *et al.* 2010) with only a few using GM techniques (Marchán-Rivadeneira *et al.* 2010, Hedrick 2021). However, these studies did not consider the effect of residual allometry, i.e. they did not account for the influence of residual size (centroid-size) on shape (Klingenberg 2016). Given the substantial size difference between small and large *Artibeus* species, the importance of analyses considering the effect of residual allometry becomes evident. Our findings, based on GM techniques using shape data, revealed significant differences between *Artibeus* species. However, with regression scores, these species are only partially discriminated in shape without any size effects.

We also found that the cranial shape and size of *A. planirostris* vary according to geographical distribution (biomes). This variation had not previously been explored for *A. planirostris*.

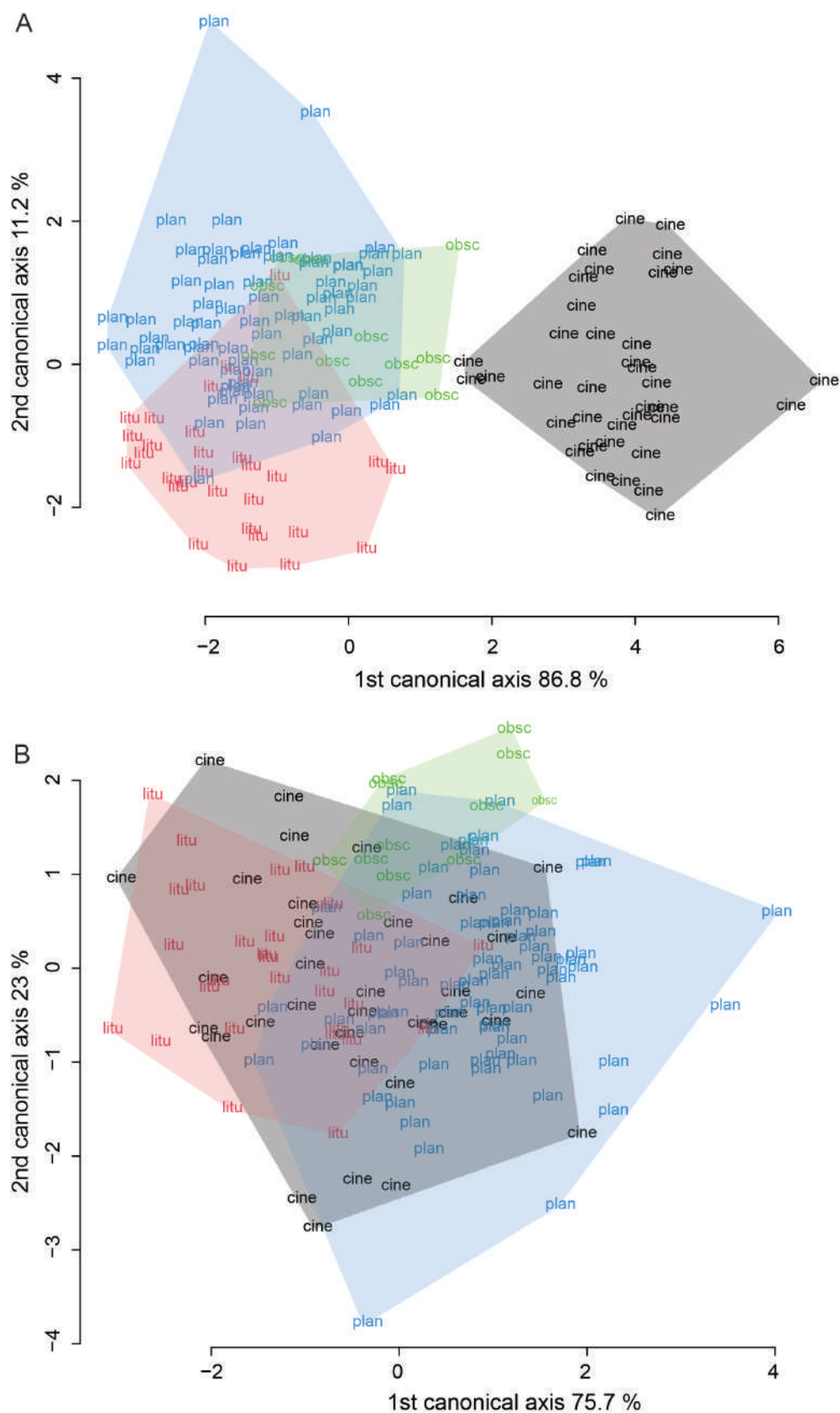


Figure 6. A, canonical variate analysis using dorsal view cranial shape data; and B, with regression scores (residuals) for evaluated *Artibeus* species.

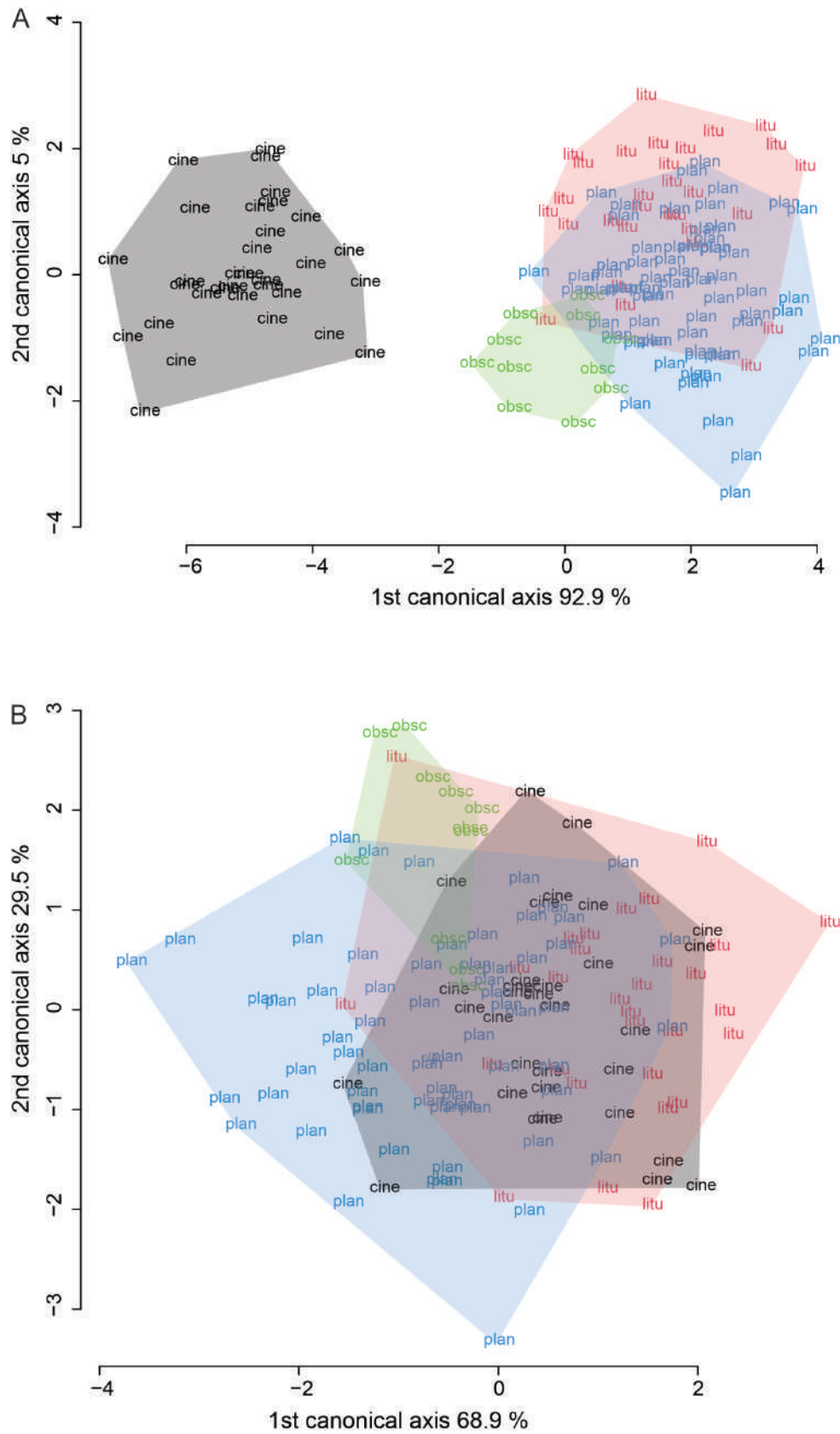


Figure 7. A, canonical variate analysis using ventral view cranial shape data; and B, with regression scores (residuals) for evaluated *Artibeus* species.

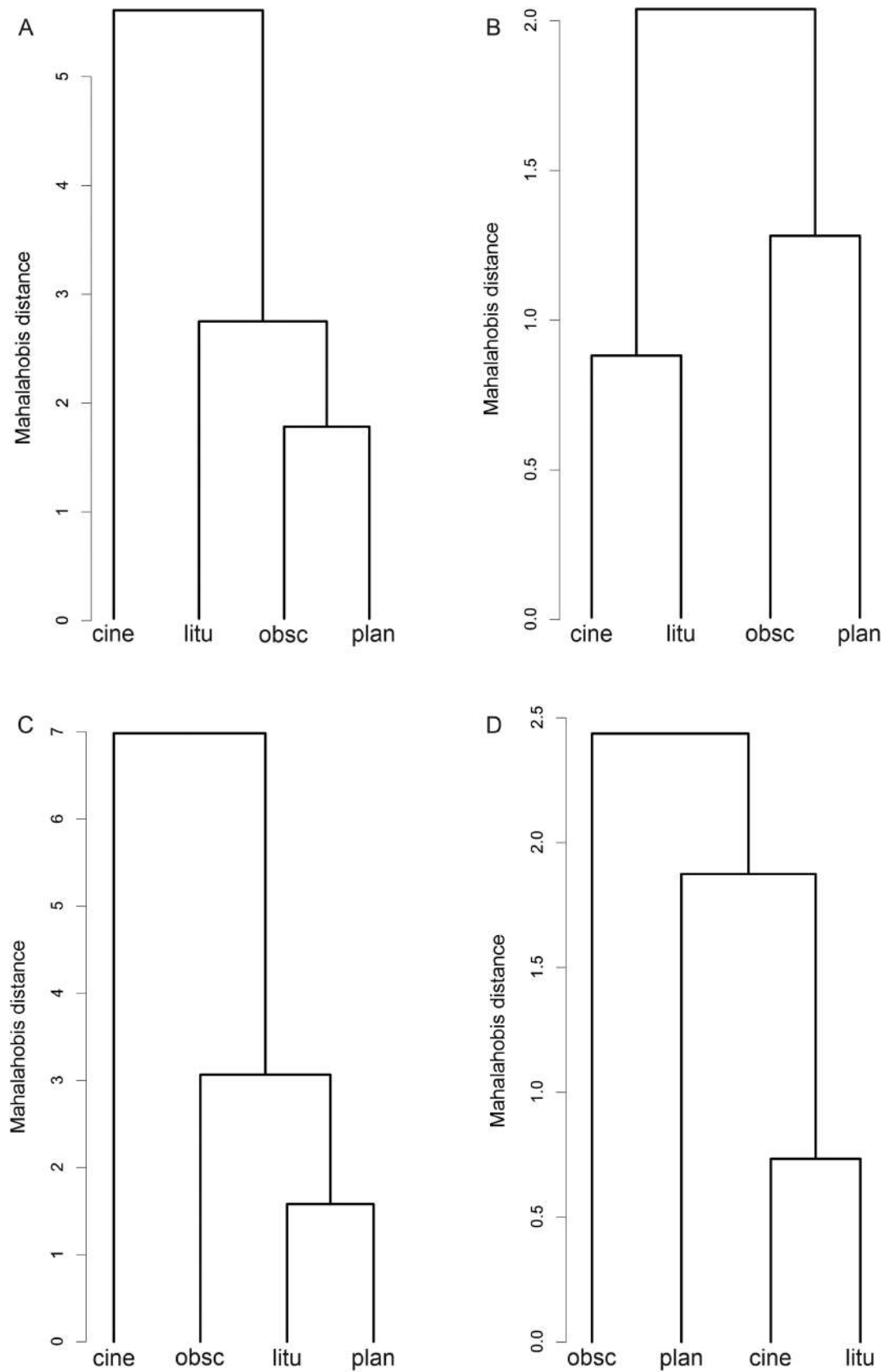


Figure 8. Mahalanobis distance of dorsal view cranial morphology: shape data scores (A) and regression scores (B); and ventral view: shape data scores (C) and regression scores (D) for evaluated *Artibeus* species.

