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CENTRO DE BIOCIÊNCIAS
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FERNANDA ITO DOS SANTOS

ANÁLISE DA ESTRUTURA GENÉTICA DE MORCEGOS EM *BAT CAVES* DO NORDESTE BRASILEIRO.

RECIFE
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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, da Universidade Federal de Pernambuco, como parte dos requisitos parciais para a obtenção do título em Doutora em Biologia Animal.

Área de concentração: Zoologia

Orientador: Prof. Dr. Enrico Bernard

Co-orientador: Dr. Thomas Lilley

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RESUMO

Conhecidas como *bat caves*, algumas cavernas encontradas na região Neotropical se caracterizam por abrigar populações excepcionais de morcegos (>>10.000 indivíduos). As populações destas cavernas têm grande importância ecológica, participando de uma variedade de processos essenciais para a manutenção do ecossistema cavernícola e também prestando diversos serviços ecossistêmicos no ambiente externo à caverna. Apesar de o conhecimento a respeito destas cavernas ainda ser incipiente, estudos recentes no Brasil apontam que as grandes colônias das bat caves do Nordeste podem chegar a 200.000 indivíduos, são formadas por morcegos do gênero *Pteronotus* e experienciam grandes flutuações no tamanho populacional em curtos períodos de tempo. Tal variação sugere que existam movimentações entre estes abrigos e que o uso das bat caves é muito mais dinâmico do que antes imaginado. Avanços científicos e tecnológicos permitiram novas abordagens para o estudo do deslocamento de morcegos Neotropicais, entre eles a análise molecular da estrutura genética de populações. Uma variedade de fatores, assim como a combinação deles, têm influência na estrutura populacional de uma espécie. Entre eles pode-se citar a distância geográfica entre as populações, diferenças na paisagem, capacidade de dispersão, história evolutiva e até características ecomorfológicas da espécie. Portanto, a análise da conectividade genética entre as subpopulações de duas espécies de morcegos presentes em cavernas geograficamente isoladas ajudaria a preencher importantes lacunas de conhecimento a respeito do uso das bat caves e de grandes movimentações de morcegos no Brasil. Assim, foi aplicada a metodologia ddRADseq para analisar e comparar a estrutura genética populacional de *Pteronotus gymnonotus* e *P. personatus* coletados em nove bat caves do Nordeste brasileiro, distantes até 700 km entre si. Meus resultados mostram padrões de estruturação distintos entre as espécies, o que sugere um uso bastante dinâmico das cavernas. *Pteronotus gymnonotus* se apresenta como uma população altamente conectada, enquanto *P. personatus* apresenta populações estruturadas, porém sem relação com a distância geográfica. Isso indica que diferenças na ecologia e biologia das espécies refletem em seus padrões de estrutura genética populacional. Além disso, os resultados sugerem que as espécies realizam movimentações entre as cavernas de forma dinâmica e relacionada com a reprodução, o que torna as bat caves áreas prioritárias para a conservação tanto de morcegos quanto do patrimônio espeleológico no país, e indicam que o manejo efetivo para a conservação destes abrigos deve considerá-las como uma única unidade.

Palavras-chave: ddRADseq; fluxo gênico; isolamento por distância; fragmentação; conservação; Caatinga.

ABSTRACT

Known as bat caves, some caves found in the Neotropical region roosts exceptional bat populations (>>10,000 individuals). Such populations are of great ecological importance, being involved in a variety of essential processes for the maintenance of the cave ecosystem and also providing several ecosystem services in the caves´ surrounding environment. Although the knowledge on those caves is still incipient, recent studies in Brazil indicate that colonies formed by species of the genus *Pteronotus* in bat caves in the Northeast can reach 200,000 individuals, and experience large fluctuations in population size in short periods of time. Such variations suggest that there are movements between these shelters and that their use is more dynamic than previously imagined. Scientific and technological advances have allowed new approaches to study the movement of Neotropical bats, including the molecular analysis of populations´ genetic structure. A variety of factors, as well as their combination, may influence the population structure of a given species. Among them are the geographic distance between populations, differences in the landscape, the species´ dispersal capacity, its evolutionary history, and even ecomorphological characteristics of the species. Therefore, the analysis of the genetic connectivity between subpopulations of two bat species in geographically isolated caves could fill important knowledge gaps on the use of bat caves and on large distance movements of bats in Brazil. Thus, the ddRADseq methodology was applied to analyze and compare the population genetic structure of *Pteronotus gymnonotus* and *P. personatus* sampled in nine bat caves in the Northeastern Brazil, distant up to 700 km from each other. My results indicated distinct structuring patterns among the species, which suggests a very dynamic use of the caves. *Pteronotus gymnonotus* presented a highly connected population, while *P. personatus* presented structured populations, however not related to geographic distance. My results indicated that differences in the ecology and biology of species may influence their genetic population structure patterns. In addition, the results suggest that the observed movements between caves are dynamic and related to reproduction, pointing bat caves as priority areas for the conservation of both bats and the speleological heritage in the country, and suggesting those caves should be considered as a single unit for an effective conservation management.

Keywords: ddRADseq; gene flow; isolation by distance; fragmentation; conservation; Caatinga.

SUMÁRIO

1 INTRODUÇÃO

Pteronotus é um gênero da família Mormoopidae composto por morcegos insetívoros que se distribuem de forma ampla na região Neotropical (PAVAN; WILSON; MITTERMEIER, 2019). Estudos recentes sobre a filogenia e filogeografia do gênero apontam para uma diversidade muito maior que a reconhecida atualmente, com espécies antes consideradas como amplamente distribuídas sendo, na verdade, complexos de espécies a serem analisados (PAVAN; MARROIG, 2016; 2017). Além disso, o gênero possui uma história evolutiva complexa e diversificação recente (PAVAN; MARROIG, 2017), portanto o entendimento da sua biogeografia, demografia e estrutura populacional ainda precisa ser melhor acessado. Assim como as outras espécies do gênero, *Pteronotus gymnonotus* e *P. personatus* são duas espécies estritamente cavernícolas (DE LA TORRE; MEDELLÍN, 2010; PAVAN; TAVARES, 2020), e no Brasil são encontradas formando grandes colônias em *bat caves*, constituídas por dezenas ou até centenas de milhares de indivíduos (*e.g.* ROCHA et al., 2011; VARGAS-MENA et al., 2018; OTÁLORA-ARDILA et al., 2019). O monitoramento sistematizado de algumas destas cavernas aponta que o tamanho destas colônias pode variar bastante ao longo do ano, com tais flutuações podendo estar relacionadas com períodos reprodutivos das espécies (OTÁLORA-ARDILA et al., 2019; LEAL; BERNARD, 2021).

Estudos populacionais com morcegos cavernícolas no Brasil são bastante raros, especialmente na Caatinga, região historicamente negligenciada pela comunidade científica por anos (SILVA; LEAL; TABARELLI, 2018; ANTONGIOVANNI et al., 2020). Contudo, um número crescente de estudos mostra que, além de abrigar uma elevada biodiversidade e casos de endemismo (SILVA; LEAL; TABARELLI, 2018), a Caatinga também apresenta um elevado potencial espeleológico (CECAV, 2021), que inclui também as *bat caves* (*e.g.* ROCHA et al., 2011; FEIJÓ; ROCHA, 2017; PAVAN; TAVARES, 2020; LEAL; BERNARD, 2021; PIMENTEL et al., 2022). De fato, cerca de 100 das 181 espécies de morcegos do Brasil têm ocorrência na Caatinga (SILVA et al., 2018; DELGADO-JARAMILLO et al., 2020), e 73 das espécies com ocorrência no país já foram registradas em cavernas (OLIVEIRA; OPREA; DIAS, 2018). Desta forma, as *bat caves* da região nordeste oferecem oportunidades de se acessar informações a respeito de morcegos cavernícolas, podendo preencher lacunas de conhecimento apontadas para o país (BERNARD et al., 2012).

A estrutura genética populacional de uma espécie pode ser influenciada por uma grande gama de fatores (*e.g.* WANG; BRADBURD, 2014; BRADBURD; COOP; RALPH, 2018), e a análise desta diversidade genética pode gerar dados importantes não só relacionados à biologia e ecologia das espécies, como também à sua conservação e manutenção da dinâmica da paisagem. Além do mais, a comparação da estrutura genética populacional de espécies que ocorrem em simpatria permite o melhor entendimento da influência do ambiente na distribuição espacial da variação genética das espécies, e também de como as diferenças ecomorfológicas entre as espécies influenciarão este padrão (*e.g.* BURLAND; WILMER, 2001; CAMPBELL et al., 2006).

Nesta tese busquei atingir três principais objetivos: o primeiro consistiu em analisar e descrever a estrutura populacional genética de duas espécies de morcegos cavernícolas encontrados em *bat caves*; o segundo englobou a comparação dos padrões de conectividade populacional observados para as espécies em estudo; e, por fim, o terceiro buscou entender as implicações conservacionistas destes resultados. Esta tese está então estruturada na forma de um Referencial Teórico inicial, seguido por dois capítulos na forma de artigos científicos, e as Considerações Finais. No Referencial Teórico contextualizo a relação entre morcegos e cavernas, discorro sobre o gênero *Pteronotus*, abordo a riqueza de espécies de morcegos na Caatinga e conecto as informações sobre ecologia do movimento, genética de populações e flutuações populacionais observadas em *bat caves* monitoradas ao longo de vários anos na Caatinga, dando também um enfoque conservacionista. No primeiro capítulo eu usei dados moleculares para analisar a estrutura genética de *Pteronotus gymnonotus* em nove cavernas de morcegos ao longo de 700 km. No segundo capítulo, acessei a estrutura populacional de *P. personatus* e comparei-a com os dados de *P. gymnonotus* obtidos no capítulo anterior. Esta foi uma rara oportunidade de analisar duas espécies intimamente relacionadas, que dividem os mesmos abrigos, forrageiam nos mesmos ambientes, e experimentam as mesmas flutuações no tamanho de suas colônias. Por fim, nas Considerações Finais, apresento as principais conclusões de meu estudo.

2 REFERENCIAL TEÓRICO

2.1 MORCEGOS: DIVERSIDADE E IMPORTÂNCIA ECOLÓGICA

Morcegos constituem a segunda ordem mais diversa entre os mamíferos, com cerca de 1450 espécies descritas (SIMMONS; CIRRANELLO, 2022). Presentes em todos os ecossistemas vegetados do mundo, estes animais também seguem o padrão dos outros mamíferos e apresentam maior diversidade e abundância nos trópicos (FENTON et al., 1992; PEREIRA; PALMEIRIM, 2013; WILLIG, 2003). E neste cenário, o Brasil se destaca por abrigar 181 espécies de morcegos, sendo oito delas endêmicas, distribuídas em nove famílias e 68 gêneros (GARBINO et al., 2020). Tamanha riqueza e diversidade estão refletidas em sua morfologia e, também, em seus hábitos alimentares (Figura 1), havendo espécies que se alimentam de frutos (frugívoras), néctar e pólen (nectarívoras), artrópodes (insetívoras), peixes e outros vertebrados (piscívoras e/ou animalívoras), e até de sangue (sanguinívoras; FENTON, 1992).

Figura 1. Exemplos de espécies de morcegos que ocorrem no Brasil. A) *Sphaeronycteris toxophyllum*, espécie frugívora. B) *Diphylla ecaudata*, hematófaga. C) *Diclidurus scutatus*, de hábito insetívoro. D) *Noctilio leporinus*, morcego piscívoro. Fonte: Morcegos do Brasil. Imagens de Guilherme Garbino (a) e Roberto Novaes (b,c,e d).

Por ocuparem tantos nichos tróficos, os morcegos participam de diversos processos ecológicos-chave e proveem importantes serviços ecossistêmicos (KUNZ; MURRAY; FULLER, 2011). Na região

Neotropical, os morcegos fitófagos são responsáveis pela polinização de mais de 570 espécies de plantas e pela dispersão de, ao menos, 550 espécies, incluindo espécies utilizadas comercialmente por humanos (MUSCARELLA; FLEMING, 2007; FLEMING; MUCHHALA, 2008; LOBOVA et al., 2009; KUNZ; MURRAY; FULLER, 2011). Com isso, morcegos auxiliam no sucesso reprodutivo destas plantas, garantindo sua variabilidade genética e conectando populações fragmentadas. Já os morcegos insetívoros, que representam a maioria das espécies neotropicais, são considerados os principais consumidores noturnos de artrópodes, controlando populações de lepidópteros, coleópteros, dípteros, homópteros e hemípteros, incluindo espécies consideradas pragas agrícolas e vetores de zoonoses (*e.g.* KALKA; SMITH; KALKO, 2008; BOHMANN et al., 2011; BOYLES et al., 2011; KUNZ; MURRAY; FULLER, 2011; MASLO et al., 2017). No Brasil, estudos indicam que morcegos insetívoros são importantes no controle de espécies pragas tanto no ambiente urbano quanto em áreas de agricultura, gerando uma economia estimada em mais de 390,6 milhões de dólares por safra (AGUIAR et al., 2021). Desta forma, estes animais desempenham um papel importante na manutenção da biodiversidade e da complexidade do ambiente, através da dispersão/polinização de espécies-chave, do controle populacional de espécies de artrópodes, da redistribuição e ciclagem de nutrientes entre os ambientes, e dos processos de sucessão e regeneração florestal (KUNZ; MURRAY; FULLER, 2011).

2.2 MORCEGOS E CAVERNAS

Cavernas constituem o tipo de abrigo mais utilizado por morcegos, e, por apresentarem maior estabilidade, fornecem proteção contra predadores e condições ambientais adversas, além de serem utilizadas para interações sociais, reprodução e cuidado da prole (FUREY; RACEY, 2016). Nestes ambientes os morcegos são classificados, sob a óptica da bioespeleologia, como organismos trogloxenos, ou seja, são organismos encontrados em cavernas, mas que precisam emergir dela em algum momento para completar seu ciclo de vida (KUNZ 1982; FUREY; RACEY, 2016).

Estudos recentes apontam os morcegos como bioengenheiros (PILÓ et al., 2022), e ao retornarem à caverna após se alimentarem durante a noite, os morcegos exercem um papel chave na manutenção do ecossistema cavernícola, através da deposição de guano (GNASPINI; TRAJANO, 2000; FUREY; RACEY, 2016; PILÓ et al., 2022). Visto que a produção primária nestes ambientes é muito baixa ou ausente, o guano produzido por morcegos é responsável pelo *input* de energia e matéria orgânica, já que é rico em nitrogênio e fosfatos (GNASPINI; TRAJANO, 2000; DEHARVENG; BEDOS, 2012; PILÓ et al., 2022). Cavernas estão entre os maiores *hotspots* de diversidade de invertebrados subterrâneos e algumas destas espécies são totalmente dependentes da deposição de guano (MYERS et al., 2000; GILBERT; DEHARVENG, 2002; DEHARVENG; BEDOS, 2012), de tal forma que comunidades inteiras podem desaparecer caso o *input* de guano seja definitivamente interrompido (GILBERT; DEHARVENG, 2002; FERREIRA, 2019).

Na região Neotropical, algumas cavernas exibem características especiais: apresentam temperatura elevada e constante (entre 28-40°C), alta umidade relativa (> 90%), baixa circulação de ar, com entrada única e relativamente pequena, e abrigam uma alta densidade de morcegos com, em geral, dezenas ou centenas de milhares indivíduos (DE LA CRUZ, 1992; LADLE et al., 2012). Algumas destas cavernas são aquecidas por convecção, ou seja, suas temperaturas elevadas originam-se do meio externo (FUREY; RACEY, 2016). Outras, conhecidas como *hot caves* ou *bat caves*, dependem da alta densidade de morcegos para a manutenção de seu microclima, já que as altas temperaturas são causadas pelo calor irradiado do corpo destes animais e pela decomposição do guano (LADLE et al., 2012). O conceito de *bat cave* precisa ser melhor definido, visto que uma caverna que apresenta uma grande colônia de morcegos não necessariamente constitui uma caverna quente, pois a manutenção de altas temperaturas depende não apenas da presença de morcegos, como também da arquitetura da caverna (FERREIRA, 2019). Portanto, nem toda *bat cave* é uma *hot cave*, mas toda *hot cave* é uma *bat cave*. Uma possível definição para o termo *bat cave* seria "cavernas que abrigam colônias de morcegos que modificam, devido à sua presença, todo o ambiente trófico e climático da caverna, independentemente do número absoluto de morcegos" (FERREIRA, 2019). Porém, a excepcionalidade do tamanho daquela população em relação às demais populações conhecidas também pode ser considerada ao definir uma *bat cave*. De qualquer forma, uma *bat cave* chama a atenção pelo número elevado de morcegos em seu interior.

Registros – tanto atuais, quanto fósseis – apontam para a existência de *bat caves* desde os EUA até o Brasil, contudo estas cavernas ainda estão subestimadas e são pouco conhecidas (LADLE et al., 2012), sendo melhores estudadas no Caribe, Cuba, Porto Rico e México (*e.g.* DE LA CRUZ, 1992; RODRÍGUEZ-DURÁN, 1998; TEJEDOR; TAVARES; RODRÍGUEZ-HERNÁNDEZ, 2005; MEDELLIN; WIEDERHOLT; LOPEZ-HOFFMAN, 2017). No Brasil o conhecimento a respeito das *bat caves* ainda é incipiente, porém estudos mais recentes apontam que elas são mais frequentes no Nordeste do país do que se imaginava, e que algumas destas cavernas contêm populações que ultrapassam 200.000 indivíduos (*e.g.* ROCHA et al., 2011; FEIJÓ; ROCHA, 2017; PAVAN; TAVARES, 2020; LEAL; BERNARD, 2021; PIMENTEL et al., 2022).

É uma *bat cave*, a caverna Bracken, no Texas (Figura 2), que abriga a maior congregação de mamíferos do mundo, composta por mais de quatro milhões de indivíduos de *Tadarida brasiliensis*

(HRISTOV et al., 2010). E outras cavernas excepcionais também são conhecidas nos Estados Unidos e no México (MEDELLIN; WIEDERHOLT; LOPEZ-HOFFMAN, 2017). Na Malásia, na caverna Deer, abrigam-se cerca de dois milhões de *Chaerophon plicata* (CLEMENTS et al., 2006). Em Cuba, nas cavernas Cueva de la Ventana e Cueva de los Majaes, foram observados cerca de 50.000 indivíduos de *Phyllonycteris poeyi* em cada uma delas (TEJEDOR; TAVARES; RODRÍGUEZ-HERNÁNDEZ, 2005). Dalquest e Hall (1949) apontam uma colônia com milhares de *Pteronotus personatus* e *P. davyi* na caverna Tuxtla, no México, e estima-se que uma caverna em Porto Rico, conhecida como Cucaracha, tenha uma colônia com mais de 141.000 morcegos da espécie *Pteronotus quadridens* (RODRIGUEZ-DURÁN; LEWIS, 1987).

Figura 2. Bracken cave, no Texas – EUA. *Bat cave* que abriga a maior colônia de morcegos do mundo, estimada em mais de 4 milhões de indivíduos de *Tadarida brasiliensis*. Imagem capturada durante a emergência dos morcegos da caverna. Fonte: Bat Conservation International.

Nas *bat caves* registradas no Brasil, as grandes colônias são majoritariamente compostas por morcegos da família Mormoopidae, principalmente *Pteronotus gymnonotus* e *Pteronotus personatus* (LADLE et al., 2012; FEIJÓ; ROCHA, 2017; OTÁLORA-ARDILA et al., 2019; PAVAN; TAVARES, 2020; LEAL; BERNARD, 2021; PIMENTEL et al., 2022). Para o Rio Grande do Norte, na Caverna do Urubu, foi observada uma colônia de mais de 10.000 indivíduos de *P. gymnonotus* (VARGAS-MENA et al., 2018). Feijó e Rocha (2017) estimaram, através de estimativas fotográficas, que pelo menos 35.000 indivíduos de *Pteronotus* abrigavam-se na Gruta do Sobradinho, no Ceará. Com base na mesma metodologia, Rocha e colaboradores (2011) estimaram cerca de 50.000 indivíduos na caverna Casa de Pedra, no Sergipe, sendo que, utilizando uma metodologia de detecção de movimento em imagens termais, Pimentel e colaboradores (2022) chegaram a registrar quase 100.000 morcegos na mesma caverna. Em Pernambuco, na Caverna Meu Rei, a população pode chegar próximo de 120.000 indivíduos (OTÁLORA-ARDILA et al., 2019).

2.3 O GÊNERO *PTERONOTUS*

Endêmica da região Neotropical, a família Mormoopidae é o principal táxon associado às *bat caves* na região Neotropical (LADLE et al., 2012; PAVAN; WILSON; MITTERMEIER, 2019), podendo ser observada também compartilhando o ambiente com espécies das famílias Phyllostomidae e Natalidae (LADLE et al., 2012). Com distribuição do sudoeste dos Estados Unidos até as regiões central e nordeste do Brasil, morcegos desta família podem ser observados em ambientes desde florestas úmidas até áridos e semiáridos (PATTON; GARDNER, 2007; PAVAN; WILSON; MITTERMEIER, 2019). A família é composta apenas pelos gêneros *Mormoops* e *Pteronotus* (PAVAN; WILSON; MITTERMEIER, 2019). O gênero *Pteronotus* atualmente inclui 15 espécies (Figura 3), pertencentes a três subgêneros: *Pteronotus*, que inclui as espécies *P. davyi*, *P. fulvus* e *P. gymnonotus*; *Chilonycteris*, incluindo *P. macleayi*, *P. quadridens*, *P. personatus* e *P. psilotis*; e *Phyllodia*, abrangendo as espécies *P. parnellii*, *P. pusillus*, *P. portoricensis*, *P. mexicanus*, *P. mesoamericanus*, *P. fuscus*, *P. rubiginosus* e *P. alitonus* (PAVAN; WILSON; MITTERMEIER, 2019; SIMMONS; CIRRANELLO, 2022).

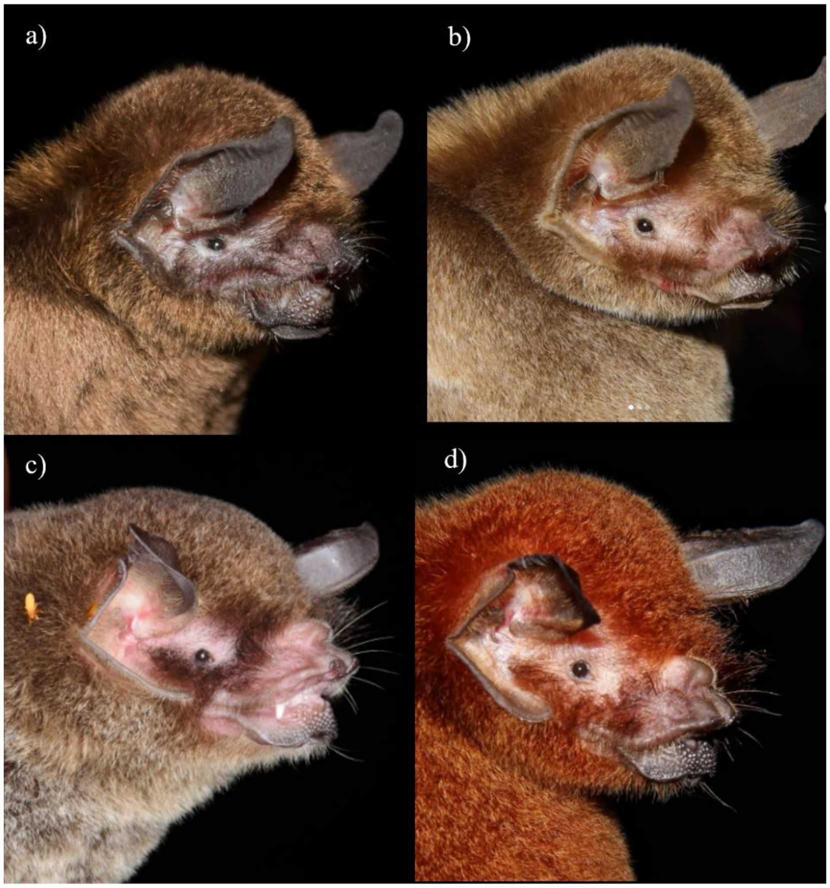

Figura 3. Representantes do gênero *Pteronotus*: a) *P. personatus* do subgênero *Chilonycteris*; b) *P. gymnonotus*, que pertence ao subgênero *Pteronotus*; c) *P. parnellii*; d) *P. rubiginosus*, ambos pertencentes ao subgênero *Phyllodia*. Fonte: Morcegos do Brasil, com imagens de Roberto Novaes, e PPGBio INPA.

Estudos filogeográficos sugerem que a origem do gênero *Pteronotus* se deu há aproximadamente 16 milhões de anos, além de estimarem uma divergência recente entre espécies proximamente relacionadas, ocorrendo há menos de 2.6 milhões de anos, no Pleistoceno (PAVAN; MARROIG, 2017). Atualmente *Pteronotus* se distribui por quase toda a região Neotropical (PAVAN; WILSON; MITTERMEIER, 2019) e, de acordo com análises biogeográficas, o ancestral do gênero também habitava uma área geográfica bastante ampla, se estendendo da América Central e Jamaica até a região amazônica (PAVAN; MARROIG, 2017). As espécies do gênero têm áreas de ocorrência bastante variadas (PAVAN; MARROIG, 2017), com algumas espécies, como *P. gymnonotus*, que possuem uma distribuição em escala continental (PAVAN; MARROIG, 2017; PAVAN; TAVARES, 2020), e outras, como *P. portoricensis*, com distribuição bastante restrita e específica de ilhas do Caribe (HERD, 1983; PAVAN; MARROIG, 2016; 2017). Em geral, muitas espécies com distribuição continental de clados diferentes dentro do gênero apresentam sobreposição de área (PAVAN; MARROIG, 2017).

Todas as espécies de *Pteronotus* são gregárias e se abrigam exclusivamente em cavernas, onde formam colônias mistas com milhares de indivíduos (PAVAN; WILSON; MITTERMEIER, 2019). A alta densidade de indivíduos nas colônias auxilia na manutenção da temperatura corporal elevada, o que é importante já que as espécies são bastante susceptíveis à quedas de temperatura (DE LA TORRE; MEDELLÍN, 2010; PAVAN; TAVARES, 2020). Mesmo todas as espécies sendo insetívoras (PAVAN; WILSON; MITTERMEIER, 2019), morfologicamente as espécies variam muito em tamanho, com *P. quadridens* apresentando um comprimento de antebraço entre 35-40 mm, e *P. paernellii* com tamanho de antebraço entre 48-66 mm (SIMMONS; CIRRANELLO, 2022).

No Brasil são registradas as espécies *P. alitonus*, *P. rubiginosos*, *P. personatus* e *P. gymnonotus* (GARBINO et al., 2020; GARBINO; BRANDÃO; TAVARES, 2022), sendo as duas últimas as principais responsáveis pelas grandes colônias das *bat caves* do Nordeste do país (Figura 4; FEIJÓ; ROCHA, 2017; OTÁLORA-ARDILA et al., 2019; PAVAN; TAVARES, 2020; LEAL; BERNARD, 2021; PIMENTEL et al., 2022). *Pteronotus personatus* é uma espécie pequena, com comprimento de antebraço médio de 43.2 mm, e que apresenta cerdas na face que se assemelham a um bigode (DE LA TORRE; MEDELLÍN, 2010). Sua distribuição se estende desde o México, abrange toda a América Central, até o norte da Bolívia e a região central do Brasil (Figura 5; DE LA TORRE; MEDELLÍN, 2010; ZÁRATE-MARTÍNEZ et al., 2018; SIMMONS; CIRRANELLO, 2022). Pode ser encontrado em diferentes habitats, desde florestas úmidas a florestas secas (SMITH, 1972; DE LA TORRE; MEDELLÍN, 2010), mas estudos realizados na América Central indicam que a espécie forrageia principalmente em áreas de vegetação densa (DE LA TORRE; MEDELLÍN, 2010). Já *P. gymnonotus* é uma das maiores espécies do gênero, com antebraço medindo entre 50 e 56 mm (PAVAN; TAVARES, 2020). Esta espécie tem a aparência de ter as costas nuas, devido ao fato de ter a membrana das asas unida no dorso (SMITH, 1972; PAVAN; TAVARES, 2020). *P. gymnonotus* tem a distribuição mais ampla entre os mormoopideos, desde o sudeste do México ao Brasil, onde é registrado em áreas abertas de ambientes quentes e áridos (PAVAN; TAVARES, 2020).

Figura 4. Espécimes de *Pteronotus gymnonotus* (à esquerda) e *Pteronotus personatus* (à direita) representando as principais diferenças morfológicas entre as duas espécies: a junção da membrana da asa e o tamanho. Fonte: a autora.