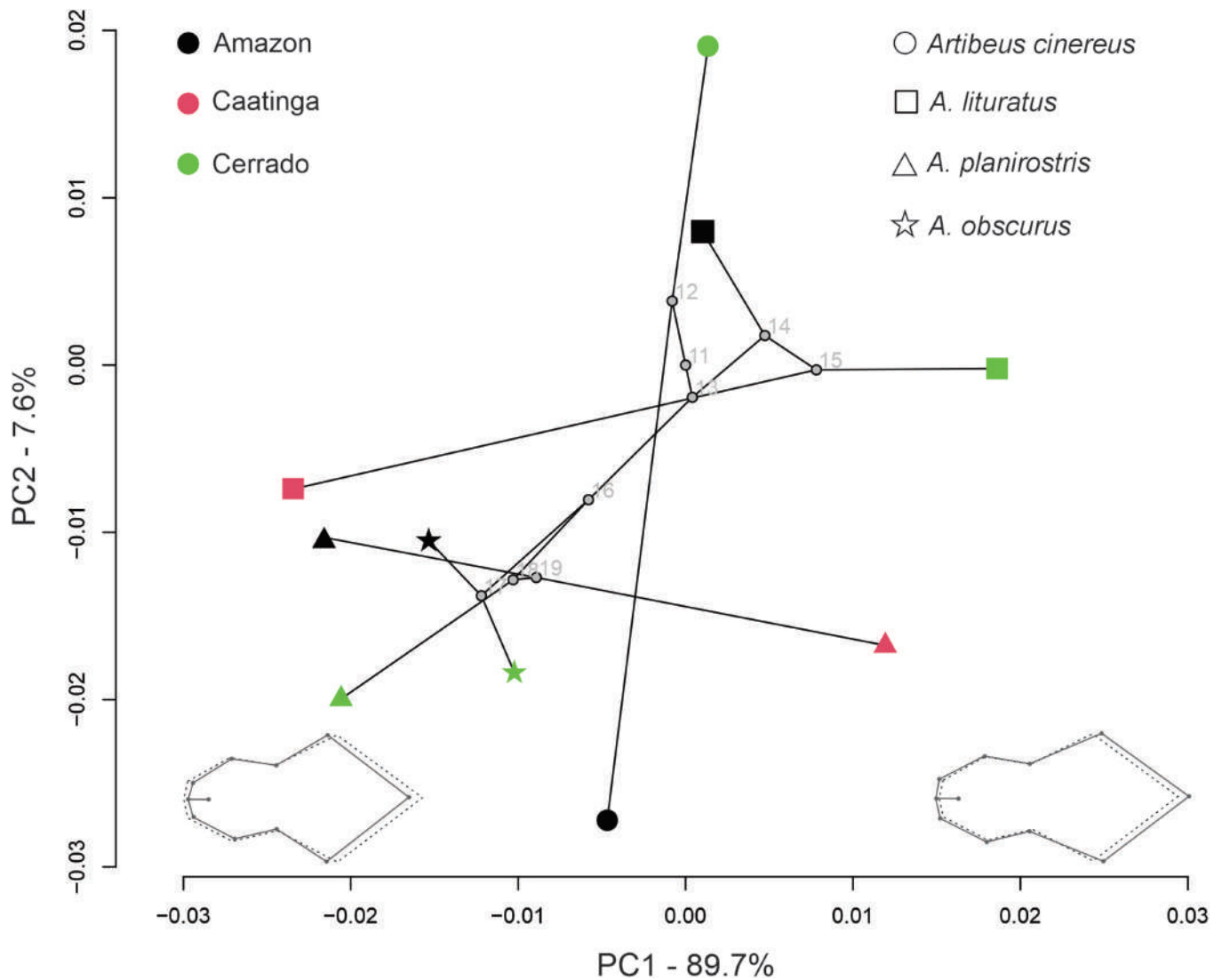


Figure 9. Phylomorphospace based on dorsal view cranial shape of *Artibeus* species. The dotted lines represent the extreme variations in cranial shapes in relation to the mean shape (solid line). The four species are represented with their respective colours as indicated in the figure.

Furthermore, based on inter- and intra-MOTU molecular delimitation the four species of *Artibeus* were well defined.

Molecular delimitation

The ABGD and ASAP molecular delimitation methods, utilizing the COI gene, demonstrated effectiveness in identifying species. These methods estimated four lineages corresponding to the species (*A. lituratus*, *A. planirostris*, *A. obscurus*, and *A. cinereus*) defined based on morphology and recovered in the Bayesian tree (Fig. 3). This is consistent with data from the genetic distance matrix, which exhibited significant inter- and intra-MOTU average values (Table 1); these data delimited large and small species of *Artibeus*, the highest inter-MOTU values being between large and small species, and the lowest inter-MOTU values being among large species. These values indicate a considerable level of genetic differentiation between small and large *Artibeus* species, although not to the extent of considering them as representing different genera, as argued by Guerrero *et al.* (2008).

The results from the GMYC model revealed inconsistencies with morphological data, indicating the presence two different

lineages in accordance with the clades recovered in *A. planirostris* shown in the BI tree. Such divergences serve as a reminder that molecular delimitation methods should be used with caution, as they may either show accurate results congruent with morphology data, as observed in the ABGD and ASAP methods, or they may eventually present incongruent results or erroneous results (Puillandre *et al.* 2020). This is particularly relevant when inter- and intraspecific divergences are low, as observed for *A. planirostris* in the present study.

Our findings indicate that the MOTUs of large species of *Artibeus* were closely related, providing evidence of this relationship after uplift of the Andes during the last 2.5 Myr, and flooding of the Amazon basin at the end of the Pleistocene. These events had a significant influence on species diversification and evolutionary patterns in *Artibeus* bats in South America (Patten 1971, Lim 1997, Lim *et al.* 2004, Larsen *et al.* 2007, Redondo *et al.* 2008). Larsen *et al.* (2013), in a study on the evolutionary history of the genus using mitochondrial and nuclear markers, identified two groups. One group comprised the species *A. aequatorialis* Andersen, 1906 and *A. jamaicensis* Leach, 1821, while the other included *A. amplus* Handley, 1987, *A. lituratus*, *A. obscurus*, *A.*

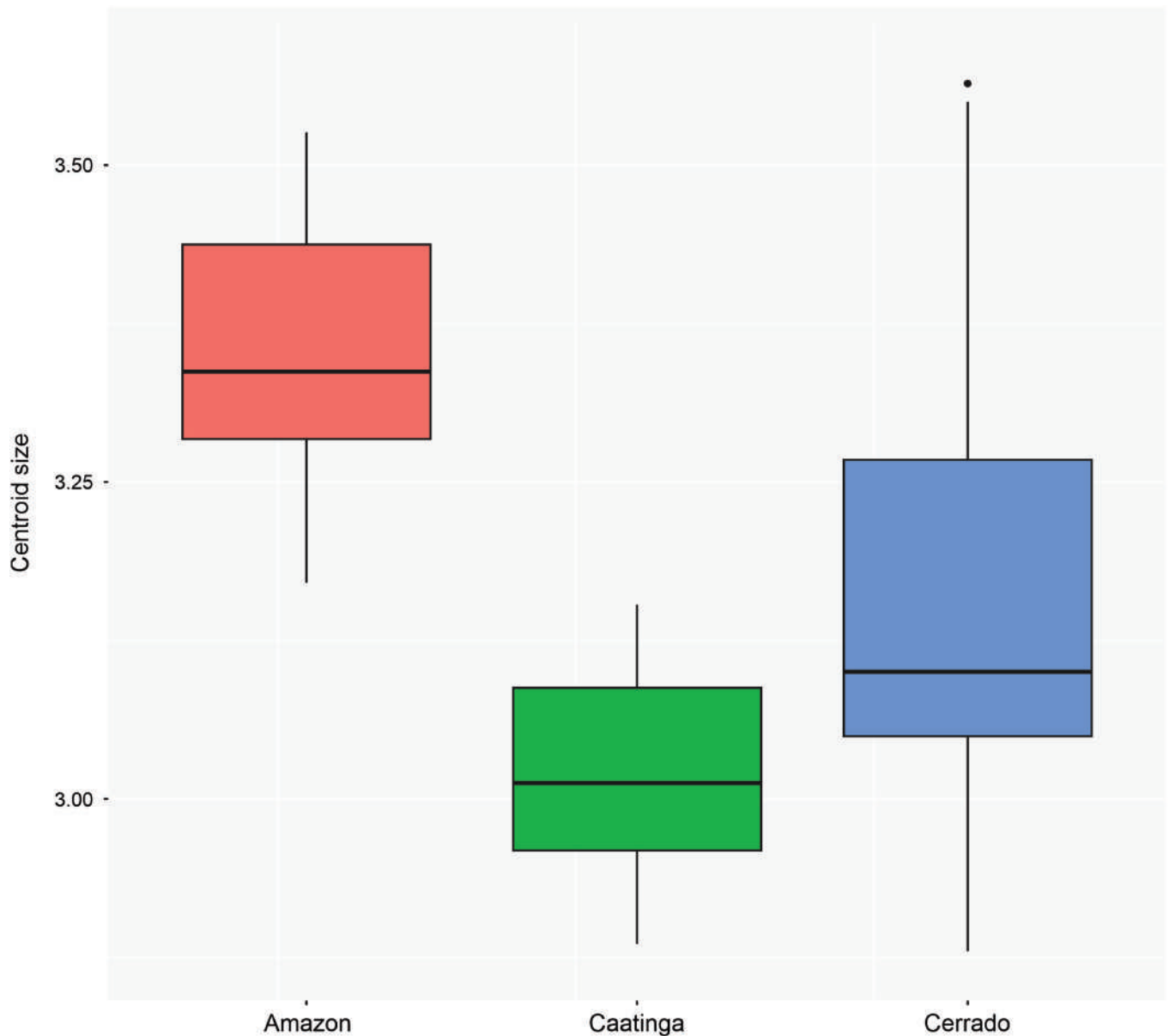


Figure 10. Boxplot of dorsal view centroid-size of *Artibeus planirostris* across sampled biomes.

planirostris, and *A. schwartzi* Jones, 1978. This supports the hypothesis of vicariance resulting from the influence of uplift of the Andes on the diversification of *Artibeus* species. The three large *Artibeus* species studied here (*A. lituratus*, *A. planirostris*, and *A. obscurus*) are widely found on the east side of the Andes in Brazil inhabiting the Cerrado, Amazon, and Caatinga biomes and living in sympatry.

The MOTU of *A. obscurus* was more closely related to the MOTU of *A. planirostris* with a posterior probability (PP) value of 0.99. *Artibeus obscurus* and *A. planirostris* exhibit morphological similarities, particularly in terms of size. Lim *et al.* (2004) and Redondo *et al.* (2008) also recovered *A. obscurus* as sister to *A. planirostris* in their molecular studies. The MOTU of *A. cinereus* formed a strongly supported clade, with a PP value of 1 (Fig. 3), aligning with the morphological identification. The taxonomic history of small species of *Artibeus* is confusing; these species are generally distinguished based on size with

morphological characteristics such as dental characters (variation in the number of molars) being crucial in species discrimination (Reis *et al.* 2013). Mitochondrial and nuclear DNA analyses have recovered the monophyly of the group as sister of all large species of the genus (Solari *et al.* 2009).

Interspecific cranial shape variation

The ANOVA results revealed significant variations in cranial shape among *Artibeus* species, supporting findings from Hedrick (2021). This suggests that evaluating cranial morphology through GM techniques constitutes a valuable tool for distinguishing species within the genus. Additionally, centroid-size of the dorsal view exhibited significantly different values among species (see Fig. 4). Moreover, the effect of centroid-size on cranial shape was noteworthy, signifying that species exhibit diverse cranial shapes relative to their sizes. Large *Artibeus* species displayed a shorter and wider braincase region, along

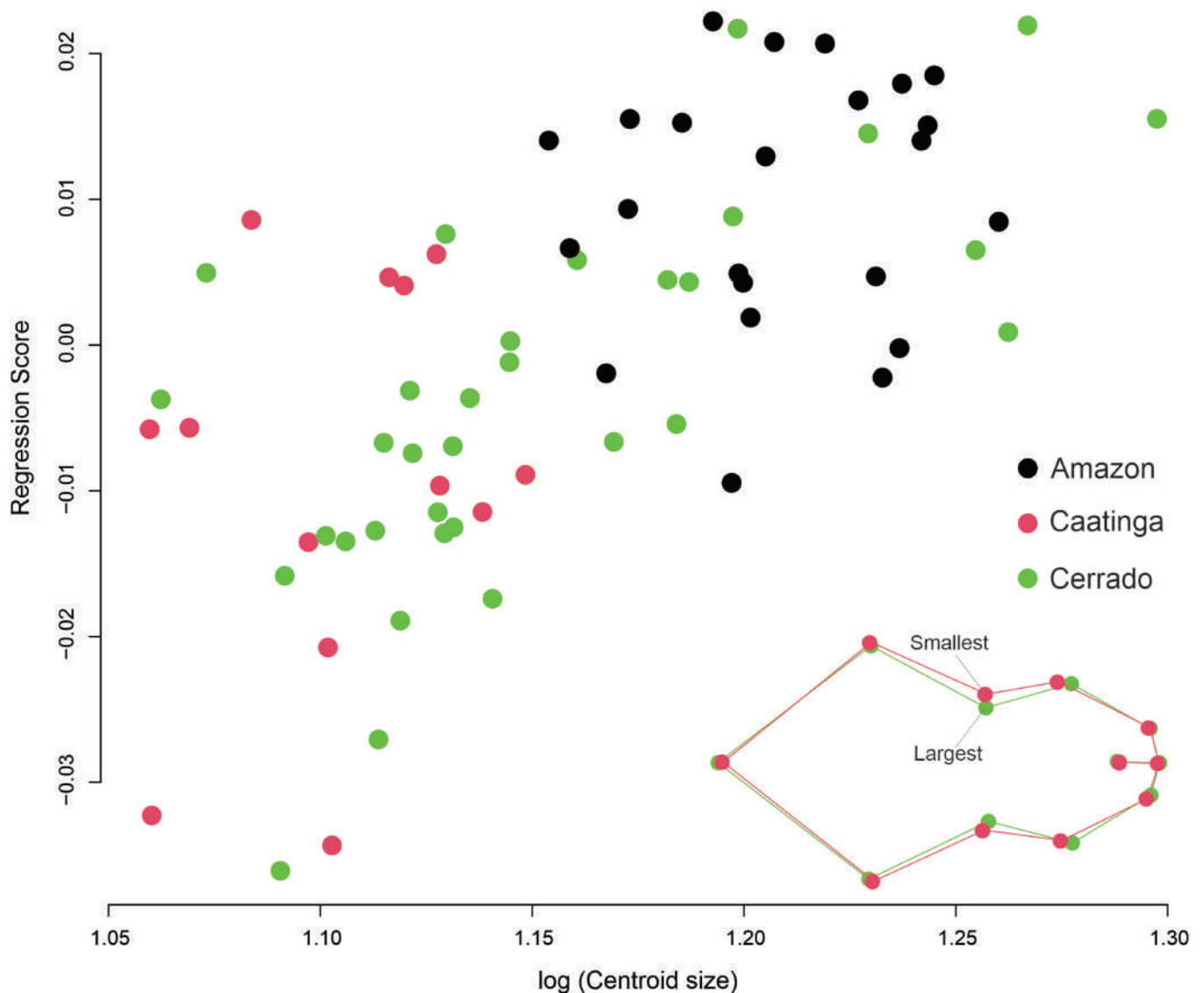


Figure 11. Regression analysis of dorsal view cranial shape scores and log centroid-size of *Artibeus planirostris*, highlighting biome variations. Shapes on the x-axis indicate the cranial shape variation related to centroid-size.

with a more protruding rostrum, compared to their smaller counterparts (see Fig. 5). Previous research on other bat species (Sztencel-Jablonka *et al.* 2009, Santana *et al.* 2012, Shi *et al.* 2021) has demonstrated the utility of cranial morphological structures in understanding ecological aspects and dietary differences driven by functional morphological demands, indicating an evolutionary shift in dietary specialties. This may be reflected in morphological structures related to chewing, such as canine teeth, where the functional importance of the space between teeth is considered, aligning with the role of these structures in the chewing process (Marchán-Rivadeneira *et al.* 2010).

Fruit-eating bats of the genus *Artibeus* exhibit a specialization in consuming *Ficus* spp. fruits (Handley *et al.* 1992, Giannini and Kalko 2004). However, larger *Artibeus* species may feed on fruits larger than the typical figs found in the Cerrado, Amazon, and Caatinga biomes, such as those from Anacardiaceae, Arecaceae, Cactaceae, and other families (Parolin *et al.* 2016, Laurindo and Vizentin-Bugoni 2020). The consumption of these larger

foods necessitates a broader skull with a more protruding rostrum. The shape and hardness of the consumed foods could significantly influence skull morphology through changes in bite force (Marchán-Rivadeneira *et al.* 2010, Santana *et al.* 2012). In contrast, *A. cinereus* possesses a more elongated skull with a narrower rostrum (see Fig. 5), suggesting a possible preference for smaller fruits. We demonstrated that the variation in cranial shape of *A. cinereus*, when compared to other larger *Artibeus* species, is closely linked to centroid-size variation. In other words, it is strongly driven by residual allometry (Klingenberg 2016) (see Fig. 6A, B).

CVAs using shape data of both views of the skull showed a high discriminatory power between large and small species of *Artibeus* resulting in high correct classification rates following cross-validation (Figs 6A, 7A). Despite some overlap, cranial shape assessed through GM techniques retains substantial potential for discriminating among *Artibeus* species. However, in CVAs performed with regression scores

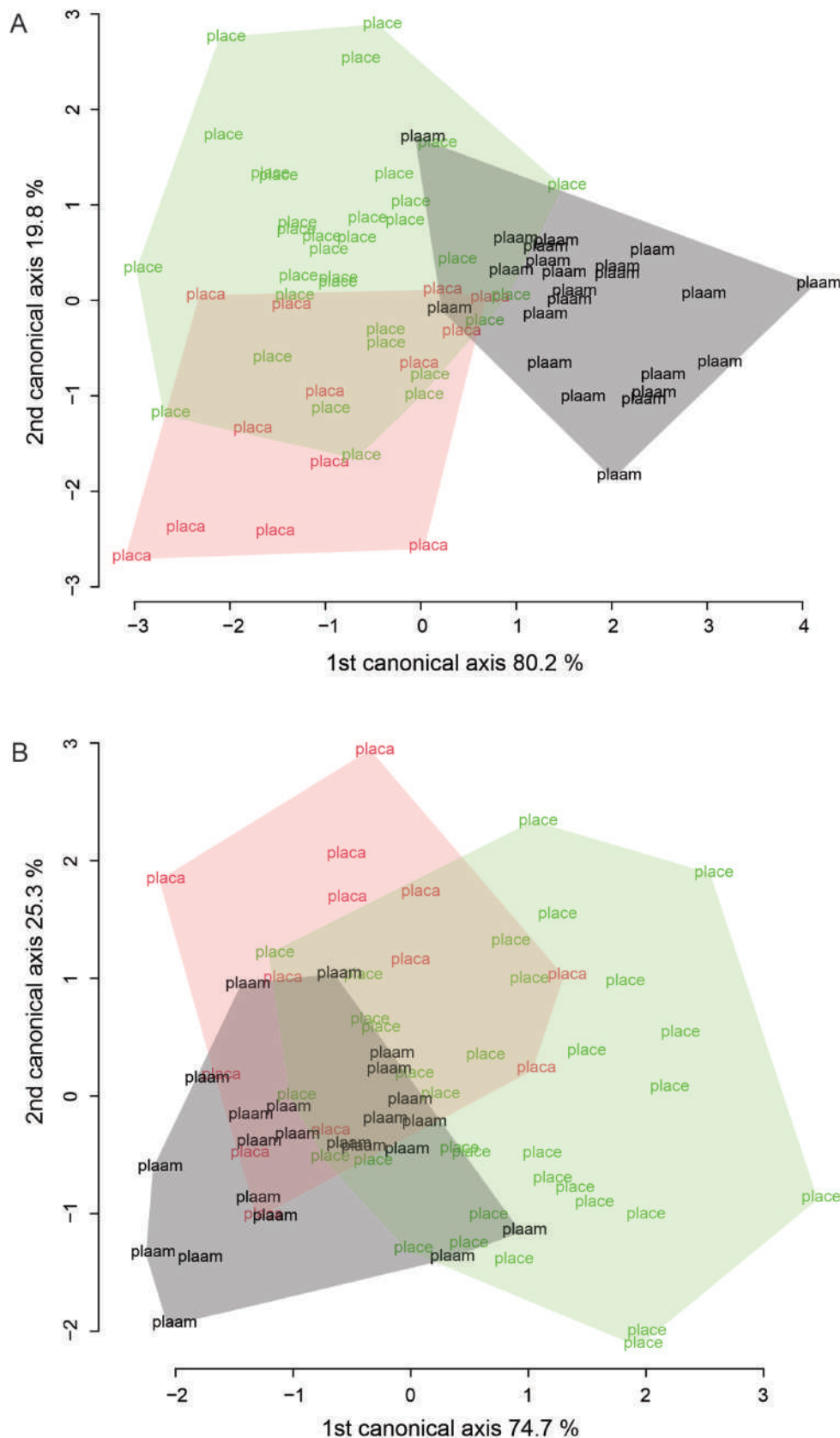


Figure 12. Canonical variate analysis using dorsal view cranial shape data of *Artibeus planirostris* (A) and with regression scores (residuals) (B).

(allometry-free data), *A. cinereus* exhibited a cranial shape similar to that of large species (see Figs 6B, 7B). This suggests that if all *Artibeus* species were similar in size, there

would be minimal variation in cranial shape. This trend was evident in the Mahalanobis distances of the cranial shape of *A. cinereus* and *A. lituratus*, where both species appeared

morphologically similar, without any size residual effect on shape (Fig. 8B, 8D).

Our hypothesis to elucidate the similarity in cranial shape between *A. cinereus* and *A. lituratus* is that these species may feed on fruits of different sizes but with similar consistency. Marchán-Rivadeneira *et al.* (2010), utilizing geometric and linear morphometric techniques on the ventral view of the skull in other *Artibeus* species, also observed cranial shape similarities among large *Artibeus* species and a small *Artibeus* species (*A. phaeotis* Miller, 1902). In contrast, significant cranial shape variations were noted between *A. concolor* and *A. anthonyi* (Wołoszyn and Silva-Taboada, 1977). Our findings suggest that the three evaluated large *Artibeus* species exhibit a high percentage of correct classification after cross-validation, based on cranial shape in both dorsal and ventral views, with or without the influence of residual allometry (see Figs 6, 7). This indicates a potential diversification process linked to dietary habits as a strategy to mitigate competition among large species, particularly considering their sympatry.

Our assessment of the degree of phylogenetic signal indicates that there is no significant effect of phylogeny on cranial shape variation in dorsal view. This implies that the cranial shape of the *Artibeus* species examined in this study is not notably affected by their phylogenetic relationships (see Fig. 9). Instead, the observed cranial shape variation is probably shaped by external and historical ecological factors, aligning with our proposed hypothesis. Differences in morphological aspects of the skull related to feeding play a crucial role in species discrimination, as demonstrated in fruit-eating bats in this study, as well as in other mammals facing intense competition for resources in their environment (Bonaccorso *et al.* 2007, Rex *et al.* 2010). As a strategic response to mitigate interspecific competition, bat species expand their niche, potentially driving the evolution of morphological characteristics over time among sympatric species. This phenomenon is recognized as a pivotal factor in evolutionary diversification (Santana *et al.* 2012).

Intraspecific cranial shape variation in *A. planirostris*

Investigating intraspecific morphological variation across biomes, each characterized by different environmental factors, is a valuable approach for understanding phenotypic characteristics directly influenced by these environmental factors (Binning *et al.* 2010). Our findings employing this approach indicate substantial variation in cranial shape and size of *A. planirostris* across different biomes (see Figs 10–12). This suggests that the availability and type of food resources play a crucial role in differentiating cranial size and shape of *A. planirostris* populations. Specimens from the Caatinga biome are geographically characterized by smaller skull phenotypes, those from the Amazon biome have larger skull phenotypes, and those from the Cerrado biome have a range of skull phenotypes in terms of size. This supports the hypothesis that phenotypes within a species are not uniform in space, as local environmental conditions can directly influence morphological differences (Nascimento *et al.* 2023).

The observed geographical variation pattern in *A. planirostris* aligns with previous findings by Davis (1984) and Marchán-Rivadeneira *et al.* (2012), who reported that smaller phenotypes of the *A. lituratus* complex are predominantly distributed in semiarid regions, whereas larger phenotypes are found in humid

regions. Marchán-Rivadeneira *et al.* (2012) also noted skull size variation in continental Central American species, with *A. intermedius* (small skull phenotype) mainly distributed along the Pacific coast and *A. lituratus* (large skull phenotype) predominantly along the Atlantic coast.

Figs (*Ficus* spp.) constitute the primary diet for *A. planirostris*, fulfilling most of their nutritional needs (Handley *et al.* 1992, Andrade *et al.* 2013). Consequently, seasonality emerges as a crucial factor influencing the availability of these fruits (Bianconi *et al.* 2006, Teixeira *et al.* 2009). Therefore, variations in cranial size and shape are expected based on the geographical distribution with distinct environmental characteristics affecting the availability of these food resources (Marchán-Rivadeneira *et al.* 2012, Macedo *et al.* 2023). For instance, the Caatinga biome, characterized by semiarid conditions and poor soil, directly influences food productivity and availability, potentially resulting in a smaller skull size for *A. planirostris* due to resource scarcity or seasonal fluctuations in the availability of different fruit species.

On the other hand, more perennial or productive regions, such as the Cerrado, known for its heterogeneity and variability depending on annual climatic conditions, have the potential to produce larger body and skull sizes. Moreover, a larger skull may also be associated with regions featuring a warm climate (Davis 1984), where there is a greater availability of food resources throughout the year. This is particularly relevant for *A. planirostris*, as it can supplement its diet with nectar, pollen, leaves, insects, etc. (Cordero-Schmidt *et al.* 2016), and these resources are abundantly present in the Amazon biome. Our findings strongly support the hypothesis that the variation in cranial size and shape in *A. planirostris* is probably influenced by the availability of food resources in each biome. The observed patterns in body and skull size appear to be a response to the diverse ecological conditions and resource availability in the distinct geographical regions inhabited by *A. planirostris*.

CONCLUSION

Herein, we demonstrate significant cranial size and shape variation among species of fruit-eating bats of the genus *Artibeus* through integration of morphological and molecular datasets. Large species within the genus exhibit considerable cranial shape variation, which can be useful for discrimination among them. However, when comparing the small species with the large *Artibeus* species, we found a strong effect of residual allometry acting on shape. Cranial size and shape variation are not significantly related to phylogeny; the sympatric species are probably diversifying due to environmental forces influencing dietary needs present in the Caatinga, Cerrado, and Amazon biomes, which may directly drive the skull morphology of these species. In *A. planirostris*, occurring across the Caatinga, Cerrado and Amazon biomes, we observed cranial shape and size variation that may be associated with environmental conditions in these biomes, potentially related to the availability of food resources.

SUPPLEMENTARY DATA

Supplementary data are available at *Biological Journal of the Linnean Society* online.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

All data are available in NCBI GenBank (see [Information Table S1](#)).