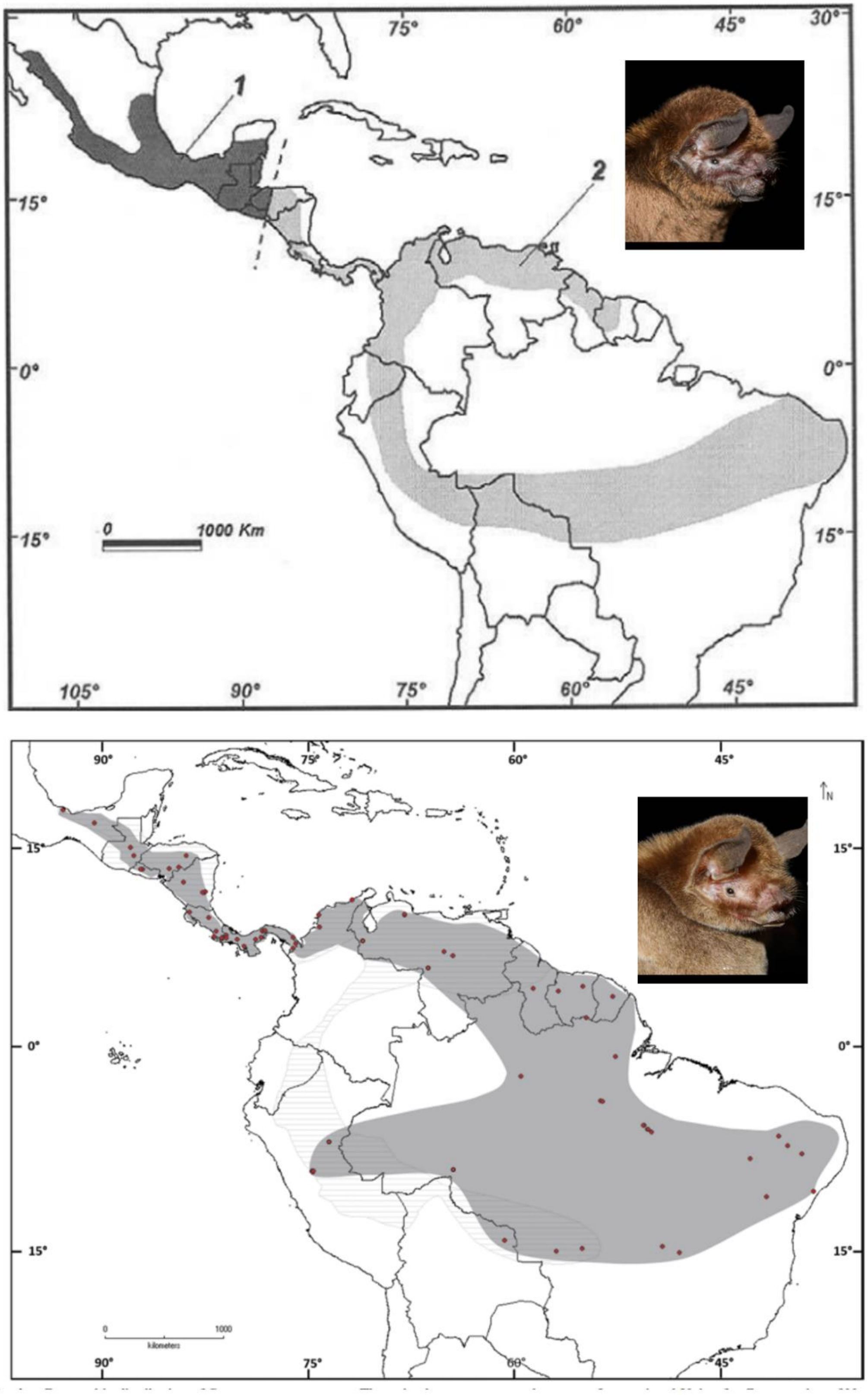

Figura 5. Distribuição das duas espécies do gênero *Pteronotus* responsáveis pela formação das grandes colônias nas *bat caves* do Nordeste brasileiro: *Pteronotus personatus* (acima) e *Pteronotus gymnonotus* (abaixo). Mapas retirados de de la Torre e Medellín (2010) e de Pavan e Tavares (2020). Fotos de Roberto Novaes.

2.4 A CAATINGA

Grande parte das *bat caves* conhecidas no Brasil estão localizadas em área de Caatinga no nordeste do país (JANSEN; CAVALCANTI; LAMBLÉM, 2012; CECAV, 2021). Esta área é conhecida por abranger o maior bloco de floresta tropical sazonalmente seca (FTSS) da América do Sul (PENNINGTON; RATTER, 2006; SILVA; LEAL; TABARELLI, 2018). Porém, evidências recentes apontam que a Caatinga representa, na verdade, um sistema ecológico bastante complexo, composto por um mosaico de paisagens em que seus componentes interagem entre si (Figura 6; DE ARAÚJO et al., 2022). Assim, além das florestas secas, são observadas áreas de florestas tropicais úmidas, savana e campos rupestres (QUEIROZ et al., 2017; DE ARAÚJO et al., 2022). Mais ainda, as FTSS podem ser divididas em cinco diferentes subtipos de vegetação: caatinga cristalina, caatinga sedimentar, florestas decidual e semi-decidual, afloramentos rochosos, e comunidades de plantas aquáticas (QUEIROZ et al., 2017; DE ARAÚJO et al., 2022). Cada subtipo é caracterizado por seus atributos florísticos e estruturais, podendo ser subdivididos em pelo menos onze categorias fisionômicas e diversas outras áreas geo-ambientais únicas (SILVA et al., 2000; PRADO, 2003; QUEIROZ et al., 2017; DE ARAÚJO et al., 2022).

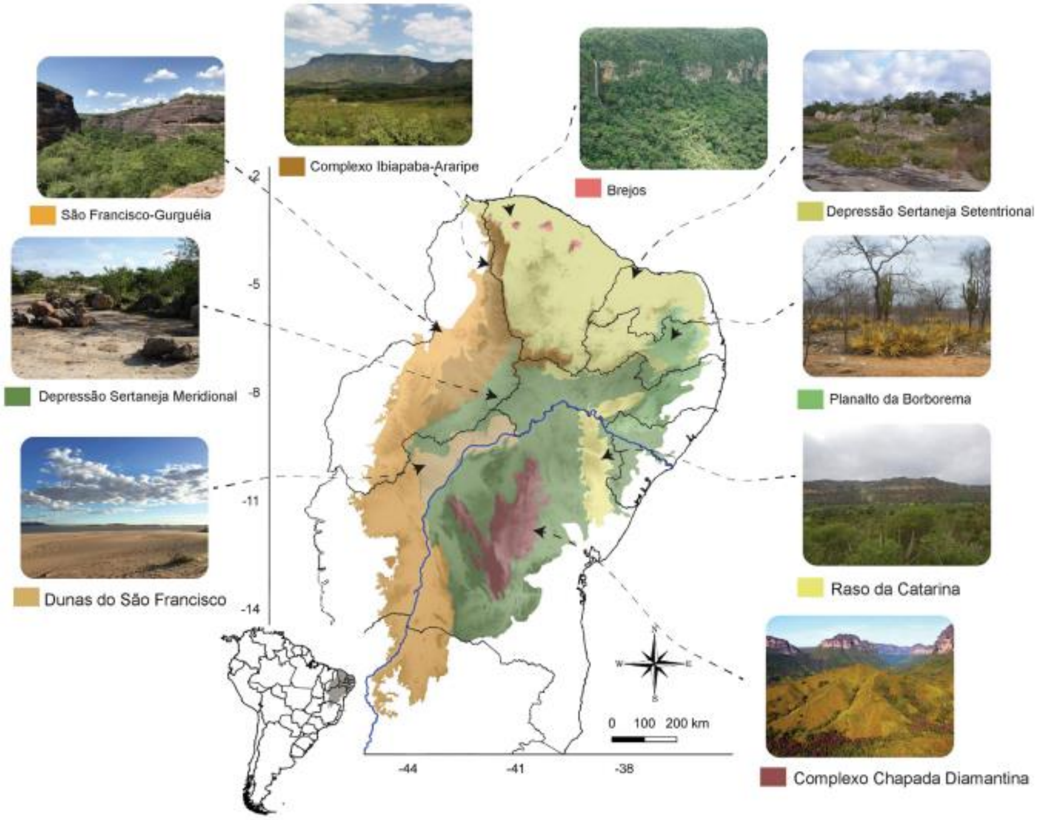

Figura 6. Mapa da Caatinga com imagens de algumas das fitofisionomias encontradas neste sistema ecológico. A linha azul representa o Rio São Francisco. Retirado de Uchôa et al. 2022.

Tamanha heterogeneidade é refletida na biodiversidade da região, que abriga 3150 espécies de plantas, 386 peixes, 98 anfíbios, 79 répteis, 548 aves e 183 espécies de mamíferos (SILVA; LEAL; TABARELLI, 2018), destas cerca de 100 espécies são de morcegos (SILVA et al., 2018; DELGADO-JARAMILLO et al., 2020). Além de muitas destas espécies serem endêmicas da região (SILVA; LEAL; TABARELLI, 2018), os organismos registrados na Caatinga apresentam adaptações para a sazonalidade característica da região, principalmente relacionada aos padrões de chuva (SILVA; BARBOSA, 2017; SILVA; LEAL; TABARELLI, 2018). Morcegos que ocorrem em regiões de FTSS, por exemplo, apresentam adaptações para as restrições sazonais na disponibilidade de água e recursos alimentícios, como dieta mais flexível, migrações curtas e períodos reprodutivos sincronizados com períodos de maior disponibilidade de alimentos (STONER; TIMM, 2011).

Apesar de sua inegável importância ecológica (SILVA; LEAL; TABARELLI, 2018; DE ARAÚJO et al., 2022), a Caatinga é a terceira região mais degradada do Brasil, com mais da metade de sua área original alterada por impactos antrópicos (ANTONGIOVANNI et al., 2020), e também uma das regiões menos protegidas do Neotrópico, com pouco mais de 1% de sua área total sob proteção integral (LEAL et al., 2005; SILVA; BARBOSA, 2017). Estudos apontam que a vegetação original restante se apresenta de maneira dividida em cerca de 47 mil fragmentos de tamanho variáveis (ANTONGIOVANNI; VENTICINQUE; FONSECA, 2018; ANTONGIOVANNI et al., 2020) e que, além de os distúrbios antrópicos estarem concentrados em alguns pontos da paisagem, praticamente toda a área da Caatinga está exposta ao uso humano (Figura 7; ANTONGIOVANNI; VENTICINQUE; FONSECA, 2018; ANTONGIOVANNI et al., 2020).

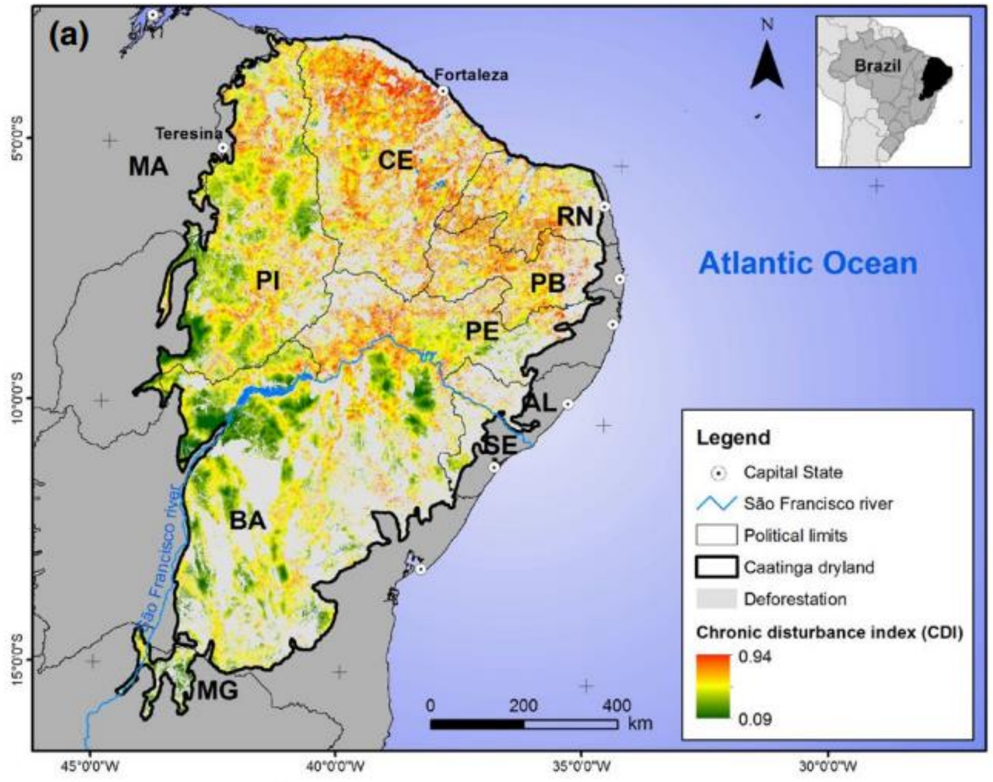

Figura 7. Mapa da Caatinga indicando os níveis do índice de distúrbios antrópicos crônicos na região. Retirado de Antongiovanni et al. 2020.

Assim, frente ao processo de perda e degradação de habitats experimentado pela Caatinga, entender quais e como se desenvolvem os processos ecológicos que mantém a dinâmica desse sistema é fundamental para a manutenção e preservação de sua biodiversidade (DE ARAÚJO et al., 2022). Evidências sugerem, por exemplo, que processos como seleção de habitat e habilidade de dispersão são determinantes para a adaptação e sobrevivência de espécies em uma região (MYERS; HARMS, 2009; CHASE; MYERS, 2011; MORI; ISBELL; SEIDL, 2018). Existem enormes lacunas de informações básicas sobre a história natural e ecologia para a maior parte das espécies de morcegos no Brasil (BERNARD et al., 2012). Estas lacunas são ainda mais acentuadas em uma região como a

Caatinga, que durante anos foi negligenciada pela comunidade científica e apresenta diversas lacunas de conhecimento (SILVA; LEAL; TABARELLI, 2018; ANTONGIOVANNI; VENTICINQUE; FONSECA, 2018; DE ARAÚJO et al., 2022). Portanto, o desenvolvimento de estudos com morcegos do gênero *Pteronotus* ganha importância, já que as grandes colônias observadas nas *bat caves* do nordeste brasileiro são formadas por duas espécies de morcegos insetívoros e estritamente cavernícolas (DE LA TORRE; MEDELLIN, 2010; PAVAN; TAVARES, 2020).