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3.2 CAPÍTULO 2

Artigo aceito para publicação na revista *Mammalian Biology*, com JCR (1.9)
intitulado: **Population genetics and demographic history of flat-faced fruit-eating bat**
Artibeus planirostris (Spix, 1823) (Chiroptera: Phyllostomidae)



Population genetics and demographic history of flat-faced fruit-eating bat *Artibeus planirostris* (Spix, 1823) (Chiroptera: Phyllostomidae)

Samira Brito Mendes¹ · Fabiano Stefanello² · Gerson Paulino Lopes^{3,4,6} · Tamily Carvalho Melo Santos⁴ · Elmary da Costa Fraga⁵ · Maria Claudene Barros^{1,5}

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Abstract

Studies on population genetic differentiation are essential for understanding the ecological, biological, and behavioral aspects of species, as well as their diversification patterns and how populations evolve over time. The species *Artibeus planirostris*, one of the best-known Neotropical fruit bats, has a wide distribution. This species exhibits high dietary plasticity and plays a significant ecological role. While *A. planirostris* has been used as a model organism in various studies, no comprehensive research has yet explored its population dynamics. Here, we analyzed fragments of the mitochondrial Cytochrome b (Cyt b) and Cytochrome c Oxidase 1 (COI) genes to investigate the genetic diversity, population structure and demographic history of *A. planirostris* in the Neotropics. Our findings reveal a weakly structured geographic population pattern. Nevertheless, in peripheral populations, we have observed limited maternal gene flow due to isolation by distance or potentially geographic barriers. It suggests that local adaptations, shaped by specific environmental pressures and Pleistocene climatic fluctuations, may have influenced the dispersal and colonization abilities of *A. planirostris* in these regions. The demographic history of *A. planirostris* indicates a recent population expansion during the late Pleistocene, approximately 50,000–60,000 years ago.

Keywords Dispersion · Genetic diversity · Mitochondrial DNA · Neotropics · Pleistocene · Phylogeography

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✉ Samira Brito Mendes
britosamira503@gmail.com

Fabiano Stefanello
stefanellof@gmail.com

Gerson Paulino Lopes
gersonlps@hotmail.com

Tamily Carvalho Melo Santos
tamily-lfv@hotmail.com

Elmary da Costa Fraga
elmaryfraga@yahoo.com.br

Maria Claudene Barros
mbdene@yahoo.com.br

¹ Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede Bionorte, Universidade Estadual do Maranhão, Cidade Universitária Paulo VI, Avenida Lourenço Vieira da Silva, nº 1000. Jardim São Cristóvão, São Luís, Maranhão 65055-310, Brazil

² Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Campus Soane Nazaré de Andrade, Rod. Jorge Amado, Km 16 - Salobrinho, Ilhéus, Bahia 45662-900, Brazil

³ Laboratório de Evolução e Genética Animal, Universidade Federal do Amazonas, Avenida Rodrigo Otávio, Manaus, Amazonas 69080-005, Brazil

⁴ Grupo de Pesquisa em Ecologia de Vertebrados Terrestres and Grupo de Pesquisa em Medicina da Conservação e Saúde Única, Instituto de Desenvolvimento Sustentável Mamirauá, Estrada do Bexiga, Tefé, Amazonas 69553-225, Brazil

⁵ Programa de Pós-Graduação em Biodiversidade, Ambiente e Saúde, Universidade Estadual do Maranhão, Campus Caxias, Praça Duque de Caxias S/N, Bairro Morro do Alecrim, Caxias, Maranhão 65604-38065604-380, Brazil

⁶ Centro Educacional Governador Gilberto Mestrinho, Coordenadoria Regional de Tefé, Secretaria de Estado de Educação e Desporto Escolar, Estrada do Aeroporto, 1241, São Francisco, Tefé, Amazonas 69552-105, Brazil

Introduction

Phylogeography focuses on the principles and processes driving the geographic distribution of genealogical lineages, particularly at the intraspecific level (Avice 2000). One of its main objectives is to integrate phylogeny and population genetics to better understand the relationships between micro- and macro-evolutionary processes. This helps explain how historical events have shaped the present-day geographic distribution of genes within populations and species (Avice 2000; Papadopoulou and Knowles 2016; Thomé and Carstens 2016; Zamudio et al. 2016).

A major challenge in biology is establishing connections between ecology and species evolution, a link that can be explored through the relationships between dispersal capability, genetic differentiation, and the spatial scale at which populations diverge. Population genetic differentiation studies are fundamental in this regard, as they allow for inferences about the interaction of microevolutionary forces over a species' history. They also provide insights into potential future local adaptation or speciation (Bohanak 1999; Anderson et al. 2018; Galetti 2023).

Understanding the geographic distribution of a species is a key source of evidence in studies of population dynamics across various animal groups. In bats, for example, species with high dispersal capability tend to exhibit less genetic structure over finer geographic scales. Several factors can influence this, such as mating outside breeding areas and long-distance movements (Moussy et al. 2012). However, several historical events, including ecological and biogeographic barriers, also can shape genetic structure in bats (Guevara-Chumacero et al. 2010; Loureiro et al. 2020), as well as in other organisms (Joly and Bruneau 2006; Moussalli et al. 2009; Bell et al. 2012; Carnaval et al. 2014; Zamudio et al. 2016; Stefanello et al. 2020).

The dispersal capability of bats surpasses those of all other land mammals, enabling them to colonize even remote islands (Ziegler et al. 2016; Speer et al. 2017). Bats of the family Phyllostomidae are good dispersers and quite diverse in the Neotropics (Simmons 2005). One of the most diverse genus in the family, *Artibeus* Leach, 1821, stands out as a radiation of relatively large frugivorous bats, comprising 23 species across the Neotropics (Solari et al. 2019; Taylor 2019).

Several species within *Artibeus* are still considered components of a species complex (Davis 1984; Lim et al. 2004; Larsen et al. 2007; Redondo et al. 2008; Marchan-Rivadeneira et al. 2012; Mendes et al. 2024). For instance, *Artibeus planirostris* (Spix, 1823) was once regarded as a subspecies of *Artibeus jamaicensis* (Leach, 1821). However, multiple studies based on morphological and

molecular data (Phillips et al. 1989; Guerrero et al. 2004; Lim 1997; Lim et al. 2004) have identified *A. jamaicensis* and *A. planirostris* as distinct species. In a phylogeographic study covering all subspecies within the *A. jamaicensis* complex, Larsen et al. (2007) used mitochondrial Cytochrome b (Cyt b) gene sequences to further confirm that *A. planirostris* is a distinct species from *A. jamaicensis*. These studies provide valuable insights into the genetic diversity and relationships between *A. planirostris* and *A. jamaicensis* and their subspecies. However, to gain a more comprehensive understanding of the population genetics of *A. planirostris*, further research and genetic analyses across different populations and regions throughout its range are still needed.

The flat-faced fruit-eating bat *A. planirostris* is one of the best-known Neotropical bats, with a distribution spanning much of South America, including Brazil, Bolivia, Colombia, Guyana, French Guiana, Paraguay, Peru, Suriname, Venezuela and Caribbean islands such as Grenada, Trinidad and Tobago (Lim 1997; Lim and Engstrom 2001; Larsen et al. 2013). This species exhibits high dietary plasticity and plays a crucial ecological role. As a primary frugivore, this bat plays a crucial role in seed dispersal for a wide variety of plants, promoting forest regeneration and maintaining biodiversity. Thus, *A. planirostris* is considered a key agent in sustaining the ecological balance and resilience of tropical ecosystems (Fleming 1988; Hollis 2005).

Artibeus planirostris has served as a model organism in various molecular studies (Lim et al. 2004; Larsen et al. 2007; Lino et al. 2021; Mendes et al. 2024), as well as in research of medical significance, such as investigations into *Hepatitis virus* (De Moraes Pires et al. 2024) and leishmaniasis (De Castro Ferreira et al. 2017). Additionally, it has been the subject of morphological studies using both linear morphometry (De Medeiros Filho et al. 2018) and geometric morphometrics (Mendes et al. 2024). Despite this, no comprehensive study has been conducted on the population dynamics of *A. planirostris*, making it an ideal candidate for population genetic research on a broad geographic scale.

In this study, we used the mitochondrial markers Cyt b and Cytochrome c Oxidase 1 (COI) to investigate the population structure, genetic diversity, and demographic history of *A. planirostris* across its geographic distribution. Understanding these phylogeographic aspects will contribute to a deeper comprehension of the species population dynamics and inform potential conservation strategies. Our hypotheses are as follows: (i) given the high flight capability and wide distribution of *A. planirostris*, we expect a low level of population genetic structure; (ii) additionally, due to its flight capability, we anticipate that the effect of isolation by distance will not be significant; (iii) the absence of geographic barriers in South America (except the Andes Mountains) is likely to facilitate gene flow, promoting genetic connectivity

among populations and consequently influencing the genetic structure of *A. planirostris*; and (iv) Pleistocene climatic events may have influenced the effective population size, with interglacial periods potentially favoring demographic expansion.

Methods

Sampling

Bats were collected from three biomes and different states and municipalities in northern and northeastern Brazil: Caatinga [Piauí (PI)], Cerrado [Maranhão (MA)], and Amazon [Maranhão and Pará (PA)]. The Caatinga biome samples were collected from the municipalities of Picos/PI and Milton Brandão/PI. For the Cerrado biome, samples were collected from Caxias/MA, Chapadinha/MA, and Carolina/MA. The Amazon biome samples came from Cândido Mendes/MA, Turiaçu/MA, Augusto Corrêa/PA, Viseu/PA, Uarini/AM, Tefé/AM, Santo Antônio do Içá/AM, Jutai/AM, Japurá/AM, Tefé/AM and Juruá/AM. The collections were authorized by the Brazilian authorities of the Chico Mendes Institute for Biodiversity Conservation—ICMBIO/SISBIO under license numbers 42670–1, 54,384–1, 68,047–1, 74,512–1, 42,111-1, 42,111-2, 42,111-3, 42,111-5, 42,111-6, 68,801-1, 68,801-2, and 68,801-3.

The collections were conducted through multiple field expeditions using mist nets set up near shelters for bats between 2014 and 2022. The captured bats were transported to an improvised laboratory near the collection sites, where they were photographed, euthanized, weighed, and measured using a manual caliper (300 mm). The bats were then labeled, and muscle tissue was removed and stored in microtubes with 70% ethyl alcohol for molecular analysis. After the collections were completed, the specimens were transported to the GENBIMOL complex, which houses the Genetics and Molecular Biology laboratories at the Universidade Estadual do Maranhão, Campus Caxias. Species identification was performed using taxonomic classification keys (Reis et al. 2013; 2017; Díaz et al. 2021) and later confirmed by additional experts. Vouchers are deposited in the Coleção de História Natural da Universidade Federal do Piauí (CHNUFPI), Campus Amílcar Ferreira Sobral (CAFS), in Floriano, Piauí, Brazil and Coleção de Mamíferos do Instituto de Desenvolvimento Sustentável Mamirauá (IDSM), Amazonas, Brazil (see Supplementary Materials, TableS3 and TableS4).

Molecular procedures

The genomic DNA was extracted from muscle tissue using the Wizard Genomic® DNA Purification Kit (Promega),

following the manufacturer's instructions. Two mitochondrial DNA fragments were amplified via Polymerase Chain Reaction (PCR): the COI gene, as described by Folmer et al. (1994), and the Cyt b gene, as detailed by Martins et al. (2007). Sequencing was conducted using the dideoxy terminator method (Sanger et al. 1977) on an ABI Prism™ 3500 automated DNA sequencer (Applied Biosystems, USA). Sequence edits were performed using Geneious R7 (Kearse et al. 2012), and alignments were carried out using the default parameters of the MUSCLE algorithm (Edgar 2004).

A total of 372 sequences of the COI gene (634 base pairs) and 223 sequences of the Cyt b gene (1,000 base pairs) were analyzed. Of these, 106 sequences were obtained from the present study—50 for the COI gene and 56 for the Cyt b gene. The remaining sequences were sourced from GenBank and BOLD Systems v3. Our final dataset covers samples from various countries in the Neotropical region: Venezuela, Ecuador, Colombia, Guyana, Suriname, and Brazil, as well as the islands of Grenada and Trinidad and Tobago (see Supplementary Materials, TableS1 and TableS2). The GenBank and BOLD Systems v3 sequences included in this study were derived from the works of Lim et al. (2004), Larsen et al. (2007), Redondo et al. (2008), Clare et al. (2011), Benítez et al. (2021), and Lino et al. (2021).

For species identification and comparison, the sequences from the present study were analyzed using BLAST (Basic Local Alignment Search Tool—<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) platform (Altschul et al. 1990). Our generated sequences also were deposited in the GenBank database (see Supplementary Materials, TableS3 and TableS4).

Genetic diversity and population structure

To estimate the genetic clusters, we employed Bayesian hierarchical clustering (BHC) using the FastBAPS algorithm (Heller and Ghahramani 2005; Tonkin-Hill et al. 2019) in the R software 4.2.3 (R Core Development Team 2024). This approach enables us to assess the similarities between genomic sequences and determine their grouping into clusters (K) (Tonkin-Hill et al. 2019).