2.5 ECOLOGIA DO MOVIMENTO E FLUTUAÇÕES POPULACIONAIS NAS *BAT CAVES*

O entendimento dos processos que mantém a dinâmica de um sistema ecológico no espaço e tempo é fundamental (DE ARAÚJO et al., 2022). Estudos ecológicos e biogeográficos apontam que processos como a interação entre biotas, seleção de habitat, distúrbios antrópicos e dispersão são essenciais para a manutenção do sistema e para a preservação da biodiversidade local (*e.g.* LEIBOLD et al., 2004; MYERS; HARMS, 2009; CHASE; MYERS, 2011; CORNELL; HARRISON, 2014; DE ARAÚJO et al., 2022). O monitoramento sistematizado de algumas *bat caves* na Caatinga aponta que enquanto algumas delas parecem manter populações residentes ao longo de todo o ano, em outras as populações podem experimentar flutuações de mais de 600% no número de indivíduos em curtos períodos de tempo (Figura 8; OTALORA-ARDILA et al., 2019). Descartada a possibilidade de mortandade e recolonização – pois, em caso de mortandade, as variações observadas exigiriam um tempo de reposição de indivíduos maior do que os intervalos já constatados – tais flutuações sugerem a existência de movimentos de deslocamento maciço de indivíduos entre diferentes abrigos.

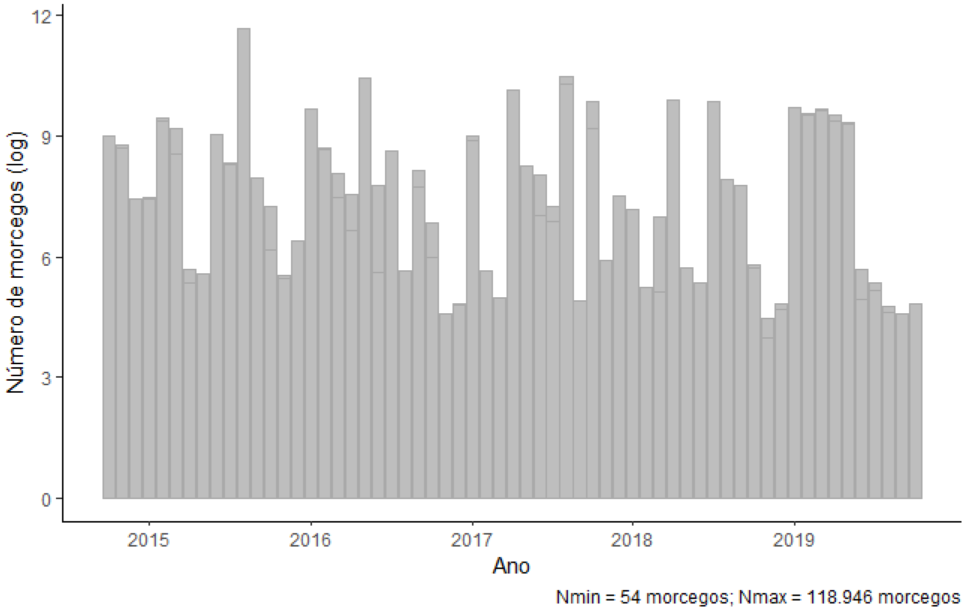

Figura 8. Variação na abundância de morcegos entre 2015 e 2019 na *bat cave* Meu Rei, em Pernambuco, Brasil. Fonte: Enrico Bernard.

Com base na sazonalidade observada na Caatinga, Otálora-Ardilla e colaboradores (2019) analisaram a relação das flutuações na abundância de morcegos de uma *bat cave* com os padrões de precipitação da região. Neste estudo, os autores testaram a hipótese de que os padrões de chuva, e consequentemente a disponibilidade de alimento, influenciariam no deslocamento dos indivíduos e, também, coincidiriam com o período reprodutivo das espécies (OTÁLORA-ARDILA et al., 2019). Tal hipótese se baseou em evidências de que espécies que ocorrem em FTSS realizam pequenas migrações que coincidem com a disponibilidade de recursos (STONER; TIMM, 2011). E que, além disso, se reproduzem sazonalmente, com períodos de lactação e nascimento ocorrendo no início da época chuvosa (STONER, 2001; STONER; TIMM, 2011). Contudo, a presença da colônia de *Pteronotus* não apresentou relação explícita com a precipitação na região e seu período reprodutivo também não apresentou sazonalidade marcada (OTÁLORA-ARDILA et al., 2019), indicando que outras variáveis estão associadas às flutuações populacionais observadas nas *bat caves* monitoradas.

Apesar de não ter sido observada sazonalidade marcante relacionada com os períodos chuvosos na Caatinga (OTÁLORA-ARDILA et al., 2019), as movimentações dos indivíduos podem ser orientadas pela disponibilidade de recursos (FLEMING et al., 2003; FLEMING, 2018). Por exemplo, no México, Burke e colaboradores (2019) apontam que as espécies nectarívoras *Choeronycteris mexicana* e *Leptonycteris yerbabuenae* se movimentam entre os chamados “corredores de nectar” da região, orientadas pela disponibilidade de recursos alimentares e abrigos temporários. Na África subsaariana, um estudo recente mostrou que, similar ao observado na bat cave da Caatinga (OTÁLORA-ARDILA et al., 2019), apesar de não haver uma correlação significante com a precipitação, as movimentações das grandes colônias do morcego frugívoro *Eidolon helvum* coincidem com os picos de crescimento da vegetação, um fenômeno conhecido como "surfar na onda verde" (do inglês, *surfing the green wave*; HURME et al., 2022). Em ambos os casos, as distâncias percorridas pelas populações são variáveis, podendo chegar a até 2.000 km (BURKE et al., 2019; HURME et al., 2022).

Na análise realizada por Otálora-Ardila e colaboradores (2019) também não foi descartada a possibilidade de as variações extremas no número de morcegos dentro da caverna estarem relacionadas, de alguma forma, com a reprodução da espécie. No caso das espécies *L. yerbabuenae* e *E. helvum*, por exemplo, os autores sugerem que apesar de as movimentações estarem relacionadas com fatores ambientais, a reprodução destes morcegos desempenha um papel chave na decisão de quando e para onde as grandes colônias irão se deslocar (BURKE et al., 2019; HURME et al., 2022). Movimentos relacionados com a reprodução são observados em várias espécies, onde indivíduos de diferentes subpopulações se deslocam por quilômetros até abrigos temporários com o único propósito de acasalar (*e.g.* RIVERS; BUTLIN; ALTRINGHAM, 2005; ROSSITER et al., 2002; GALINDO et al., 2006; HUA et al., 2013; LLAVEN-MACÍAS et al., 2021). Em alguns casos esta distância percorrida pelos indivíduos pode ser relativamente pequena, como no caso do morcego vespertilionídeo *Tylonycteris pachypus*, que apresenta sinais de mistura genética entre subpopulações a até 3 km de distância (HUA et al., 2013). Ou ainda do morcego filostomídeo *Leptonycteris curasoae*, em que foram reportadas distâncias de sete e oito quilômetros sendo percorridas por indivíduos adultos (GALINDO et al., 2006). Contudo, para o morcego molossídeo migratório *Tadarida brasiliensis* já foi registrada a movimentação de indivíduos por mais de mil quilômetros (RUSSEL; MEDELLIN; MCCRACKEN, 2005). No caso de *Pteronotus gymnonotus*, Leal e Bernard (2021) relatam machos adultos percorrendo distâncias de até 15 km entre duas *bat caves* em Pernambuco. Em todos os exemplos citados anteriormente, estas movimentações não apresentam uma periodicidade marcante e os locais de acasalamento geralmente não correspondem com as áreas de uso das espécies (*e.g.* RIVERS; BUTLIN; ALTRINGHAM, 2005; ROSSITER et al., 2002; GALINDO et al., 2006; HUA et al., 2013; LLAVEN-MACÍAS et al., 2021).

2.6 GENÉTICA DE POPULAÇÕES: DESAFIOS PARA A PESQUISA COM MORCEGOS

O entendimento da capacidade de movimentação dos organismos é importante para determinar como as espécies se distribuem no espaço e o grau de conectividade entre suas populações (CLOBERT et al., 2001). No caso dos morcegos, por serem animais noturnos e capazes de voar, estimativas diretas da capacidade de dispersão são desafiadoras, especialmente em grandes distâncias. As técnicas tradicionais, como observações em campo e marcação-e-recaptura, além de serem difíceis de aplicar ou requererem aplicação maciça para se obter bons resultados, geralmente apenas conseguem detectar a movimentação de indivíduos, sem determinar a efetividade da dispersão (CLOBERT et al., 2001). O uso de marcadores genéticos, portanto, permitiria não só a identificação de movimentos de dispersão, como também estimar a quantidade de migrantes que se reproduziram e contribuíram com o fluxo gênico de forma efetiva (BURLAND; WILMER, 2001; CLOBERT et al., 2001). Caracterizando-se, assim, como importantes ferramentas complementares nos estudos populacionais, principalmente em situações de escassez de informações e recursos.

Estudos de genética de populações de morcegos apontam que, em escalas macro geográficas, espécies migratórias tendem a apresentar baixos níveis de estruturação, com fluxo gênico acentuado entre as populações (WILKINSON; FLEMING, 1996; PETIT; MAYER, 1999; BURLAND; WILMER, 2001; RUSSEL; MEDELLIN; MCCRACKEN, 2005). Contudo, para espécies não migratórias, diversos padrões de estruturação já foram observados, podendo ser relacionados com vários fatores (BURLAND; WILMER, 2001). Para *Nyctalus noctula*, presente em praticamente toda a Europa e partes da Ásia, evidências sugerem que essa espécie migratória se apresenta como uma população panmítica em uma área de mais de 1600 km, ou seja, quase não há diferenciação genética entre os indivíduos, apesar da grande distância (PETIT; MAYER, 1999; PETIT; EXCOFFIER; MAYER, 1999). De maneira similar, *Tadarida brasiliensis* é um morcego que realiza grandes migrações entre os Estados Unidos, México, América Central e Caribe, por distâncias que ultrapassam milhares de quilômetros, e também apresenta baixos níveis de diferenciação genética em toda a sua distribuição (RUSSEL; MEDELLIN; MCCRACKEN, 2005). *Myotis chiloensis*, entretanto, é uma espécie de morcego que ocorre na América do Sul e tem uma distribuição norte-sul de cerca de 2800 km. Porém um estudo populacional mostra que a espécie apresenta uma clara estrutura populacional genética fortemente relacionada com a distância geográfica (LILLEY et al., 2020).

Padrões de estruturação populacional fortemente relacionados com a distância geográfica são relatados para diversas espécies de morcegos, como por exemplo *Macroderma gigas* na Austrália (WILMER et al., 1994; 1999), *Plecotus auritus* na Escócia (BURLAND et al., 1999), *Rhinolophus affinis* na Indonésia (MAHARADATUNKAMSI et al., 2000) e *Eidolon helvum* na região da África Central (JUSTE; IBÁÑEZ; MACHORDOM, 2000). Contudo, a distância geográfica não é o único fator possível influenciando a estrutura genética das espécies. Martins e colaboradores (2007) sugerem que a estrutura populacional observada em *Desmodus rotundus* no Brasil é um reflexo tanto da filopatria das fêmeas como da história evolutiva da espécie, que experienciou eventos vicariantes. Ao comparar espécies de *Cynopterus* na Malásia e Tailândia, Campbell e colaboradores (2006) verificaram que além da capacidade dispersiva, as preferências de habitat também influenciam na estrutura populacional das espécies, inclusive no passado. Neste estudo eles avaliaram se espécies de áreas abertas teriam uma estrutura genética populacional mais marcante do que as de floresta, por terem experienciado uma expansão populacional durante as oscilações climáticas no Pleistoceno (CAMPBELL et al., 2006). Já para *Myotis bechsteinii*, na Europa, foi observado que o padrão de estruturação genética é resultado do comportamento social da espécie, que vive em sociedades fechadas, onde cerca de 10 a 45 fêmeas adultas vivem juntas durante toda a sua vida, fazendo com que a migração de indivíduos seja praticamente inexistente (KERTH; VAN SCHAIK, 2012).

Grande parte dos estudos de genética de populações foi realizada aplicando marcadores mitocondriais (*e.g.* BURLAND et al., 1999; RUSSEL; MEDELLIN; MCCRACKEN, 2005; MARTINS et al., 2007). Contudo, evidências recentes sugerem combinar este tipo de dado com os gerados por marcadores nucleares, já que podem existir diferenças entre os padrões de estruturação revelados por cada tipo de marcador (e.g. CASTELL; RUEDI; EXCOFFIER, 2001; MARTINS et al., 2009; KERTH; VAN SCHAIK, 2012; HUA et al., 2013; PLATT et al., 2018). E neste cenário o uso de marcadores genômicos ganhou importância, pois além de terem herança biparental, também fornecem uma quantidade de informação substancialmente maior (DOOL, 2020). O gênero *Myotis* constitui um bom exemplo da aplicação de dados genômicos. É um gênero com distribuição em quase todo o mundo, exceto as regiões polares, e com grande diversidade: mais de 100 espécies descritas (*e.g.* STADELMANN et al., 2007; LARSEN et al., 2012; PLATT et al., 2018). Diversos estudos utilizando principalmente marcadores mitocondriais sugerem uma diversidade filogenética para o gênero que não coincide totalmente com aquela observada ao se analisar também marcadores nucleares (PLATT et al., 2018). Assim, o uso de dados genômicos permite inferências mais refinadas a respeito de sua diversificação, seus processos demográficos e da estrutura populacional das espécies, mesmo quando um número pequeno de indivíduos é amostrado (SUNDE et al., 2020).

Apesar da importância dos estudos de genética de populações e da grande riqueza e diversidade de morcegos observadas no Brasil, este tipo de estudo é bastante raro, com a grande maioria dos estudos moleculares já realizados até hoje focando em problemas taxonômicos (*e.g.* PAVAN; MARROIG, 2016; PAVAN; MARROIG, 2017; MORAS et al. 2018; GARBINO; LIM; TAVARES, 2020). Além disso, grande parte dos estudos realizados utilizam apenas marcadores mitocondriais (*e.g.* PAVAN; MARROIG, 2016; LINO et al., 2019). Esta situação é bastante preocupante diante do atual cenário de impactos antrópicos sobre a biodiversidade, já que de acordo com a teoria da genética da conservação populações pequenas e isoladas tendem a apresentar diversidade genética reduzida, o que as torna mais suscetíveis a mudanças no ambiente (DOOL, 2020).