Genetic diversity within populations was estimated based on the number of haplotypes (H), haplotypic diversity (h) (Nei 1987), and nucleotide diversity (π) (Nei and Li 1979), using the DnaSP v.5 program (Librado and Rozas 2009). Haplotype networks were constructed in the POPART 1.7 program (Leigh et al. 2015) using the median-joining (MJ) algorithm (Bandelt et al. 1999) with 1000 interactions. This analysis facilitates inferences about the spatial distribution of haplotypes and helps verify genealogical relationships among the clusters identified by FastBAPS.

Analysis of Molecular Variance (AMOVA) was performed using Arlequin v3.5 (Excoffier and Lischer 2010) to assess genetic variation within and between the clusters identified by

FastBAPS. This analysis was based on *F*-statistics, using fixation indices (*F_{st}*) following the approach of Weir and Cockerham (1984). The significance of *F_{st}* values was determined through 10,000 random permutations. Pairwise genetic differentiation indices between populations (*F_{st}*) (Neigel 2002) were calculated for the established populations, and significance levels (*P*-values) were assessed for all *F_{st}* values. This analysis was also performed in Arlequin v. 3.5, based on 10,000 random permutations.

Genetic isolation by distance (IBD) was assessed using the Mantel test, which evaluated the correlation between the linearized genetic distance matrix (calculated as $F_{st}/(1 - F_{st})$ according to Slatkin 1993) and the geographic distance matrix. This analysis was performed using the GenAlEx v. 6.5 program (Peakall and Smouse 2012), with pairwise population differentiation (ϕ_{PT}) and statistical significance determined through 9,999 random permutations.

Demographic history

Demographic history analyses were conducted using the whole dataset. Neutrality tests *D* (Tajima 1989) and *F_s* (Fu 1997) were conducted using the Arlequin v. 3.5 program (Excoffier and Lischer 2010), while the mismatch distribution was estimated with DnaSP v.5 (Librado and Rozas 2009). The Bayesian Skyline Plot (BSP) method (Pybus et al. 2000) was implemented using BEAST v1.10.4 (Drummond and Rambaut 2007), with an average substitution rate of 0.020 substitutions per site per million years, estimated for mitochondrial genes (Larsen et al. 2013). The BSP analysis estimates changes in the effective population size over time (in generations) and evaluates historical patterns of population size from genealogies (Turchetto-Zolet et al. 2013), providing a graphical representation of the demographic history of *A. planirostris*. The best algorithm for BSP analysis was determined using jModelTest2 (Darriba et al. 2012), with GTR + I + G identified as the most suitable model based on Akaike information criterion (AIC), with no other model having a ΔAIC value below 2. The analysis involved two runs of 100 million MCMC generations with a 10% burn-in for each gene. Convergence and burn-in adequacy were assessed by examining log files in Tracer v1.6 (Rambaut et al. 2014), with convergence considered adequate when the Effective Sample Size was greater than 200. The BSP and confidence intervals were generated using Tracer v1.6.

Results

Genetic diversity and population structure of *Artibeus planirostris*

Based on FastBAPS, the optimal cluster configurations were identified as *K* = 10 for the COI gene and *K* = 7 for the Cyt b gene. The 10 populations for COI gene were structured as follows: pop1: Colombia (Córdoba); pop2: Brazil (Amazonas), Guyana and Suriname; pop3: Guyana and Suriname; pop4: Brazil (Mato Grosso do Sul and São Paulo) and Guyana; pop5: Brazil (Mato Grosso do Sul, São Paulo, Piauí, Maranhão and Pará), Guyana and Suriname; pop6: Ecuador, Guyana and Suriname; pop7: Colombia, Guyana and Suriname; pop8: Brazil (Mato Grosso do Sul, Maranhão), Ecuador, Guyana and Suriname; pop9: Brazil (Piauí, Maranhão and Pará), Guyana and Suriname; and pop10: Brazil (Mato Grosso do Sul, São Paulo, Piauí and Maranhão), Guyana and Suriname (Fig. 1).

The seven populations for Cyt b gene were structured as follows: pop1: Venezuela, Grenada, Trinidad and Tobago; pop2: Brazil (Pernambuco, Paraíba, Maranhão and Piauí) and Venezuela; pop3: Brazil (Amazonas, Minas Gerais, Bahia, Pernambuco, Paraíba, Maranhão, Pará and Piauí) and Venezuela; pop4: Brazil (Amazonas) and Venezuela; pop5: Brazil (Amazonas, Pernambuco and Maranhão), Venezuela and Ecuador; pop6: Brazil (Amazonas, Pernambuco and Maranhão), Venezuela and Ecuador; and pop7: Brazil (Amazonas, Bahia, Pernambuco, Piauí, Pará and Paraíba) and Venezuela (Fig. 2).

A total of 131 haplotypes were identified for the COI gene, and 97 for the Cyt b gene (see Supplementary Materials, TableS3 and TableS4). For the COI gene, haplotypic and nucleotide diversity was $h = 0.977$ and $\pi = 0.01444$, whereas for the Cyt b gene, the values were $h = 0.960$ and $\pi = 0.01148$, respectively (Table 1). The genetic diversity and haplotype distribution for the COI gene among the different populations showed that pop8 had the highest haplotypic and nucleotide diversity values ($h = 0.939$; $\pi = 0.00797$; Table 1). In contrast, pop2 exhibited the lowest haplotypic and nucleotide diversity values ($h = 0.596$; $\pi = 0.00187$). The number of haplotypes across populations varied from three in pop7 to 28 in pop5 (Table 1).

For the Cyt b gene, the population with the highest haplotypic and nucleotide diversity was pop6, with a haplotypic and nucleotide diversity values of $h = 0.990$ and $\pi = 0.00984$. In contrast, the population with the lowest haplotypic and nucleotide diversity was pop1, with haplotypic and nucleotide diversity values of $h = 0.686$ and $\pi = 0.00200$. The number of haplotypes across populations varied, ranging from four in pop4 to 40 in pop3 (Table 1).

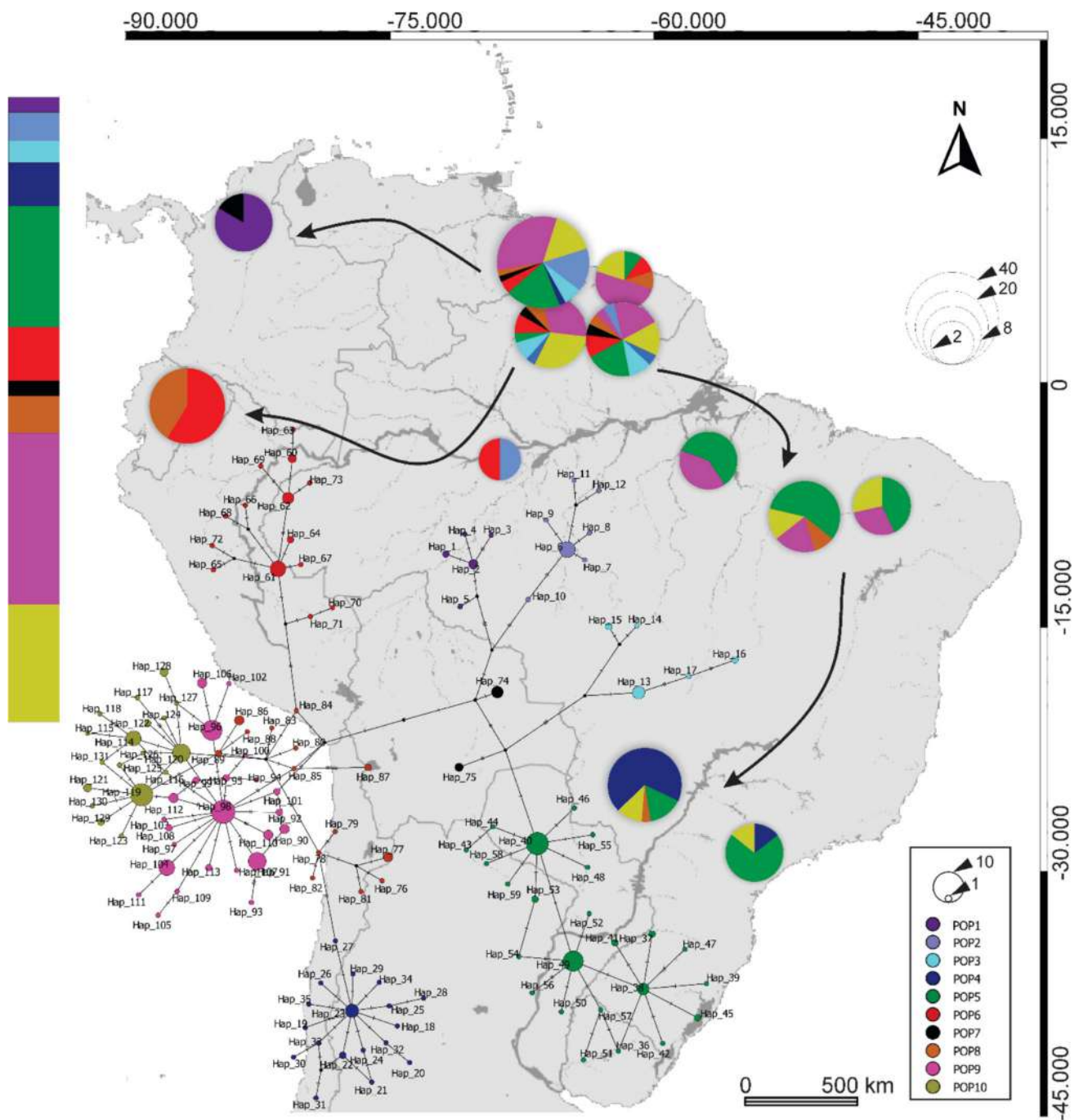


Fig. 1 Network and geographic distribution of *Artibeus planirostris* haplotypes in the Neotropical region, based on the mitochondrial COI gene. The size of the circles is proportional to the frequency of the haplotypes in each location. The colors of the nodes and the bar on

the left correspond to the population (Pop) clusters established by FastBAPS. Arrows indicate the potential dispersal of *Artibeus planirostris* in the Neotropical region

The AMOVA results revealed a high genetic population structure, with the majority of the genetic variation observed among populations (K-clusters from FastBAPS) for both the COI and Cyt b genes (COI with 76.42%, $F_{st}=0.764$, $P<0.001$; Cyt b with 69.51%, $F_{st}=0.695$, $P<0.001$; Table 2). The pairwise F_{st} values calculated

for the populations of *A. planirostris* revealed high values of genetic differences for both molecular markers. For the COI gene, the lowest genetic differentiation was observed between populations 10 and 9 ($F_{st}=0.399$), while the highest was between populations 10 and 2 ($F_{st}=0.876$) (Table 3). For the Cyt b gene, the lowest F_{st} value was found

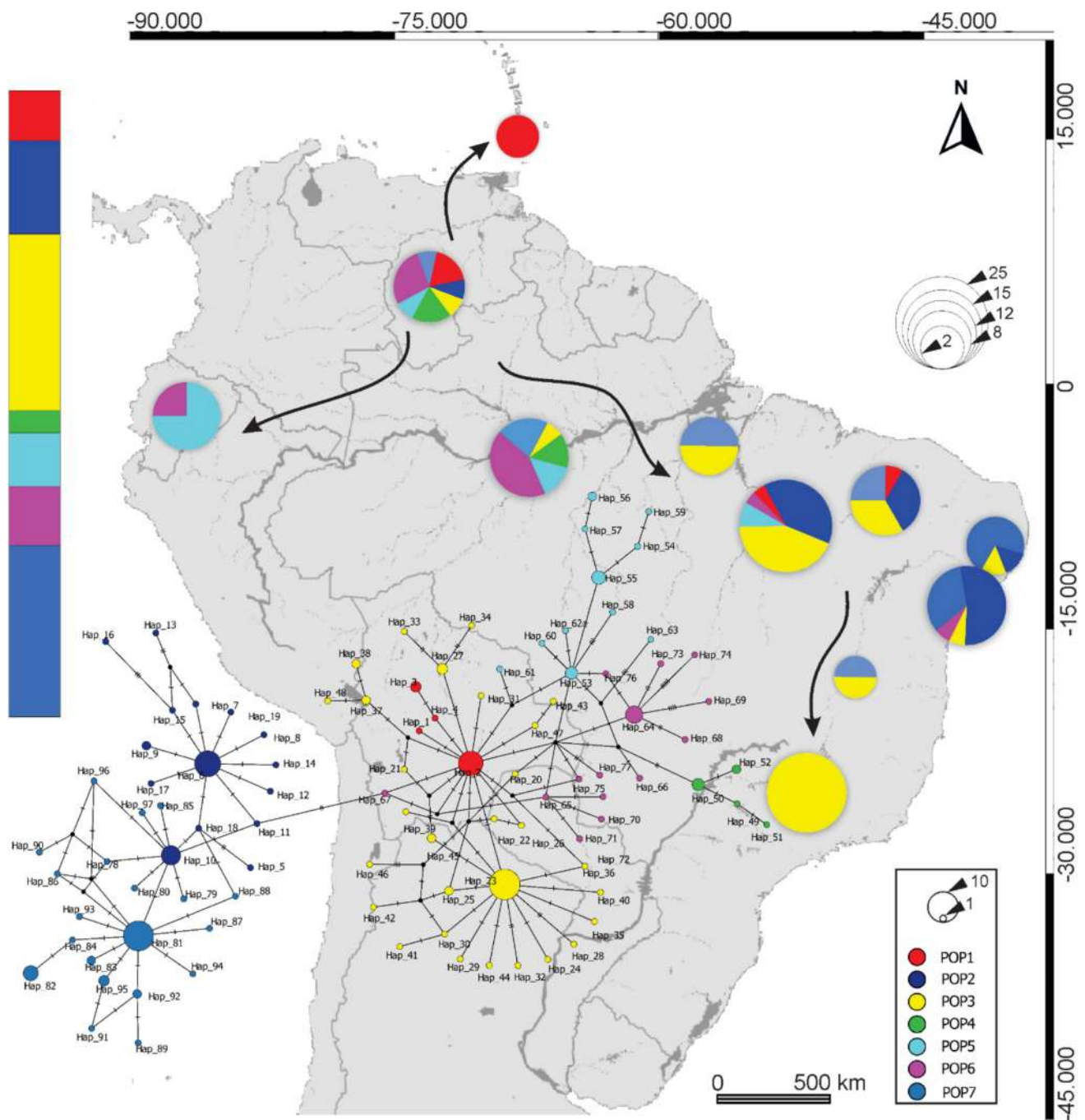


Fig. 2 Network and geographic distribution of *Artibeus planirostris* haplotypes in the Neotropical region, based on the mitochondrial Cyt b gene. The size of the circles is proportional to the frequency of the haplotypes in each location. The colors of the nodes and the bar on

the left correspond to the population (Pop) clusters established by FastBAPS. Arrows indicate the potential dispersal of *Artibeus planirostris* in the Neotropical region

between populations 3 and 1 ($F_{st}=0.308$), and the highest value for between populations 4 and 2 ($F_{st}=0.829$) (Table 4).

For the COI gene, our analysis of the spatial distribution and genealogical relationships of haplotypes in *A. planirostris* revealed clusters with nearly all populations (pop2, pop3, pop4, pop5, pop6, pop7, pop8, pop9, and pop10) in

northern South America, specifically in Guyana (northern and southern regions) and Suriname (northern and southern regions). This indicates significant genetic diversity, highlighting the crucial role of these areas in the species' genetic diversity in the Neotropical region. In contrast, Colombia and Ecuador exhibited cluster with only two populations

Table 1 Genetic diversity of populations (Pop) of *Artibeus planirostris* from various locations across the Neotropics, based on the COI and Cyt b genes. Population clusters were identified using the program FastBAPS

Gene	Population	N	S	NH	Genetic diversity	
					<i>h</i>	π
COI	Pop1	9	6	5	0.806	0.00237
	Pop2	17	8	7	0.596	0.00187
	Pop3	13	7	5	0.705	0.00376
	Pop4	26	15	18	0.911	0.00306
	Pop5	72	26	28	0.863	0.00319
	Pop6	32	23	19	0.938	0.00483
	Pop7	9	7	3	0.750	0.00552
	Pop8	22	21	14	0.939	0.00797
	Pop9	102	24	24	0.886	0.00366
	Pop10	70	21	22	0.861	0.00314
Total	10	372	94	131	0.977	0.01444
Cyt b	Pop1	18	13	7	0.686	0.00200
	Pop2	33	23	21	0.943	0.00457
	Pop3	63	60	40	0.901	0.00493
	Pop4	8	12	4	0.750	0.00428
	Pop5	19	39	17	0.988	0.00857
	Pop6	21	45	19	0.990	0.00984
	Pop7	39	26	19	0.842	0.00437
Total	07	223	70	97	0.960	0.01148

N=sampling size, S=polymorphic sites, NH=estimated number of haplotypes, *h*=haplotypic diversity, and π =nucleotide diversity

Table 2 AMOVA results for populations of *Artibeus planirostris* from various locations across the Neotropics, based on the COI and Cyt b genes. Population clusters were identified using the program FastBAPS

Gene	Source of variation	Variance components	Variation (%)	F_{ST}	P*
COI	Among populations	3.178	76.42	0.764	P<0.001
	Within populations	0.980	23.58		
Cyt b	Among populations	4.222	69.51	0.695	P<0.001
	Within populations	1.851	30.49		

**p*-values, calculated randomly with 10.000 permutations

each: in Colombia (pop1 and pop7) and in Ecuador (pop6 and pop8) (Fig. 1). In the mid-northern region of Brazil (Maranhão, Piauí, and Pará), we identified cluster with pop5, pop8, pop9, and pop10, while in the southern (São Paulo) and central-western (Mato Grosso do Sul) Brazil, the clusters were with pop4, pop5, pop8, and pop10. The Amazon region, however, only presented clusters with pop2 and pop6 (Fig. 1). Thus, while clusters with several populations of *A. planirostris* were distributed across the Neotropical region, Colombia, Ecuador, and the Amazon—each exhibited clusters

with only two populations. Notably, Colombia had an exclusive cluster with pop1.

Similarly, our analysis of the spatial distribution and genealogical relationships of haplotypes of *A. planirostris* for the Cyt b gene revealed clusters with all populations in Venezuela and from populations pop3, pop4, pop5, and pop6 in the Amazon (Fig. 2). This suggests that these regions play a significant role in the species' genetic diversity. The difference in clusters observed between the Amazon region for the two markers may be attributed to the larger sample size, which provided a more representative dataset for the Cyt b gene. The islands of Grenada and Trinidad and Tobago (located in the Caribbean, north of Venezuela) each had a single cluster (pop1), which was found only in Venezuela and Brazil (Maranhão and Piauí). In Ecuador, only clusters with populations 5 and 6 were identified (Fig. 2).

In Brazil, the states of Pará and Bahia exhibited the same population clusters (pop3 and pop7) as Piauí and Paraíba, with the exception of pop1, which was found only in Piauí and Maranhão. Maranhão and Pernambuco also shared the same clusters (pop2, pop3 and pop6), except for pop1 and pop5, being the last one exclusive to Maranhão and Amazonas. Minas Gerais (in southeastern Brazil) had only the pop3 cluster (Fig. 2).

Isolation by distance analysis revealed a significant positive correlation for the COI gene ($r=0.361$, $P<0.001$) and for the Cyt b gene ($r=0.126$, $P<0.001$). Low pairwise *F_{st}*

Table 3 Pairwise *Fst* values showing genetic differentiation among *Artibeus planirostris* populations from different locations in the Neotropics based on the COI gene. Population (Pop) clusters were identified using the program FastBAPS

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10
Pop1	–									
Pop2	0.862	–								
Pop3	0.770	0.859	–							
Pop4	0.814	0.865	0.804	–						
Pop5	0.832	0.837	0.734	0.828	–					
Pop6	0.753	0.820	0.753	0.706	0.798	–				
Pop7	0.735	0.782	0.669	0.742	0.619	0.660	–			
Pop8	0.626	0.725	0.648	0.468	0.744	0.537	0.502	–		
Pop9	0.810	0.845	0.820	0.759	0.820	0.769	0.748	0.602	–	
Pop10	0.817	0.876	0.824	0.748	0.851	0.769	0.800	0.536	0.399	–

Table 4 Pairwise *Fst* values showing genetic differentiation among *Artibeus planirostris* populations from different locations in the Neotropics based on the Cyt b gene. Population (Pop) clusters were identified using the program FastBAPS

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7
Pop1	–						
Pop2	0.822	–					
Pop3	0.308	0.775	–				
Pop4	0.760	0.829	0.650	–			
Pop5	0.626	0.756	0.630	0.628	–		
Pop6	0.425	0.690	0.454	0.491	0.394	–	
Pop7	0.796	0.614	0.767	0.796	0.736	0.655	–

values were observed up to approximately 2,500 km for both genes (Fig. 3). The only high pairwise *Fst* values were found in populations from Colombia for the COI gene, and in Grenada and Minas Gerais for the Cyt b gene.

Historical demographic changes

The overall Tajima's *D* and Fu's *F_s* neutrality tests for both genes showed negative values. However, the *p*-value for the COI gene was not significant ($P > 0.10$), while the *p*-value for the Cyt b gene was significant ($P < 0.05$) (Table 5). When analyzed separately, populations for the COI gene (pop1 and pop8) exhibited negative values, but with non-significant *p*-values ($P > 0.10$). Conversely, populations pop3 and pop7 showed positive values with non-significant *p*-values ($P > 0.10$) (Table 5). For the Cyt b gene, populations pop2 and pop5 displayed negative values with non-significant *p*-values, while populations pop1 and pop4 showed positive values, also with non-significant *p*-values ($P > 0.10$). In both cases, these results suggest that the populations remained stable, without evidence of rapid population growth (Table 5).

However, the COI cluster (pop2, pop4, pop5, pop6, pop9, and pop10) exhibited negative values with significant *p*-values ($P < 0.05$). Similarly, Cyt b cluster (pop3, pop6, and pop7) also showed significant *p*-values ($P < 0.05$), indicating recent population expansion events (Table 5).

The global mismatch distribution analysis revealed a bimodal curve for the COI gene, suggesting that populations remained stable before undergoing expansion over time (Fig. 4a). In contrast, the analysis for the Cyt b gene showed a unimodal curve, indicating recent population expansion (Fig. 4b). Bayesian Skyline Plot (BSP) analysis for both markers indicated that populations of *A. planirostris* experienced recent expansion during the late Pleistocene, approximately 50 to 60 thousand years ago (Fig. 5a and 5b). However, the BSP analysis for the COI gene also revealed periods of population instability before the recent population increase (Fig. 5a).

Discussion

Population structure of *Artibeus planirostris* in the Neotropics

Population genetic studies of bats have revealed diverse phylogeographic patterns, with some populations showing low genetic structure (Wilkinson and Fleming 1996; Miller-Butterworth et al. 2014; Gorman et al. 2022; Grimshaw et al. 2024), while others exhibit strong population structure (Miller-Butterworth et al. 2003; Martins et al. 2007; Lopes et al. 2009; Ferreira et al. 2014). Using the most extensive sampling for *A. planirostris*, we have not detected a strongly structured geographic population pattern, consistent with the

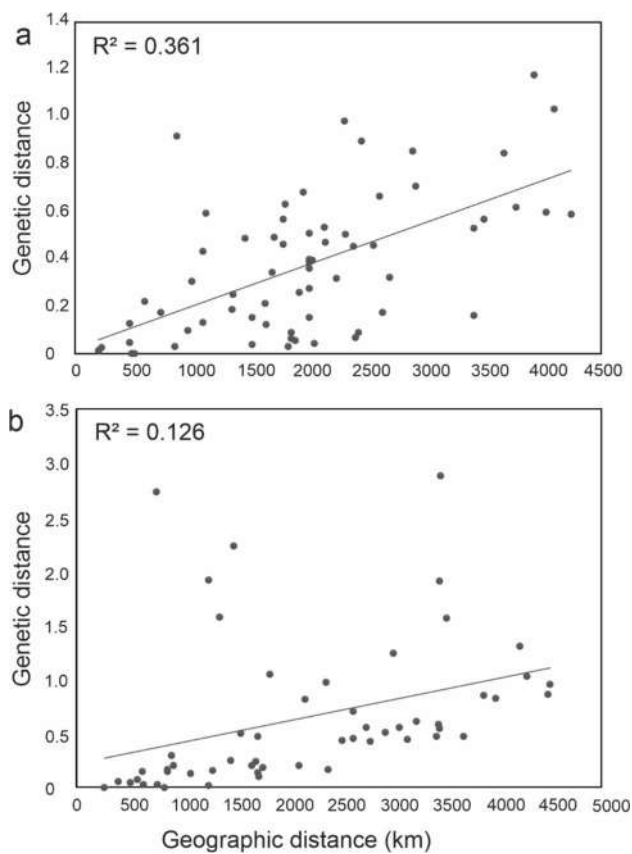


Fig. 3 Isolation by distance (IBD) analysis using the Mantel test, showing the positive correlation between genetic Slatkin's linearized distances and geographic distance for *Artibeus planirostris* in the Neotropics. In **a** results based on the COI gene. In **b** results based on the Cyt b gene

findings of Larsen et al. (2007) for this species within the *A. jamaicensis* complex with a substantially smaller dataset. However, in peripheral populations, we have observed limited maternal gene flow due to isolation by distance or potential geographic barriers, which have shaped the population genetic structure of *A. planirostris*. Furthermore, during the late Pleistocene, interglacial periods may have facilitated an increase in the effective population size of *A. planirostris*.

The Guiana Shield seems to have acted as a crucial center of diversification, playing a key role in the dispersal of this species based on COI data. This suggests that local adaptations and isolation by distance may influence the dispersal and colonization patterns of *A. planirostris*. This adaptive dynamic highlights the complex interplay between ecological and evolutionary factors, emphasizing the Guiana Shield's significance as a key source of genetic diversity and a central hub for the species' radiation across distinct Neotropical ecosystems. A study conducted by Lim and Engstrom (2001) examined bat diversity in the Guyana region and its surroundings, focusing on species richness and the critical role of tropical habitats in bat conservation. They

Table 5 Neutrality tests for populations of *Artibeus planirostris* from various locations in the Neotropical region, based on the COI and Cyt b genes. Population (Pop) clusters were identified using the program FastBAPS

Gene	Populations	Neutrality tests	
		Tajima's <i>D</i>	Fu's <i>F_s</i>
COI	Pop1	−1.398	−1.504
	Pop2	−1.976*	−3.782*
	Pop3	0.216	0.368
	Pop4	−1.911*	−19.535*
	Pop5	−1.878*	−22.088*
	Pop6	−1.557*	−6.553*
	Pop7	1.520	4.475
	Pop8	−0.690	−4.932
	Pop9	−1.761*	−17.157*
	Pop10	−1.676*	−14.953*
Total	10	−1.111	−8.566
Cyt b	Pop1	0.023	2.476
	Pop2	−1.777	−6.368
	Pop3	−2.260*	−17.957*
	Pop4	0.171	2.088
	Pop5	−1.332	−0.471
	Pop6	−1.408*	−2.112*
	Pop7	−1.920*	−9.844*
Total	07	−1.214*	−4.598*

Significance levels for neutrality tests: * $P < 0.05$ (significant) according to DnaSP

documented 14 new bat species for Guyana, also they identified the Iwokrama Forest in central Guyana as a biodiversity hotspot, with 86 bat species recorded, representing the highest bat diversity previously observed in any protected area globally.