2.7 CONSERVAÇÃO: LIGANDO ECOLOGIA DO MOVIMENTO, GENÉTICA DE POPULAÇÕES E AS *BAT CAVE*S DA CAATINGA

Dados genômicos já foram sugeridos como o futuro da genética da conservação para morcegos, devido ao potencial que possuem de gerar dados importantes para o preenchimento de diversas lacunas do conhecimento sobre morcegos (DOOL, 2020). Bernard e colaboradores (2011) realizaram uma análise de horizontes sobre a conservação de morcegos no Brasil e citam a necessidade de ampliar o número de estudos aplicando técnicas moleculares para análises populacionais, assim como o melhor entendimento sobre a ecologia de movimentos realizados no país e a aplicação destes dados em projetos de genética da conservação. Além disso, Mammola e colaboradores (2020) também incluem a geração de dados genéticos no guia para a conservação de biomas subterrâneos.

O Brasil, além de ter um potencial espeleológico bastante elevado (CECAV, 2021), registra 73 das 182 espécies que ocorrem no país utilizando cavernas como abrigos (GARBIRNO et al., 2020; GARBIRNO; BRANDÃO; TAVARES, 2022). Apenas nas *bat caves* do Nordeste brasileiro pode-se observar ao menos três das seis espécies ameaçadas no país, como *Natalus macrourus*, *Lonchorhina aurita* e *Furipterus horrens* (DELGADO-JARAMILLO; BARBIER: BERNARD, 2018; VARGAS-MENA et al., 2020; LEAL; BERNARD, 2021). Além disso, cavernas também são apontadas como hotspots de fluxo gênico (HUA et al., 2013), de diversidade de artrópodes endêmicos (FERREIRA, 2019) e de diversidade de fungos (CUNHA et al., 2020). Entretanto, estes também são abrigos bastante vulneráveis, susceptíveis ao impacto de várias atividades, e no Brasil em especial a mineração (FERREIRA et al., 2022), de tal forma que proteger cavernas é apontado como essencial para a conservação dos morcegos brasileiros (BERNARD et al., 2012).

Centenas de cavernas no país estão inseridas dentro de áreas que experimentam exploração mineral. A Resolução Conama nº 347/2004 determina no § 2º que, nestas situações, “a área de influência sobre o patrimônio espeleológico será definida pelo órgão ambiental competente que poderá, para tanto, exigir estudos específicos, às expensas do empreendedor”. Mas logo no § 3º, ela também especifica que “até que se efetive o previsto no parágrafo anterior, a área de influência das cavidades naturais subterrâneas será a projeção horizontal da caverna acrescida de um entorno de duzentos e cinquenta metros, em forma de poligonal convexa”. Na prática, o que se tem observado é que tem prevalecido este buffer de 250 m ao redor da caverna, e raros são os estudos que se propuseram a investigar de fato o tamanho real da área de influência das cavidades. Morcegos apresentam uma mobilidade notável e os 250 m estabelecidos por lei não são suficientes para representar tal influência, assim o entendimento legal sobre área de influência de uma caverna precisa ser revisto.

Portanto, este estudo analisou, de forma inédita para morcegos cavernícolas neotropicais, a estrutura genética populacional de duas espécies de *Pteronotus* que formam as colônias excepcionais das bat caves no Nordeste brasileiro. Utilizando, para tanto, dados genômicos obtidos com a metodologia ddRADsequencing e contando com uma amostragem ímpar nestas cavernas, a fim de comparar os padrões populacionais das duas espécies e entender as implicações conservacionistas dos resultados diante de um cenário de intenso impacto antrópico.

3 ARTIGO 1

High genetic connectivity among large populations of *Pteronotus gymnonotus* in bat caves in Brazil and its implications for conservation

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High genetic connectivity among large populations of *Pteronotus gymnonotus* in bat caves in Brazil and its implications for conservation

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Abstract

Bat caves in the Neotropical region shelter exceptional bat populations (some > 100,000 individuals). Their populations have a wide role in ecological interactions, are vulnerable due to their restriction to caves, and have a disproportionate conservation value. Current knowledge on bat caves in Brazil is still small. However, the systematic monitoring of some bat caves in Northeastern Brazil shows they experience strong population fluctuations in short periods of time, suggesting large scale movements between roosts and a much broader use of the landscape than previously considered. A spatio-temporal reproductive connectivity between distant populations would change our understanding of roost use among bat species in Brazil, and important gaps on the knowledge about large-distance bat movements in the country would be filled. Here, we used ddRADseq data to analyze the genetic structure of *Pteronotus gymnonotus* across nine bat caves along 700 km. Our results indicate lack of a clear geographic structure with gene flow among all of the analyzed caves, suggesting that *P. gymnonotus* uses a network of geographically segregated bat caves hundreds of kilometers away. Facing strong anthropogenic impacts and an underrepresentation of caves in conservation action plans worldwide, the genetic connectivity here demonstrated confirms that bat caves are priority sites for bat and speleological conservation in Brazil and elsewhere. Moreover, our results are a warning call: applied aspects of the environmental licensing of the mining sector and its impact must be reviewed, not just in Brazil, but wherever this licensing affects caves with exceptional bat populations.

Introduction

Caves are among the most used shelters by bats and are critical to the survival of several species worldwide (Furey and Racey 2016). Although the number of bats in a cave can vary drastically, the largest aggregation of warm-blooded vertebrates is found in caves, reaching millions of individuals at a single site (Betke et al. 2008; Medellín et al. 2008). Locations providing shelter for these large bat populations are known as bat caves and have been documented in some places across the world (Ladle et al. 2012; Furey and Racey 2016). In the Neotropics, bat caves have been documented in Mexico, Central America and the Caribbean, and in some countries in South America (e.g. Corso et al. 2010; Ladle et al. 2012; Medellín et al. 2017; Otálora-Ardila et al. 2019).

In Brazil, the large colonies found in bat caves are formed predominantly by *Pteronotus gymnonotus* (e.g. Rocha et al. 2011; Vargas-Mena et al. 2018; Otálora-Ardila et al. 2019), an insectivorous and strictly cave-dwelling species from the Mormoopidae family (de la Torre and Medellín 2010; Pavan and Tavares 2020). The systematic monitoring of some of these caves points out that while some of them appear to maintain resident populations throughout the year, in others, populations may experience large fluctuations within short periods of time (Otálora-Ardila et al. 2019; Leal and Bernard 2021). Such variations suggest the existence of massive displacement of bats between different shelters. Thus, bats from one cave could leave the shelter and move as a group to another bat cave, leading to a far broader use of the landscape than previously considered. In fact, mark/recapture studies have proved displacements of up to 15 km between bat caves in Pernambuco state, in Brazil (Leal and Bernard 2021). But could those bats be moving further away? Data for *Leptonycteris yerbabuenae* and *Tadarida brasiliensis* have indicated movements between summer bat caves in USA and winter bat caves in Mexico of 1,000 – 1,600 km for the first species and > 2,500 km for the second (Cole and Wilson 2006; Wiederholt et al. 2013; Medellín et al. 2017). With such dynamic roost site use, some bat caves should be treated as a single management unit for both genetic, conservation and environmental services purposes (e.g. Wiederholt et al. 2015, 2017; López-Hoffman et al. 2017a, 2017b; Medellín et al. 2017).

Sites with large animal congregations – such as bat caves – are considered exceptional ecological and conservation locations because 1) they have a disproportionate value for the survival of the species in question, 2) species can be particularly vulnerable when present in such large congregations, and 3) because they are numerically abundant, those individuals participate in a wide range of ecological interactions (Mittermeier et al. 2003). Bat caves have all such characteristics and their ecological and conservation importance is recognized in a few countries. In Mexico, for instance, bat caves are key-sites for the conservation of nectarivorous species like *Leptonycteris curasoae* and the endangered *L. yerbabuenae* (Arita and Santos-Del-Prado 1999; Stoner et al. 2003; Galindo et al. 2004); in Brazil, bat caves have legal protection status (Brasil 2022) and special attention during environmental licensing; and the millions of Mexican free-tailed bats at bat caves in the United States provide an ecosystem service of pest control worth $3.42 billion per year (Betke et al. 2008; Medellín et al. 2017) justifying their economical protection.

Although research on caves and their conservation status is increasing worldwide (Medellín et al. 2017), reliable scientific information that could help identify priority areas for cave conservation is highlighted by the conservation roadmap for the subterranean biome (Mammola et al. 2020; Wynne et al. 2021). This is especially true in Brazil, where caves are at high risk due to relaxation of the protection laws (Bernard et al. 2021). Moreover, studies on cave bats – and especially from bat caves – are still scarce in Brazil, despite the species richness and ecological importance of these animals (De Oliveira et al. 2018; Silva et al. 2018; Barros et al. 2020; Delgado-Jaramillo et al. 2020). Caves are also very susceptible to various impacts, such as mining and agricultural activities (Furey and Racey 2016), both economically important and widespread sectors in Brazil and Latin America (Bernard et al. 2012; Bernard et al. 2021). For instance, cave protection has a central role in the licensing of mining activities in Brazil, setting such habitats under strong pressure. Furthermore, recent attempts to dismantle environmental regulations in Brazil have made matters worse, further stressing the urgent need to better understand and protect the Brazilian cave heritage (Bernard et al. 2021; Ferreira et al. 2022). This is especially true for Northeastern Brazil, a region that simultaneously harbors high bat species richness (potentially 100 species – Silva et al. 2018; Delgado-Jaramillo et al. 2020), great potential for the occurrence of caves (Jansen et al. 2012), but in which the main biome (Caatinga) has already lost ~50% of its original natural cover, and is suspect to chronic anthropogenic disturbance (Antongiovanni et al. 2020). Moreover, more than 90% of the wind turbines in operation in Brazil are installed in the Northeast, setting conflicts for biodiversity conservation (Neri et al. 2019) and bats in particular (Bernard et al. 2014; Valença and Bernard 2015).

Although population structuring in bats is often relatively low due to their ability to fly and cross some ecological barriers impossible for other animals (Laine et al. 2013), at large geographical scales, distance can significantly correlate with genetic distance, thus population structure can be observed (e.g. Martins et al. 2007; Hua et al. 2013; Lilley et al. 2020). In fact, phylogeographic and population studies of bats in the Neotropical region reveal several cases of genetic structure and cryptic diversity (e.g. Martins et al. 2007; Pavan and Marroig 2016, 2017; Moras et al. 2018; Garbino et al. 2020). In this scenario, genomic data have the potential to assist in conservation studies (Dool 2020), and shed some light on genetic structure and diversity of a species, as well as large-scale movements of individuals at a landscape level (e.g. Sovic et al. 2016; Gong et al. 2019; Pinzari et al. 2020; Luna et al. 2021).

Here we used genomic data to assess the genetic structure of *P. gymnonotus* colonies from nine bat caves along 700 km in northeastern Brazil. We hypothesized that, due to the strong population fluctuations observed in some bat caves, bats were using networks of caves as roosts and, therefore, no strong population structure would be observed. If a spatio-temporal reproductive connectivity is identified between populations hundreds of kilometers away, important gaps on the knowledge about large-distance bat movements in Brazil would be filled. Moreover, the legal status of those caves and their populations would have to be revised, since they have to be considered as priority areas for the conservation of biological and speleological heritage of Brazil.

Methods

Sample collection and library construction

Nine colonies of *P. gymnonotus* from bat caves in the Brazilian states of Ceará, Rio Grande do Norte, Pernambuco and Sergipe were sampled in July of 2019 (Fig1, Table 1). The distance to the nearest bat cave varied from 15 km (PEMR to PEFM) up to 196 km (RNGA to RNCU) (Table 1), and the distance from the southernmost cave to the northernmost cave was 700 km. Estimates of the colony size were made using a non-invasive thermal detection system (see Otálora-Ardila et al. 2019, Pimentel et al. 2022), with two counts per cave. Bats were captured with a hand-net inside the cave, euthanized and liver tissue samples were collected from around 20 adult individuals in each cave, following the guidelines of the American Society of Mammalogists (Sikes et al. 2016). Tissue samples were stored in 1.5 mL tubes with 95% EtOH and stored at -80°C until further analysis. All specimens are deposited at the Mammal Collection of UFPE as vouchers. Project activities were conducted under the SISBIO/ICMBio permit 68992-1, registered on SisGen under the protocol A974BB7 and approved by the Commission on Ethics and Animal Use of Federal University of Pernambuco (CEUA-UFPE 114/2019).

Genomic DNA was extracted using Biopur Mini Spin Plus kit (Biometrix) following the manufacturers' protocols. All the extractions were done at Instituto Aggeu Magalhães, FIOCRUZ/Pernambuco and stored at -80°C before being sent to LUOMUS, Helsinki/Finland, for the following analyses. The amount of DNA of each sample was tested and quantified using the Thermo

Scientific Nanodrop spectrophotometer, and all samples were diluted into a 12 ng/mL solution, following Lilley et al. (2020).

DNA was prepared for genotyping-by-sequencing using a double digestion RAD-seq method adapted from Lemopoulos et al. (2018) and Elshire et al. (2011) for the use of low-concentration samples. Therefore, 180 ng of each DNA extract were used in a reaction together with 20 U of each of the restriction enzymes PstI-HF™ and BamHI (New England Biolabs) and 2 µl of CutSmart Buffer. The PstI-BamHI-digested libraries were prepared and sequenced using the Illumina Novaseq6000 over two lanes with 100 bp single-end reads by Bioname Oy. Details of the library preparation methods and sequencing run are provided as Supplementary Information and the raw data is available from the NCBI SRA archive under bioproject PRJNA824143.

Bioinformatic processing

The processing of the resulting reads followed Lilley et al. (2020) and the complete scripts and pipelines are available at https://github.com/itofs/Pgymno_radseq. Demultiplexing and quality filtering was carried out using Stacks 1.48 (Catchen et al. 2013) 'process_radtags'. The trimming of barcodes, low-quality regions and uncalled bases, followed by a minimum read length filter of 30 bp was carried out. After quality control, reads were mapped against *Pteronotus mesoamericanus* genome (Scheben et al. 2021), using BWA mem 0.7.17 with parameters -B 3 -O 5 -k 15 (Li and Durbin 2009). Stacks 1.48 (Catchen et al. 2013) pipeline 'ref_map.pl' was used for SNP calling and filtering. Several Stacks depths were tested in order to optimize the analyses (Catchen et al. 2013), with a value of 3 being used for downstream analysis. Samples were assigned to nine different populations, representing the bat caves where they were collected. SNPs with low (≤ 5) or high (≥ 125) coverage were excluded and only biallelic SNPs were used in the subsequent analyses (Lilley et al. 2020).