Although bats have a high dispersal capability, several factors, such as mountain ranges and large continental or marine water bodies, can limit this activity in different species (Davalos 2004; Speer et al. 2017; Andriollo et al. 2018). The cluster present in individuals from the Lesser Antilles (pop1) also was found in Venezuela, Maranhão, and Piauí (Fig. 2), suggesting a possible intraspecific lineage geographically restricted to these regions, but currently dispersed toward northeastern Brazil. This genetic differentiation also could be linked to the presence of subspecies (*Artibeus planirostris trinitatus* and *Artibeus p. grenadensis*) (Lim et al. 2004; Larsen et al. 2007), whose distribution includes the islands of Trinidad and Tobago, Grenada, and Venezuela (Lim et al. 2004; Larsen et al. 2007). Climatic fluctuations during the Pleistocene likely facilitated contact between some islands, influencing population dynamics and diversification (Larsen et al. 2011; Velazco and Patterson 2013; Pavan and Marroig 2017). Rising sea levels may have

Fig. 4 Mismatch distribution for *Artibeus planirostris* populations based on the COI gene (a) and the Cyt b gene (b). The solid line represents the observed pairwise distribution, while the dotted line indicates the expected distribution under the sudden expansion model. The X-axis represents the number of differences between the haplotypes, while the Y-axis the frequency of each number of differences

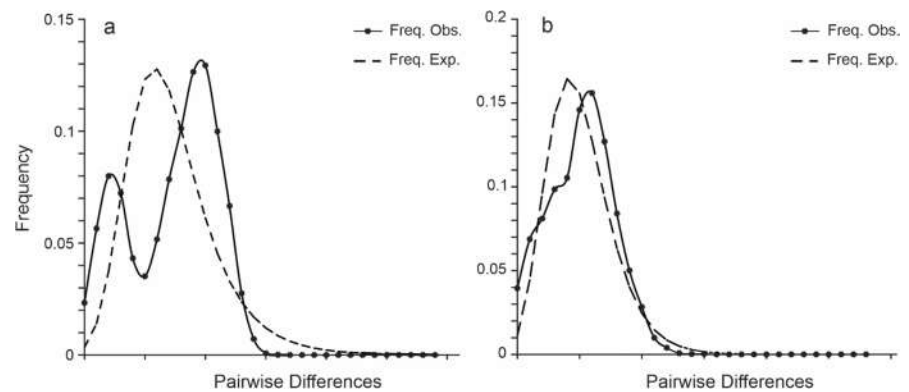
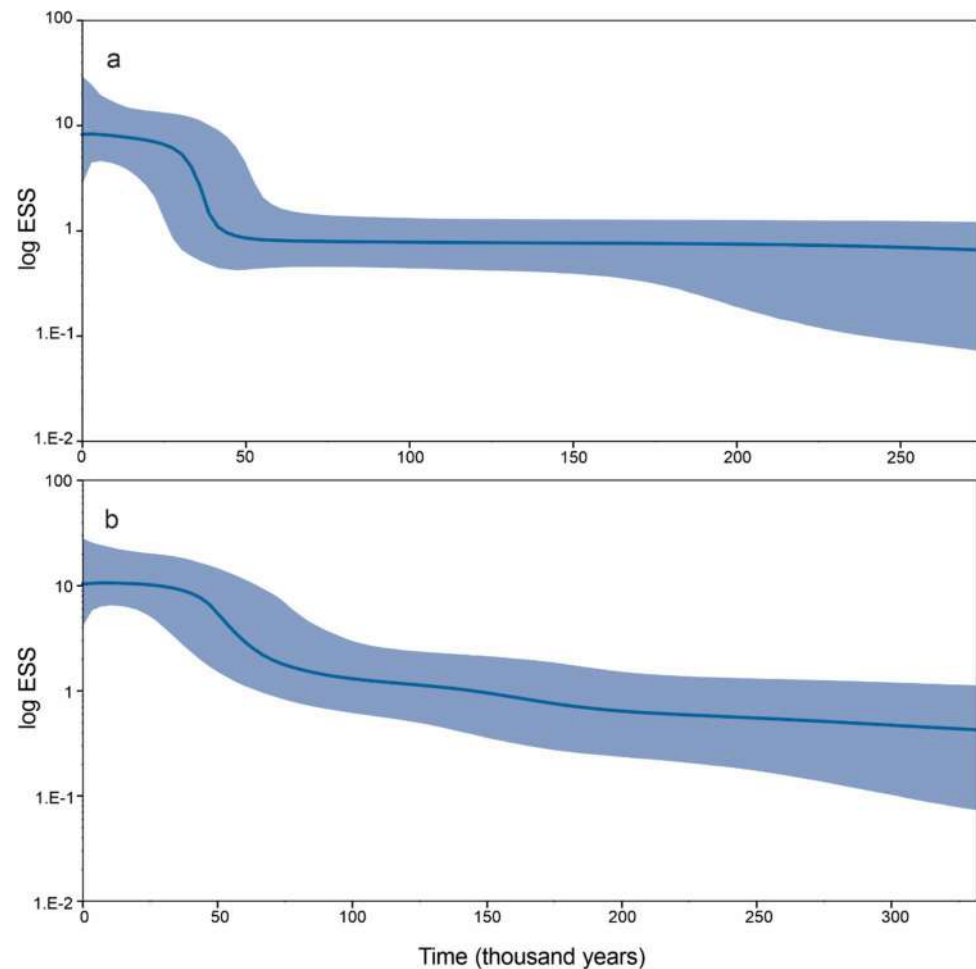


Fig. 5 Bayesian Skyline Plot estimating the demographic history of *Artibeus planirostris* over time. In a based on the COI gene, in b based on the Cyt b gene. The x-axis represents time in thousand years, and the y-axis shows the log of estimated effective population size (ESS). The middle line represents the median, while the shaded area indicates the 95% confidence intervals



subsequently blocked gene flow between the mainland and adjacent islands, promoting allopatric speciation, as seen in the genus *Micronycteris* in the Lesser Antilles (Larsen et al. 2011).

The Cerrado and Caatinga biomes are characterized by more open vegetation and drier climates compared to tropical forests. Along with the Chaco, they form the “dry diagonal”, which extends from the southwest to the northeast of South America and separates the Amazon from the Atlantic

Forest (Prado and Gibbs 1993; Ledo et al. 2020). Pleistocene climatic fluctuations in this region may have facilitated local ecological adaptations and played a significant role in the dispersal of *A. planirostris* from the mid-northern region of Brazil to the northeast, southeast, and central-west regions. In mid-northern Brazil, where the Amazon, Cerrado, Caatinga, and Atlantic Forest biomes predominate, clusters were shared across these biomes, suggesting a potential historical connection between the Amazon and the other biomes

(Figs. 1 and 2). Although tropical forests in these regions are not currently connected, past connections likely existed, allowing the dispersal of various animal species through the dry diagonal, reinforcing the idea of historical links between the Atlantic Forest and the Amazon (Ledo et al. 2020). These findings corroborate Costa (2003), who observed that small non-flying mammals (rodents and marsupials) collected in Cerrado areas exhibited phylogenetic affinities with populations originating from the Amazon or the Atlantic Forest. Our data suggest a possible phylogeographic pattern of population expansion for *A. planirostris* in these regions (Figs. 1 and 2), underscoring the significance of these biomes for the genetic diversity and evolution of the species.

The Amazon, Cerrado, Caatinga, and Atlantic Forest biomes possess several characteristics that may facilitate the dispersal of *A. planirostris*. One key factor is the abundance of food resources, as these biomes offer a wide variety of fruit-bearing plants throughout the year and provide diverse habitats, ranging from dense forests to more open areas, which offer secure refuges and suitable environments for feeding and reproduction (Willig et al. 1993; Zortéa and Alho 2008; Marques et al. 2012; Cordero-Schmidt et al. 2016; Vargas-Mena et al. 2020; Owen et al. 2022).

Although *A. planirostris* is a generalist species with a high capability for environmental adaptation, figs (*Ficus* spp.) constitute its primary diet (Oliveira and Lemes 2010; Martins et al. 2014). These fruits, found in abundance across aforementioned biomes, serve as a crucial food source, aiding in the bat's dispersal. Additionally, the presence of ecological corridors, which act as transitional zones between biomes, can further facilitate bat movement, enabling them to traverse different regions in search of food (Puth and Wilson 2001; Hilty et al. 2012). This movement enhances gene flow and genetic diversity among populations. Greater genetic diversity offers populations an improved capability to adapt to environmental changes and selective pressures (Carvalho 1993; Barrett and Schluter 2008), which is vital for the long-term survival and evolution of *A. planirostris*. Furthermore, it is important to consider that species of *Artibeus* coexist in sympatry, and competition for food resources may influence their spatial distribution, as observed in *A. planirostris* (Marchán-Rivadeneira et al. 2012; Macedo et al. 2023).

Also, environmental heterogeneity can significantly impact population structure in bats (Grant and Grant 2007; Moussy et al. 2012; Ripperger et al. 2013). In a recent study, Silva et al. (2021) investigated how forest dynamics influence patterns of genetic diversity and functional connectivity in flying mammals of eastern Amazonia. They evaluated the effects of landscape heterogeneity on the population structure of four frugivorous species of phyllostomid bats: *Artibeus gnomus* (Handley, 1987); *Artibeus obscurus* (Schinz, 1821); *Carollia perspicillata* (Linnaeus, 1758); and

Rhinophylla pumilio (Peters, 1865). The study revealed that landscapes with greater habitat availability supported populations with higher genetic diversity, indicating a substantial effect of landscape features on the population structuring of bats. These findings emphasize the importance of conserving the Cerrado, Amazon, Caatinga, and Atlantic Forest biomes to maintain genetic diversity in fruit bats like *A. planirostris*.

Bats can disperse over long distances, but gene flow may decrease as these distances increase (Bohonak 2002; Gonçalves et al. 2023), which plays a significant role in the genetic structure as observed in *A. planirostris* (Fig. 3). Notably, the northern region of Colombia (Córdoba) exhibited high levels of genetic differentiation, clusters with pop1 and pop2. Pop1 was exclusive to this region, while pop2 shared a cluster only with Guyana and Suriname. This suggests possible geographic isolation without rapid population growth, potentially influenced by the Llanos region, which affects the distribution patterns of cis-Andean species of *Artibeus* (Gregory-Wodzicki 2000; Larsen et al. 2013). Additionally, the presence of two morphologically distinct subspecies, *A. p. trinitatus* and *A. p. fallax* (Lim 1997; Larsen et al. 2007), concentrated in the regions north (Llanos, *A. p. trinitatus*) and south (Orinoco River, *A. p. fallax*), could explain this pattern (Lim 1997; Larsen et al. 2007; 2013). Another commonly suggested factor influencing the population structure of *Artibeus* is the elevation of the Andes (Gregory-Wodzicki 2000; Larsen et al. 2013; Ferreira et al. 2014). However, this factor may account for divergences between lineages that led to cis-Andean and trans-Andean species (Larsen et al. 2013), rather than the population structure observed in this study.

Isolation by distance seems to be the most plausible explanation for the genetic differentiation of *A. planirostris* in Ecuador (Figs. 1 and 2). Studies by Clare et al. (2011) and Benítez et al. (2021) using mitochondrial genes also found high levels of intraspecific differentiation in phyllostomid species from Ecuador and Colombia. These findings suggest the presence of potential cryptic species and support the hypothesis of allopatric lineages. This could explain the significant genetic differentiation observed in individuals of *A. planirostris* from these regions.

Individuals of *A. planirostris* from Mato Grosso do Sul and São Paulo, in Brazil, were more closely related to those from the northeast region, suggesting a dispersal pattern of *A. planirostris* throughout the eastern part of South America, with shared population clusters (for COI). A similar connection was observed for individuals from Minas Gerais; however, those from this region exhibited a distinct cluster (pop3), indicating another geographically restricted intraspecific lineage specific to this area (for Cyt b).

Furthermore, individuals of *A. planirostris* from southern peripheral regions of South America were more genetically

differentiated from those in the northern region (Figs. 1 and 2). This observation supports the hypothesis that isolation by distance and Pleistocene colonization may influence this distribution pattern, contrary to our hypothesis. In summary, our results suggest that the genetic structure of *A. planirostris* may have been influenced by forest expansions during the Pleistocene in the Cerrado, Caatinga, and Atlantic Forest biomes. These expansions likely isolated individuals in peripheral regions from those in the northern regions (Figs. 1 and 2), leading to increased genetic differentiation between these groups (Ledo et al. 2020).