Data analysis

After sequencing data processing and SNP filtering, a Principal Component Analysis (PCA) was performed using Plink 1.09 (Purcell et al. 2007). The inference of individual ancestry coefficients was conducted using two approaches: the first is based on sparse nonnegative matrix factorization (NMF) and least-squares optimization, implemented in sNMF software (Frichot et al. 2014); and the second one with ADMIXTURE, that is based on likelihood models with quasi-Newton convergence acceleration method (Alexander et al. 2009). Considering each of the nine caves as a population, the sNMF runs were performed for values of the number of clusters set to K = 1-10 and the regularization parameter (α) varied between 0 and 1000. The cross-entropy criterion was used to select the best K. For ADMIXTURE possible K values were evaluated ranging from one up to nine, representing the bat caves analyzed, and 10 runs were performed for each number of K.

Nei's pairwise F_{ST}, expected (H_E) and observed (H_O) heterozygosity, nucleotide diversity (π) and inbreeding coefficient were calculated with the *populations* program in Stacks 1.48 (Catchen et al. 2013), using each cave as a population. F_{ST} is a standardized variance, representing the portion of the total genetic variance that is due to among-subpopulation differences (Hartl and Clark 1997). F_{ST} values of 0 to 0.05 were considered to be of low differentiation, and 0.05 to 0.15 as moderate differentiation, respectively, whereas F_{ST} values > 0.15 were considered distinctly differentiated (Hartl and Clark 1997). Latitude and longitude coordinates of the sampling locations were used to calculate pairwise geographic distances between caves in kilometers using Haversine method assuming a spherical earth, implemented in function distm in the R package geodist v. 1.5.10. Then, isolation-by-distance was estimated with a Mantel test with complete permutations, using the pairwise F_{ST} as a measure for the genetic distances and the between-caves distances as geographic coordinates for populations and considering alfa = 0.05.

Results

Colony size estimations ranged from 5,365 bats at Gruta do Arnold (RNGA) to 98,986 bats at Casa de Pedra (SECP), confirming that all of those caves harbor large populations (Table 1). We analyzed samples from 177 individuals of *P. gymnonotus*, 84 males and 93 females (Table 1). The number of samples per cave varied from 11 for the Meu Rei cave in Pernambuco to 24 bats for the Gruta do Farias cave, Ceará, and Furna do Morcego, in Pernambuco (Table 1). In total, the ddRADseq yielded an average of 1.6 million single-end raw reads. After quality filtering and SNP calling with Stacks, we obtained 62,644 genotypes that were biallelic and 26,091 SNPs were retained after pruning the datasets of variants that were in linkage (Purcell et al. 2007).

In the principal component analysis (PCA), the first two axes explained 15.32% of the variation in the data (Fig1), and based on the two axes there is no clear geographic structure among the *P. gymnonotus* sampled in the nine bat caves, along 700 km. Although at a fine scale PC1 generated two clusters (Fig1, bottom panel at right), the variance between them is not very pronounced and does not represent a case of isolation-by-distance. In fact, cluster one covers samples from five caves, while the second cluster has samples from seven of the nine caves analyzed (FigS1). Besides, individuals from the northernmost cave (RNGA) are grouping together with bats from the southernmost bat cave (SECP). Also, individuals from caves located at intermediate points of the sampling area (PEFM and PEMR) are clustering with bats from the northern (RNCU), western (CEGS, CEBL and CEGF), and southern caves (SEUR; FigS1), pointing out that the genetic distance observed here for *P. gymnonotus* has no correlation with the geographical distances between the caves. Moreover, individuals from the caves RNCU, CEGS and SEUR are found in both clusters, despite the geographical distance between them. Therefore, bats from these three caves are connected to bats from all of the caves analyzed. The genetic connectivity between the cave populations is also observed in both sNMF and ADMIXTURE (Fig2), with K = 1 showing the lowest cross-entropy (Fig3). Thus, each individual bat belongs to the same population and has the contributions of a single ancestral population, K = 1.

Overall, pairwise F_{ST} was low when considering each cave as a population (Table 2), ranging from 0.012 (SEUR~CEGF) to 0.029 (PEMR~SECP). Since F_{ST} represents the portion of the total genetic variance that is due to among-subpopulation differences (Weir 1996), the low values observed here indicate no genetic differentiation between the colonies. This also suggests the existence of gene flow among the *P. gymnonotus* colonies, which is supported by the low values of the inbreeding coefficient in all populations analyzed, which varied from 0.003 to 0.076 (Table 1). Also, observed (H_O) and expected (H_E) heterozygosity ranged from 0.178 to 0.267 and 0.181 to 0.263, respectively. We found no strong correlation between genetic and geographical distance, as estimated by the Mantel test: r = 0.1372 (P = 0.01).

Discussion

Using an individual-based approach with genome-wide markers, we conducted population genetic analyses of *P. gymnonotus* bats sampled from nine geographically distant caves in Northeastern Brazil and our results indicate that these bats do not present a clear geographic structure. Despite some of these caves being up to 700 km apart, the large bat populations analyzed show a poor pattern of isolation by distance and present high gene flow among bat caves. Our results help to better understand the large population fluctuations reported in some bat caves (Otálora-Ardila et al. 2019) and confirmed our initial hypothesis that *P. gymnonotus* is moving between distant bat caves, using them as a network of reproductive roosts. As shown on the PCA, individuals from SEUR, CEGS and RNCU caves had close genetic proximity with individuals from all of the caves sampled, while samples from PEMR and PEFM caves are genetically similar to samples from six other caves. Although this did not happen for all caves, bats sampled at one cave are similar to populations from at least four different caves, which suggests that these animals are moving among the roosts analyzed. Based on our findings, the current understanding of how these bats use the landscape needs to be revised, as the exchange of genetic material between bats sampled at distant caves indicates a much broader use of the landscape than previously understood.

Pteronotus gymnonotus is a relatively mobile species (Pavan and Tavares 2020), with the broadest latitudinal range among mormoopid bats, and occurring in several distinct habitats (Pavan and Tavares 2020). The large distribution range could contribute to genetic population structure (Burland and Wilmer 2001), seeing as an array of factors can affect the extent of genetic partitioning among populations, including dispersal ability and geographic barriers (Burland and Wilmer 2001). In fact, phylogeographic and population studies of bats in the Neotropical region reveal several cases of genetic structure and cryptic diversity, like in the *Chiroderma*, *Myotis* and *Pteronotus* genera (e.g. Martins et al. 2007; Pavan and Marroig 2016, 2017; Moras et al. 2018; Garbino et al. 2020; Novaes et al. 2021). The genetic structure of bat populations in Brazil is poorly known, but the few data available point out to strong population structure between the populations analyzed (e.g. Carstens et al. 2004; Pavan and Marroig 2016, 2017; Moras et al. 2018; Garbino et al. 2020). However, our results point out that *P. gymnonotus* presents as a strongly connected population in Northeastern Brazil, with poor correlation between the geographic and genetic distances.

The estimates of inbreeding coefficient and genetic differentiation for *P. gymnonotus* presented here suggest the existence of strong gene flow between caves on a broad geographic scale. Thus, together with the population fluctuations (Otalora-Ardilla et al. 2019) and movements previously observed for *Pteronotus* in some of the caves contributing to our study (Leal and Bernard 2021), these results suggest a very dynamic use of the roosts. Those observed fluctuations had no correlation with the amount of rain or environmental conditions around the caves (Otálora-Ardila et al. 2019) and nursery colonies have been observed in some of the bat caves analyzed, but not in all of them (Otálora-Ardila et al. 2019; Barros et al. 2020; Leal and Bernard 2021; Pimentel 2021). Therefore, the lack of correlation between genetic and geographic distances, along with the existence of gene flow between the colonies observed here, suggest that displacements related to reproduction could be a driver for better understanding the extreme population fluctuations already observed.

Movements associated with sexual segregation are known to occur in bat species of the family Mormoopidae, with female philopatry and male-biased dispersal (Pavan 2019). Similar patterns are also observed in other species forming bat caves, such as *Tadarida brasiliensis* (Llaven-Macías et al. 2021) and *L. curasoae* (Galindo et al. 2006). These species can be found in bat caves in Mexico and their movements between the roosts are also related to reproductive periods (Galindo et al. 2006; Llaven-Macías et al. 2021). The free-tailed bat (*Tadarida brasiliensis*) forms the largest colonies ever reported for mammals - up to about 2 million individuals (Betke et al. 2008), and uses a roost-network along southwestern USA and south of Mexico (Llaven-Macías et al. 2021). The migration hypothesis for the species proposes that northward migration is mostly undertaken by female individuals, while males only migrate over short distances with mating occurring in transitional roosts (Llaven-Macías et al. 2021). Migrations between southern USA and Mexico were also evidenced by genetic analysis, proving that bats from these caves are genetically similar (Morales et al. 2016). Our study adds to the evidence that long-distance reproductive-related movements are also observed for bat species in the Neotropics, and that bat caves in the Brazilian Northeast may be considered a network of reproductive roosts used by *P. gymnonotus* bats.

Conservation implications

Our results suggest that bat caves in Northeastern Brazil are priority areas for bat conservation in the country. In a scenario of strong anthropogenic impacts, the maintenance of gene flow between bat populations in these bat caves is essential, as studies indicate losses in species and genetic diversity of bat assemblages and populations from fragmented habitats compared to those occurring in continuous habitats (Meyer et al. 2009; Struebig et al. 2011; Ripperger et al. 2012; Lino et al. 2019, 2021). Those bat caves are essential for the preservation of *Pteronotus* bats, as species of this genus are mostly cave-dwelling (de la Torre and Medellín 2010; Pavan and Tavares 2020) and the large colonies they form are essential to control both the environmental and body temperature, being a very specialized expression of eco-evolutionary adaptation (de la Torre and Medellín 2010).

Furthermore, bat caves are very important ecologically, due to the wide range of ecological interactions the large *Pteronotus* aggregations participate (Mittermeier et al. 2003; Furey and Racey 2016; Pimentel et al. 2022). For instance, recent studies show that energy input in bat caves is strongly related to their presence in the cave, with guano deposition on the ground in some bat caves reaching up 738 g/m²/96h (Pimentel et al. 2022). Thus, the presence of the bats is essential to the maintenance of the cave ecosystem, since part of the cave biota is totally dependent on guano deposition (Furey and Racey 2016), in a way that entire communities can disappear without guano input into the caves (Trajano 1995; Gilbert and Deharveng 2002; Ferreira 2004). Also, caves are hotspots of subterranean invertebrate diversity (Myers et al. 2000; Gilbert and Deharveng 2002; Deharveng and Bedos 2012; Furey and Racey 2016) and bat caves are identified as hotspots for fungal diversity (Cunha et al. 2020; Pereira et al. 2022). Along with *P. gymnonotus*, other bat species can also be found in the bat caves analyzed, including endangered species such as *Natalus macrourus* and *Furipterus horrens* (e.g. Rocha et al. 2011; Feijó and Rocha 2017; Delgado-Jaramillo et al. 2018; Vargas-Mena et al. 2018; Otálora-Ardila et al. 2019; Vargas-Mena et al. 2020; Leal and Bernard 2021). Therefore, besides their relevance as sites holding thousands of individuals, bat caves can also act as umbrella sites for bat conservation.

The finding of genetically connected populations in bat caves distant 700 km apart also has legal implications in Brazil. Currently, Brazil's national legislation on the environmental licensing of mining activities (Brasil 2017; Brasil 2022) establishes that caves within those areas has to pass a classification process and only those with “maximum relevance” would be under strict protection. Bat caves are identified as maximum relevance caves (Brasil 2022). Moreover, in the licensing process, mining companies should identify how far a cave can influence its surroundings (Brasil 2017) However, this is rarely done and most of the companies adopt a 250 m buffer surrounding the cave. Thousands of caves are within mineral exploration areas in Brazil, and this concept of area “under influence” of a cave is very important, as mining activities must stop at its limits. However, bats have remarkable mobility and the 250 m established by law are not sufficient to represent such area of influence (e.g. Bernard and Fenton 2003; Delgado-Jaramillo et al. 2018; Otálora-Ardila et al. 2019; Leal and Bernard

2021). The lack of genetic structure and the existence of gene flow between colonies in bat caves 700 km apart, as we observed, stresses that the 250 m buffer around caves adopted by the Brazilian mining companies is an underestimation and clearly insufficient to maintain all the ecological interaction those animals have with caves and their surroundings. Therefore, our data is a warning call that the current practices adopted in the licensing of mining activities in Brazil must be reviewed facing the new existing evidence.

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Figures

Fig1 Bat caves in Northeastern Brazil where *Pteronotus gymnonotus* bats were sampled (left) and the genetic structure of the species showed by a Principal Component Analysis (right). The two most important components explain 15.32% of the nucleotide variation for the species.

Fig2 ADMIXTURE analysis showing the proportion of ancestry for *Pteronotus gymnonotus* from nine bat caves in Northeastern Brazil. Each vertical bar represents an individual, ordered by cave. Vertical bars with both colors indicate individuals of mixed ancestry. Plots represent individuals from one ancestral population (K = 1; at the top) and two ancestral populations (K = 2; at the bottom). For complete cave names please see Table 1.

Fig3 sNMF plot showing the values of cross-entropy for each K (number of ancestral populations) calculated for *Pteronotus gymnonotus* from nine bat caves in Northeastern Brazil. K = 1 have the lowest cross-entropy.

Tables

Table 1 Geographical and population data for *Pteronotus gymnonotus* per bat cave in northeastern Brazil, including sampling location, number of genetic samples collected (N) and division of samples by gender (Female/Male), estimated colony size, and genetic diversity information for each cave.

Cave	Locality	Geographical Coordinates	N	Female/ Male	Colony size	Polymorphic sites	Nucleotide diversity	Unique SNPs	Inbreeding coefficient	Heterozygosity	
										Observed	Expected
CEBL - Boqueirão de Lavras	Lavras da Mangabeira /CE	06°42'45.05"S 38°57'28.1"W	22	17/5	86089	54122	0.81783	89	0.04596	0.25741 (± 0.030)	0.2631 (± 0.020)
CEGF - Gruta do Farias	Arajara Park/CE	07°19'59.0"S 39°24'45.9"W	24	13/11	11540	51382	0.82269	36	0.00379	0.26737 (± 0.033)	0.25792 (± 0.020)
CEGS - Gruta do Sobradinho	Aiuaba/CE	6°38'35.6"S 40°5'57.1"W	15	10/5	57200	49167	0.8242	59	0.02273	0.25887 (± 0.036)	0.25372 (± 0.022)
RNGA - Gruta do Arnold	João Câmara /RN	05°26'36.2"S 35°53'37.1"W	21	11/10	5365	49130	0.81868	68	0.06561	0.2485 (± 0.028)	0.26195 (± 0.021)
RNCU - Caverna do Urubu	Felipe Guerra /RN	05°34'22.8"S 37°39'08.8"W	23	13/10	22743	46296	0.82472	14	0.05572	0.2459 (± 0.028)	0.2553 (± 0.021)
PEMR - Meu Rei	PARNA Catimbau/ PE	08°29'14.1"S 37°16'48.8"W	11	8/3	13828	47989	0.82652	49	0.02045	0.2586 (± 0.040)	0.24954 (± 0.024)
PEFM - Furna do Morcego	PARNA Catimbau/ PE	08°34'14.1"S 37°22'55.4"W	24	16/8	37789	55286	0.81807	111	0.05209	0.2558 (± 0.029)	0.26337 (± 0.020)

SECP - Casa de Pedra	Campo do Brito/SE	10°50'03.0"S 37°27'03.6"W	17	2/15	98986	18510	0.88359	8	0.04088	0.17825 (± 0.025)	0.18131 (± 0.018)
SEUR - Urubu	Divina Pastora/SE	10°43'58.1"S 37°09'56.0"W	20	3/17	62149	51679	0.82049	70	0.07602	0.24473 (± 0.029)	0.25951 (± 0.021)

Table 2 Pairwise F_{ST} (above diagonal) and pairwise geographical distance (below diagonal) between subpopulations of *Pteronotus gymnonotus* from nine bat caves in Northeastern Brazil. Non strong correlation between variables was observed (Mantel test, r = 0.1372; P = 0.01). Cave name acronym as in Table 1.

	RNGA	**RNCU**	**CEGS**	**CEBL**	**CEGF**	**PEMR**	**PEFM**	**SEUR**	**SECP**
RNGA	-	0.01532	0.01842	0.01517	0.01556	0.02043	0.01577	0.01738	0.01781
RNCU	195.419	-	0.01998	0.01726	0.01479	0.02258	0.01614	0.01725	0.01354
CEGS	484.33	295.867	-	0.01681	0.0171	0.02337	0.01309	0.02024	0.0247
CEBL	367.424	192.26	126.432	-	0.0157	0.0164	0.01423	0.01742	0.02022
CEGF	442.493	276.222	107.934	85.43	-	0.01993	0.01328	0.01248	0.01699
PEMR	371.863	327.008	372.689	270.726	267.944	-	0.01789	0.02348	0.029
PEFM	384.998	335.023	368.658	270.194	262.867	14.5511	-	0.01586	0.01854
SEUR	605.26	576.886	550.05	489.042	451.937	250.291	241.867	-	0.02027
SECP	624.138	586.091	550.01	487.836	445.404	261.929	252.094	33.1912	-

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

FI contributed to concept and design, data collection, analysis and interpretation of data, and drafting the manuscript. TL contributed to analysis and interpretation of data, funding acquisition and drafting the manuscript. VGT contributed to analysis and interpretation of data, and drafting the manuscript. EB contributed to conception and design, funding acquisition, analysis and interpretation of data, drafting and revising the manuscript for publication.

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Data Availability Statement

The datasets generated and analyzed during the current study are available in the NCBI SRA repository, under SRA BioProject PRJNA824143, and the complete scripts for the analyses performed are available in the GitHub repository, https://github.com/itofs/Pgymno_radseq.

4 ARTIGO 2

Comparative population structure of *Pteronotus* (Chiroptera: Mormoopidae) from bat caves in northeastern Brazil.

Manuscrito a ser submetido para publicação.

Comparative population structure of *Pteronotus* (Chiroptera: Mormoopidae) from bat caves in northeastern Brazil.

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ABSTRACT

In addition to the geographical distance, a variety of factors can affect the gene flow among populations, including ecomorphological traits of the species. The comparison of the population structure of closely related species under similar ecological situations can provide important information about these factors. Bat caves in northeastern Brazil shelter exceptional bat populations (> 100,000 individuals) formed by two species of *Pteronotus* bats: *P. gymnonotus* and *P. personatus*. These insectivorous, strictly cave-dwelling bats from the Mormoopidae family present similar patterns of fluctuation in their colony size that can be related with their reproductive periods. Current evidence points *P. gymnonotus* as a highly connected population in the bat caves at the Brazilian northeast, despite the distance of 700 km between some of them. Thus, we accessed the population structure of *P. personatus* and compared it with the data of *P. gymnonotus*. Our initial hypothesis was that, since the species are closely related, roost together and experience the same fluctuations in their colony size, they would also present similar patterns of structuration. But, contrary to our hypothesis, the ddRADseq data analyzed revealed a clear population structure among the subpopulations of *P. personatus*, however with no correlation with the geographical distance. The genetic structure of *P. personatus* can be a result of a combination of factors, such as morphology, evolutionary history, habitat preferences and mating strategies. Our results emphasizes that even very close-related species sharing roosts under similar ecological conditions can have distinct gene flow levels and population structures. Combined data for both

Pteronotus species suggest a very complex and dynamic use of the bat caves and point those roosts as priority sites for conservation.

Key-words: ddRADseq; gene flow; isolation by environment; dispersal ability; habitat fragmentation; Caatinga.

INTRODUCTION

Genetically structured populations are a reflection of continuous and discrete processes (Bradburd et al. 2018), thus geographic barriers to gene flow, demographic and evolutionary history, as well as species biology and ecology can translate into varying patterns of population structure (Hartl and Clark 1997; Campbell et al. 2006; Bradburd et al. 2018). Due to their ability to fly, bats are highly mobile animals, which promotes long-distance dispersal and often leads to low levels of population differentiation (Laine et al. 2013). However, at larger geographic scales and in highly variable environments, structured populations can be observed (*e.g.* Martins et al. 2009; Lilley et al. 2020), mostly due to limited dispersal capability (*e.g.* Burland et al. 1999; Newton et al. 2003). The migratory bat *Tadarida brasiliensis*, for example, is the most widely distributed mammalian species in the Western hemisphere (Armstrong 2008) with highly connected populations along its distribution (Russel et al. 2005). On the contrary, *Glossophaga longirostris* is a non-migratory bat from northern South America (Webster et al. 1998), with much more limited dispersal ability and consequently stronger population genetic structure (Newton et al. 2003).

The comparison of population structure among closely related species with overlapping distribution ranges can provide important information on the interactions of extrinsic and intrinsic factors influencing the patterns of genetic differences (Hartl and Clark 1997; Avise 2000). In such cases, minor differences in the species traits and ecology may translate into significant differences in the degree and scale of population structure (*e.g.* Newton et al. 2003; Campbell et al. 2006). Studies have shown that genetic structuring in nonmigratory bats might be a result of a variety of factors, such dispersal ability, social behavior, geographical barriers and historical events, as well as of the combination of these factors (*e.g.* Newton et al. 2003; Carstens et al. 2004; Vonhof et al. 2008; Meyer et al. 2009). For example, by comparing the population structure of four sympatric *Cynopterus* species with similar dispersal abilities, Campbell et al. (2006)

observed the influence of demographic processes on the structuration of the species. However, the opportunity to analyze the population structure of ecologically similar, sympatric and very closely related species is rare.

Commonly known as mustached and naked-backed bats, the genus *Pteronotus* comprises insectivorous, strictly cave-dwelling bats from the Mormoopidae family (Smith 1972; Simmons 2005). Eight species are traditionally recognized for the genus (Smith 1972; Simmons and Conway 2001), but divergent lineages found suggest that the diversity of this genus is underestimated (Clare et al. 2011; Pavan and Marroig 2016). All species are gregarious and are commonly observed roosting together (Smith 1972; Simmons and Conway 2001; Pavan and Tavares 2020). In Northeastern Brazil, for example, *P. personatus* and *P. gymnonotus* may form exceptional colonies within bat caves, reaching more than 100,000 individuals (Pavan and Tavares 2020; Leal and Bernard 2021; Pimentel et al. 2022).

Molecular data currently suggests that *P. personatus* is a species complex with five mitochondrial lineages: three correspond to *P. psilotis* and have a geographic distribution ranging from Mexico to Honduras; and the other two are *P. personatus* lineages that occur from Costa Rica to Brazil (Pavan and Marroig 2016; Zárate-Martínez et al. 2018). Although high levels of genetic differentiation can be observed between the lineages (Zárate-Martínez et al. 2018), this has so far only been considered at mitochondrial level (Clare et al. 2011; Pavan and Marroig 2016; Zárate-Martínez et al. 2018), preventing the description of these lineages as separated species, since nuclear markers can present patterns of structuration that differ from mitochondrial lineages (*e.g.* Castella et al. 2001; Martins et al. 2009; Hua et al. 2013; Platt et al. 2018). *Pteronotus gymnonotus*, on the other hand, occurs from southeastern Mexico to central Brazil and northeastern Bolivia (Pavan and Tavares 2020), and a recent study on the population genetic structuring reveal that the species forms a highly connected population among the bat caves in Northeastern Brazil, with no correlation between genetic and geographic distance (Ito et al. 2022).

Here we describe the genetic structure of *P. personatus* from bat caves in northeastern Brazil and compared with data from *P. gymnonotus*, whose population structure in the same region was recently assessed (Ito et al. 2022). Both species have phylogenetic proximity (Pavan and Marroig 2016; Pavan and Marroig 2017), are exclusive insectivores (de la Torre and Medellín 2010; Pavan and Tavares 2020), and are frequently found coinhabiting bat caves across the landscape (Pavan and Tavares 2020; Leal and Bernard 2021; Pimentel et al. 2022). Moreover, both species present similar patterns of fluctuation in their colony size (Otálora-Ardila et al. 2019; Leal and Bernard 2021; Pimentel et al. 2022), indicating the possibility of certain similarity in their reproductive patterns. Therefore, we had the chance to assess the population structure of two very close-related species sharing roosts and habitat areas. We hypothesized that *P. personatus* movements are reproductive-related, thus exhibiting low population structure and no correlation with the geographical distance among the subpopulations, as observed for *P.gymnonotus* (Ito et al. 2022).

METHODS

Taxa

Pteronotus personatus, Wagner's mustached bat, is a small species, with a forearm lenght < 50mm, fully furred and with mustachlike bristles (de la Torre and Medellín 2010). The distribution of the species has its northern limits in Mexico and extends southward through the entire Central America and reaches South America until northern Bolivia and Brazil (de la Torre and Medellín 2010). Two subspecies are traditionally recognized in its geographical distribution: *P. p. pisilotis* and *P. p. personatus* (Pavan and Marroig 2016; Zárate-Martínez et al. 2018). However, molecular data suggest that these subspecies should be considered separate species, with *P. psilotis* occurring from Mexico to Honduras and *P. personatus* distributing from Costa Rica to Brazil (Pavan and Marroig 2016; Zárate-Martínez et al. 2018). This species roosts in caves and forms colonies that can exceed thousands of individuals, which helps maintain a relatively high and constant body temperature (de la Torre and Medellín 2010). It is considered a generalist insectivorous and can be found in different habitats, from rain forests to dry deciduous forests (Smith 1972; de la Torre and Medellín 2010), but most frequently forages in dense vegetation in areas > 3.5 km from the roost (de la Torre and Medellín 2010).

Pteronotus gymnonotus, the big naked-backed bat, is the largest species among naked-backed bats, with the length of the forearm usually being > 50 mm (Pavan and Tavares 2020). It has the wing membrane jointed in the dorsal midline, which gives the appearance of a naked back, as well as short and dense hairs in the membrane (Smith

1972; Pavan and Tavares 2020). This species has the widest latitudinal range among mormoopid bats, from southeastern Mexico to central Brazil and northeastern Bolivia (Pavan and Tavares 2020), where can be found roosting in hot caves and foraging in warm and arid environments (Pavan and Tavares 2020). It is a relatively mobile species, more abundant in dry and semi-open environments and equally occupying habitats with distinct levels of disturbance (Pavan and Tavares 2020). Specializations in the wing musculature and morphology provide the species a better performance for rapid flight in open areas (Assis et al. 2011; Pavan and Tavares 2020). Mitochondrial data suggest a shallow phylogeographic structure (Pavan and Marroig 2016; 2017) and genomic data pointed to a highly connected population in Northeastern Brazil, despite the distance of almost 700 km between the caves analyzed (Ito et al. 2022). Also, previous study points to displacements of more than 15 km that are probably reproductive-related (Leal and Bernard 2021).

Sample collection and library construction

Nine bat caves in the Brazilian states of Ceará, Rio Grande do Norte, Pernambuco and Sergipe were sampled for *Pteronotus* bats from the 1st to the 28th of July 2019 (Figure 1, Table 1). Estimates of colony sizes were made using a non-invasive thermal detection system (see Otálora-Ardila et al. 2019, Pimentel et al. 2022), with two counts per cave. Bats were captured with a hand-net inside the cave, euthanized following the guidelines of the American Society of Mammalogists (Sikes et al. 2016), and liver tissue samples were collected from around 20 adult individuals in each cave. Tissue samples were collected in the field and stored in ice IN 1.5 mL tubes with 95% EtOH, then transported and stored at -80°C until further analysis. All specimens were deposited at the Mammal Collection of Federal University of Pernambuco (UFPE) as vouchers. Project activities were conducted under the SISBIO/ICMBio permit 68992-1, registered on SisGen under the protocol A974BB7 and approved by the Commission on Ethics and Animal Use of Federal University of Pernambuco (CEUA-UFPE 114/2019).

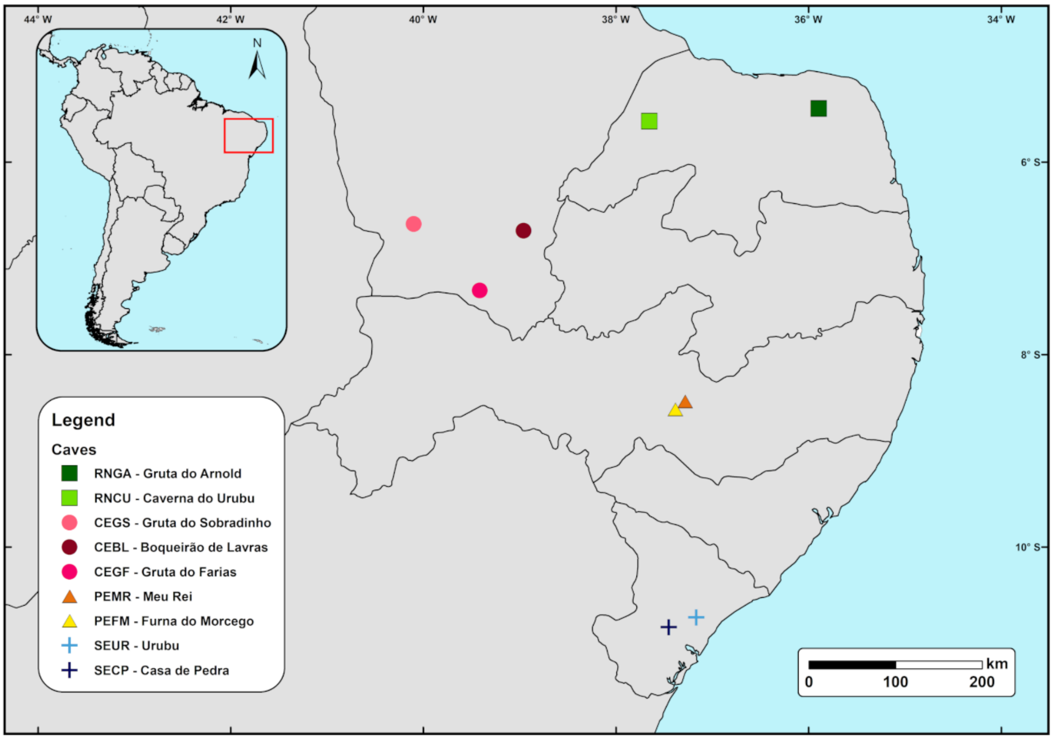

Figure 1. Bat caves in Northeastern Brazil where *Pteronotus* bats were sampled in July of 2019 to the analyses of their population genetic structure.