Demographic history of *Artibeus planirostris*

Current biodiversity patterns are the result of a long and intricate evolutionary history, shaped by ecological processes and influenced by external environmental factors such as climate change, mountain uplift, and sea-level fluctuations (Rull 2011). Both pre-Pleistocene geological events (Hoorn et al. 2010) and Pleistocene climatic shifts (Vanzolini and Williams 1981) are considered key drivers of Neotropical diversification. For example, vertebrate populations in the Americas experienced significant changes, driven by climatic oscillations during the Pleistocene and the formation of geographic barriers. These events promoted the expansion of populations of reptiles (Castoe et al. 2009), birds (Barber and Klicka 2010), and mammals (Hurtado and D'Elia 2022), including bats (Larsen et al. 2013).

Our results provide valuable insights into the demographic history of *A. planirostris* based on the Cyt b gene, indicating that populations of *A. planirostris* have undergone a late Pleistocene expansion. This hypothesis is further supported by the mismatch distribution (Fig. 4), Bayesian Skyline Plot (BSP) analysis (Fig. 5a), and neutrality tests (Table 5). For the COI gene, the distribution of incompatibilities and neutrality tests indicate that populations have remained relatively stable over time. However, BSP analysis shows that this stability was followed by a late Pleistocene population expansion (Fig. 5b), which is supported by both genetic markers (Fig. 5a, b). These phylogeographic expansion patterns, especially in northeastern populations, align with studies on Pleistocene vegetation changes in northeastern Brazil (De Oliveira et al. 1999; Pessenda et al. 2010), which suggest that favorable climatic conditions supported population growth in several species. Alternatively, these populations of *A. planirostris* may have remained stable during less favorable periods, which could explain the observed stability followed by a late Pleistocene expansion, as seen in similar studies (Ledo et al. 2020).

Climatic variations during the Pleistocene facilitated the movement of biotas between the Amazon and the Atlantic Forest, via the Cerrado and Caatinga, and vice versa, as described by Batalha-Filho et al. (2013) and Ledo and Colli

(2017). This interpretation is supported by paleoecological evidence from these regions, as documented by De Oliveira et al. (1999) and Pessenda et al. (2004; 2010). Our study further supports this hypothesis, with *A. planirostris* inhabiting the dry diagonal, as demonstrated by phylogeographic inferences (Figs. 1 and 2). These findings are consistent with those of Ferreira et al. (2014), who conducted a phylogeographic study on *A. obscurus* in the Amazon. They identified two main groups of *A. obscurus* and their correlation with the Amazon and Atlantic Forest biomes, separated by the dry diagonal of the Cerrado.

During the late Pleistocene, approximately 20,000 years ago, fossil records from caves in the Caatinga of northeastern Brazil revealed the presence of several taxa of phyllostomid bats, including species of the genus *Artibeus* (Czaplewski and Cartelle 1998). Fracasso and Salles (2005) examined the taxonomic diversity of fossil and extant bats from the Serra da Mesa region in Goiás, central-western Brazil, and also identified fossil records of *Artibeus* from the Quaternary period. Evidence suggests that the climate during this time was warmer and more humid (Czaplewski and Cartelle 1998; Fracasso and Salles 2005). These warmer and more humid conditions likely facilitated the dispersal of bats from the northern regions to the central and southern regions, as supported by our study.

Geological events, historical climate oscillations, and environmental heterogeneity have significantly influenced the extent of Neotropical rainforests. Climate change promoted cycles of tropical forest expansion during the Pleistocene (Burnham and Graham 1999; Fiaschi and Pirani 2009; Honório-Coronado et al. 2014), leading to increased growth of various plant species, including fig trees. These trees are crucial for the nutritional needs of *A. planirostris* (Oliveira and Lemes 2010; Martins et al. 2014). Although other vertebrates, such as howler monkeys and birds, visit fig trees (Shanahan et al. 2001), phyllostomid fruit bats are the primary seed dispersers for these plants (Kalko et al. 1996). This dispersal behavior may have contributed to high fig densities during the Pleistocene (Costa et al. 2017). These events likely influenced the dispersal of *A. planirostris* in forested areas associated with the Amazon and the Atlantic Forest, as evidenced by our study.

This study is the first to examine the population dynamics of *A. planirostris* using two mitochondrial markers and extensive sampling. Here, we have not detected a strongly structured geographic population pattern. Nevertheless, in peripheral populations, we have observed limited maternal gene flow due to isolation by distance or possibly geographic barriers. Also, Pleistocene climatic fluctuations likely facilitated ecological adaptations and species dispersal across various Brazilian biomes. Demographic history suggests a recent population expansion during the late Pleistocene, around 50 to 60 thousand years ago.

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Data availability All data are available in the Supplementary Materials.

Declarations

Conflict of interest None declared.

Ethical approval Bat handling and manipulation were conducted in accordance with the guidelines established by the Animal Care and Use Committee of the American Society of Mammalogists (Sikes 2016; Sikes et al. 2011), under collection permits (#42670-1, #54384-1, #68047-1, #74512-1, #42,111-1, #42,111-2, #42,111-3, #42,111-5, #42,111-6, #68,801-1, #68,801-2, and #68,801-3). The study received approval from the Ethics Committee for Animal Experimentation (CEEA) of Universidade Estadual do Maranhão, under approval number #06/2023-CEEA/CMV/UEMA, and Committee for the Ethical Use of Animals (003/2014, 001/2022, 008/2022) of the Instituto de Desenvolvimento Sustentável Mamirauá.

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4 DISCUSSÃO INTEGRADORA

O status taxonômico das espécies dentro do gênero *Artibeus* não é amplamente aceito, dada a falta de informações e as variações morfológicas significativas entre os diferentes táxons do grupo (SIMMONS, 2005; LIM *et al.*, 2008; REDONDO *et al.*, 2008; BAKER *et al.*, 2016; CIRRANELLO *et al.*, 2016). Portanto, considerando o desafio de distinguir espécies dentro desse grupo com base em uma única abordagem, nossos resultados ressaltam a relevância da integração de diferentes tipos de dados. A combinação de informações moleculares, morfológicas e populacionais permite avaliar de forma mais robusta a congruência entre a estrutura genética e a variação morfológica intraespecífica.

Diante disto, no primeiro capítulo referente ao artigo intitulado: “Morphological and molecular data combined reveal inter- and intraspecific cranial shape variations in bats of *Artibeus* Leach, 1821 (Chiroptera: Phyllostomidae)”, utilizou-se dois conjuntos de dados, um baseado nos métodos de delimitação molecular, utilizando sequências do gene COI, e o outro baseado em dados de forma craniana, usando técnicas de MG a fim investigar variações intra e interespecíficas em espécies de grandes e pequenos *Artibeus*.

Estudos anteriores que se basearam em técnicas de morfometria linear (LIM, 1997; MARCHÁN-RIVADENIERA, 2006, 2008; LIM *et al.*, 2008; LARSEN *et al.*, 2010) demonstraram que, embora as variações morfológicas possam ser detectadas, essas abordagens muitas vezes não são capazes de delimitar as espécies. A utilização de dados de forma tem sido essencial para entender a variação na morfologia craniana de *Artibeus*, especialmente quando se considera a adaptação dessas características a diferentes biomas e ambientes (HEDRICK, 2021). Além disso, uma abordagem que considera o efeito da alometria residual (KLINGENBERG, 2016), proporciona uma visão mais precisa das diferenças na forma.

Os métodos de delimitação molecular ABGD e ASAP através do gene COI, mostraram-se eficazes na identificação das espécies, tais métodos recuperaram os quatros táxons de *Artibeus* (*A. lituratus*, *A. planirostris*, *A. obscurus* e *A. cinereus*) definidos na morfologia. As análises usando a técnica de MG mostraram variações intra e interespecíficas significativas nas formas dorsal e ventral no crânio das espécies de pequenos e grandes *Artibeus*. A integração desses resultados neste estudo revelou que não existe um sinal filogenético significativo atuando sobre a variação do crânio, o que indica não haver efeito das relações filogenéticas atuando sobre essa variação. Tal cenário indica que possivelmente fatores ecológicos históricos externos podem estar influenciando diretamente na morfologia craniana dessas espécies e ainda atuarem como forças de seleção significativas no espaço geográfico gerando variações intraespecíficas em *A. planirostris*.

O segundo capítulo que trata da estrutura genética populacional de *Artibeus planirostris* intitulado: “Population genetics and demographic history of flat-faced fruit-eating bat *Artibeus planirostris* (Spix, 1823) (Chiroptera: Phyllostomidae)”, possibilitou uma melhor compreensão sobre a dinâmica populacional desta espécie na região Neotropical. Nesse estudo, analisamos fragmentos dos genes mitocondriais Citocromo b (Cyt b) e Citocromo c Oxidase 1 (COI) para investigar a diversidade genética, a estrutura populacional e a história demográfica de *A. planirostris* no Neotrópico. Nossos achados revelam um padrão populacional geográfico fracamente estruturado. No entanto, em populações periféricas, observamos um fluxo gênico materno limitado devido ao isolamento por distância ou potenciais barreiras geográficas. Isso sugere que adaptações locais, moldadas por pressões ambientais específicas e flutuações climáticas do Pleistoceno, podem ter influenciado as capacidades de dispersão e colonização de *A. planirostris* nessas regiões. A história demográfica de *A. planirostris* indica uma expansão populacional recente durante o Pleistoceno tardio, há aproximadamente 50 a 60 mil anos.

No cenário comparativo intraespecífico em *A. planirostris* verificou-se que há diferenças significativas na forma e tamanho do crânio entre os biomas Cerrado, Amazônia e Caatinga. O que sugere que a disponibilidade de alimentos interfere na dieta das espécies nestes ambientes, sendo assim, considerado um fator importante nas diferenças do tamanho e forma do crânio em *A. planirostris*. Da perspectiva da genética populacional também se observou variação de clusters entre esses diferentes biomas, ainda que muita dessa diversidade genética seja compartilhada. Essa abordagem integrada contribui para uma compreensão mais abrangente dos processos microevolutivos que atuam em *A. planirostris*.

5 CONCLUSÃO

Neste estudo, observamos variação significativa de tamanho e forma craniana entre espécies de morcegos frugívoros do gênero *Artibeus*. Grandes espécies dentro do gênero exibem considerável variação na forma do crânio, o que pode ser útil para discriminação entre elas. No entanto, ao comparar as espécies menores com *Artibeus* de maior porte, encontramos um forte efeito da alometria residual na forma craniana. Além disso, a variação no tamanho e formato do crânio não está significativamente relacionada à filogenia, sugerindo que fatores ecológicos, como as necessidades alimentares nos biomas Caatinga, Cerrado e Amazônia, possam estar influenciando diretamente a morfologia craniana dessas espécies. Em *A. planirostris*, a variação no tamanho e formato do crânio parece estar associada às condições ambientais desses biomas, possivelmente relacionadas à disponibilidade de recursos alimentares.

Quanto a dinâmica populacional de *A. planirostris*, não foi detectado um padrão geograficamente estruturado entre as populações. No entanto, observou-se fluxo gênico materno limitado em populações periféricas, provavelmente devido ao isolamento por distância ou barreiras geográficas. Essas barreiras geográficas, somadas às flutuações climáticas do Pleistoceno, podem ter facilitado tanto as adaptações ecológicas quanto a dispersão de *A. planirostris* entre diversos biomas brasileiros. A história demográfica sugere uma expansão populacional recente durante o Pleistoceno tardio, por volta de 50 a 60 mil anos atrás, evento que pode ter influenciado a morfologia craniana observada hoje, refletindo tanto a adaptação ambiental quanto a diversidade genética nas populações.

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ANEXOS-Declaração de Ética e Experimentação Animal (CEEA)



**UNIVERSIDADE
ESTADUAL DO
MARANHÃO**

**Centro de Ciências
Agrárias Curso de
Medicina Veterinária**

**Comissão de Ética e Experimentação Animal
(CEEA) Credenciamento Provisório -
CONCEA/MCT Processo 01200.002200/2015-**

06 (449) - Emissão 19/06/2015

DECLARAÇÃO – PROTOCOLO Nº 06/2023-CEEA/CMV/UEMA

Declaramos aos devidos fins, para atividade de pesquisa, que o projeto intitulado “Taxonomia Integrativa e Sistemática do Gênero *Artibeus* Leach, 1821 (Chiroptera, Phyllostomidae)” tem aprovação da Comissão de Ética e Experimentação Animal -CEEA do Curso de Medicina Veterinária da Universidade Estadual do Maranhão-UEMA, conforme protocolo nº 06/2023 e execução pela pós-graduanda do Curso de Doutorado da Rede de Biodiversidade e Biotecnologia da Amazônia Legal - BIONORTE, a bióloga, Samira Brito Mendes, sob a orientação da Profa. Dra. Maria Claudene Barros, docente do Departamento de Química e Biologia do Centro de Estudos Superiores de Caxias-CESC/UEMA. O projeto submetido atende as normas de Bem- Estar Animal da Resolução do CFMV nº 1000/2012, Lei 11.794/2008 e do CONCEA/MCTI, e será executado no período de 24 meses (10/10/23 Término: 10/10/25), em um número amostral de 10 espécimes para o gênero *Artibeus* e tem licença SISBio/ICMBio nº 68047-4 de 05/01/2022.

São Luís, 06 de outubro de
2023.

Profa. Dra. Alana Lislea de Sousa
Presidente do
CEEA/CMV/UEMA Matrícula
6503

Cidade Universitária Paulo VI - s/n - Tirirical - C.P. 09 - CEP. 65055-310 - São Luís/MA - Fone: (98) 3245-5461 / Fax: (98) 3245-5882

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