Genomic DNA was extracted using DNeasy Blood & Tissue kit following the manufacturer protocol (QIAGEN, Inc.). The amount of DNA of each sample was tested and quantified using the Thermo Scientific Nanodrop spectrophotometer, and all samples were diluted into a 20 ng/mL solution, following Lilley et al. (2020). DNA was prepared for genotyping-by-sequencing using a double digestion RAD-seq method adapted from Lemopoulos et al. (2018) and Elshire et al. (2011) for the use of low-concentration samples. Therefore, 180 ng of each DNA extract were used in a reaction together with 20 U of each of the restriction enzymes PstI-HF™ and BamHI (New England Biolabs) and 2 µl of CutSmart Buffer. The PstI-BamHI-digested libraries were prepared and sequenced using the Illumina Novaseq6000 (Elshire et al. 2011; Lemopoulos et al. 2018) by Bioname Oy. Samples were sequenced over one lane with 100 bp paired-end reads. Details of the library preparation methods and sequencing run are provided as Supplementary Information and the raw data is available at the NCBI SRA archive under bioproject PRJNA824143. Data on *P. gymnonotus* were accessed as in Ito et al. (2022).

Bioinformatic processing

The processing of the resulting reads followed Lilley et al. (2020) and Ito et al. (2022). The complete scripts and pipelines are availa/github.com/itofs/Pteronotus_radseq. All the bioinformatic processing was done using paired-end parameters. Demultiplexing and quality filtering were carried out using FastP (Chen et al. 2018). The trimming of barcodes, low-quality regions and uncalled bases, followed by a minimum read length filter of 30 bp were also carried out with FastP (Chen et al. 2018). After quality control, reads were mapped against *Pteronotus mesoamericanus* genome (Scheben et al. 2020), using BWA mem 0.7.17 with parameters -B 3 -O 5 -k 15 (Li and Durbin 2009). Stacks 1.48 (Catchen et al. 2013) pipeline ‘ref_map.pl’ was used for SNP calling and filtering. The samples were assigned to four different populations, representing the bat caves where they were collected. SNPs with low (≤ 5) or high (≥ 125) coverage were excluded and only biallelic SNPs were used in the subsequent analyses (Lilley et al. 2020).

Data analysis

After sequencing data processing and SNP filtering, a Principal Component Analysis (PCA) for all bats sampled was performed using Plink 1.09 (Purcell et al. 2007). In order to test for signs of philopatry, the genetic structure of the species was also analyzed according to the sex of the individuals, by running a PCA for each sex separately. The inference of individual ancestry coefficients was conducted using two approaches: the first is based on sparse nonnegative matrix factorization (NMF) and least-squares optimization, implemented in sNMF software (Frichot et al. 2014); and the second with ADMIXTURE, which is based on likelihood models with quasi-Newton convergence acceleration method (Alexander et al. 2009). Considering each of the four caves as a population, the sNMF runs were performed for values of the number of clusters set to K = 1-4 and the regularization parameter (α) varied between 0 and 1,000. The cross-entropy criterion was used to select the best K (Frichot et al. 2014). For ADMIXTURE possible K values were evaluated ranging from one up to four, representing the bat caves analyzed, and 10 runs were performed for each number of K. The cross-validation error (CV error) was used to select the best K value (Alexander et al. 2009).

The genetic diversity of each population was calculated with the ‘populations’ script in Stacks 1.48 (Catchen et al. 2013), which included nucleotide diversity (π), F_{ST}, expected (H_E) and observed (H_O) heterozygosity and the inbreeding coefficient. The pairwise F_{ST} was also calculated using each cave as a population. F_{ST} is a standardized variance, representing the portion of the total genetic variance that is due to among-subpopulation differences (Hartl and Clark 1997). F_{ST} values of 0 to 0.05 were considered to be of low differentiation, and 0.05 to 0.15 as moderate differentiation, respectively, whereas F_{ST} values > 0.15 were considered distinctly differentiated (Hartl and Clark 1997).

Latitude and longitude coordinates of the sampling locations were used to calculate pairwise geographic distances between caves in kilometers using Haversine method assuming a spherical earth, implemented in function distm in the R package geosphere v. 1.5.14 (Hijmans et al. 2017). Then, isolation-by-distance was estimated with a Mantel test with complete permutations, using the pairwise F_{ST} as a measure for the genetic distances and the between-caves distances as geographic coordinates for populations, with 10,000 permutations and considering alfa = 0.05.

In order to verify whether the population genetic structure in *P. personatus* is determined by continuous or discrete processes, the model-based clustering method implemented in conStruct R package (Bradburd et al. 2018) was used. If the genetic diversity in this species is strictly determined by continuous processes (Isolation by Distance – IBD), the results will strongly support the spatial model with a K value of 1. While values of K > 1 will be expected if discrete processes are influencing the genetic structure of the species (Bradburd et al. 2018). Considering each cave as a subpopulation, we tested K values of 1 to 4, and five replicates with 10,000 iterations were performed for each value of K. Models were compared using the cross-validation method and the best value of K was determined by the analysis of the contribution of each layer in the spatial model (Bradburd et al. 2018).

RESULTS

Colony size estimations confirmed that all the caves analyzed harbor large bat populations (Table 1), ranging from 5,365 bats at Gruta do Arnold (RNGA) to 98,986 bats at Casa de Pedra (SECP). *Pteronotus gymnonotus* individuals were sampled in all of the nine caves visited (Ito et al. 2022), while *P.personatus* was only sampled in Boqueirão de Lavras (CEBL), Furna do Morcego (PEFM), Casa de Pedra (SECP) and Urubu (SEUR) caves (Table 1, Fig1). Since the colonies in these caves are formed mainly by *P. gymnonotus* and *P. personatus*, we considered an estimation of 50/50 distribution between the two species, based on echolocation records made for some of the bat caves (Pimentel et al. 2022). In total, 249 bats were sampled: 177 individuals of *P. gymnonotus,* 93 females and 84 males (Ito et al. 2022); and 72 individuals of *P. personatus,* 21 females and 51 males (Table 1).

Table 2. Bat caves from the Brazilian Northeast where *Pteronotus* bats were sampled. Colony size represents an estimation of the number of bats present in the cave in the sampling night. The number of individuals collected (N) and the number of females (F) and males (M) individuals sampled are shown for each *Pteronotus* species. Data for *P. gymnonotus* as in Ito et al. (2022).

Cave	Locality	Coordinates	Colony size	*P. gymnonotus*		*P. personatus*	
				N	F/M	N	F/M
RNGA - Gruta do Arnold	João Câmara/RN	05°26'36.2"S 35°53'37.1"W	5,365	21	11/10	-	-
RNCU - Caverna do Urubu	Felipe Guerra/RN	05°34'22.8"S 37°39'08.8"W	22,743	23	13/10	-	-
CEGS - Gruta do Sobradinho	Aiuaba/CE	6°38'35.6"S 40°5'57.1"W	57,200	15	10/5	-	-
CEBL - Boqueirão de Lavras	Lavras da Mangabeira/CE	06°42'45.05"S 38°57'28.1"W	86,089	22	17/5	23	5/18
CEGF - Gruta do Farias	Arajara Park/CE	07°19'59.0"S 39°24'45.9"W	11,540	24	13/11	-	-
PEMR - Meu Rei	PARNA Catimbau/PE	08°29'14.1"S 37°16'48.8"W	13,828	11	8/3	-	-
PEFM - Furna do Morcego	PARNA Catimbau/PE	08°34'14.1"S 37°22'55.4"W	37,789	24	16/8	11	6/5
SEUR - Urubu	Divina Pastora/SE	10°43'58.1"S 37°09'56.0"W	62,149	20	3/17	24	7/17
SECP - Casa de Pedra	Campo do Brito/SE	10°50'03.0"S 37°27'03.6"W	98,986	17	2/15	14	3/11

After quality filtering and SNP calling and filtering, we kept 297,389,520 paired-end reads for *P. personatus,* from which we obtained 2,058,362 genotypes that were biallelic and 137,627 SNPs were retained after pruning the datasets of variants that were in linkage (Purcell et al. 2007). For *P. gymnonotus* we used the 26,091 SNPs generated by Ito et al. (2022). Sequencing and mapping information are available as Supplementary Material.

For *P. personatus,* the PC1 and PC2 explained 13.35% of the genetic variation in all the 72 individuals of the species and generate three clusters (Figure 2a). The first cluster covered only the southernmost caves (SEUR and SECP). The bats from PEFM, an intermediate cave within sampling area, were in the other two clusters (Figure 2a), grouping with bats from caves in the South (SEUR) and Northwest (CEBL). Although the number of male bats sampled was higher than the females, the same three clusters were found in both PCAs (Figure 2b and 2c).

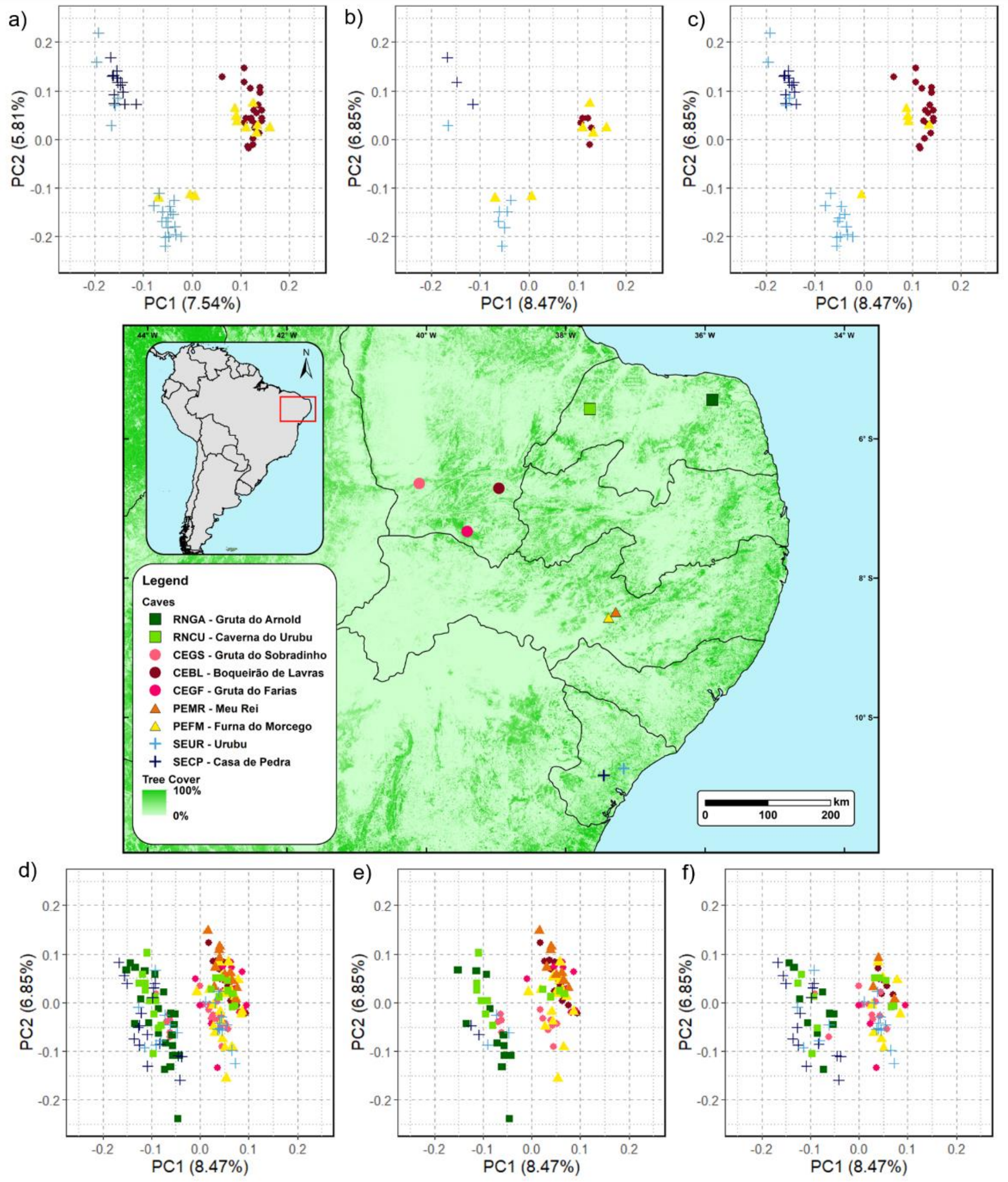

Figure 2. Genetic structure of *Pteronotus personatus* (top) and *Pteronotus gymnonotus* (bottom) sampled in nine different bat caves in Northeastern Brazil. (a, d) PCA with all individuals sampled. (b, e) PCA with only the females sampled. (c, f) PCA with only the male individuals analyzed. Data for *P. gymnonotus* as in Ito et al. (2022).

The analysis of individual ancestry with sNMF suggests high genetic connectivity among *P. personatus*, with K = 1 showing the lowest value of cross-entropy (Figure 3).

With the ADMIXTURE, on the other hand, the lowest value of CV-error was for K = 2 (Figure 3). According to this analysis, individuals from the caves in the South belong to the same ancestral population, while the bats from the other two caves belong to a different ancestral population (Figure 4). When comparing to the PCA, the two ancestral populations match clusters formed by PC1: one with the individuals from the two southernmost caves (SECP and SEUR), and the separated cluster formed by the bats from CEBL and PEFM (Figures 2 and 4).

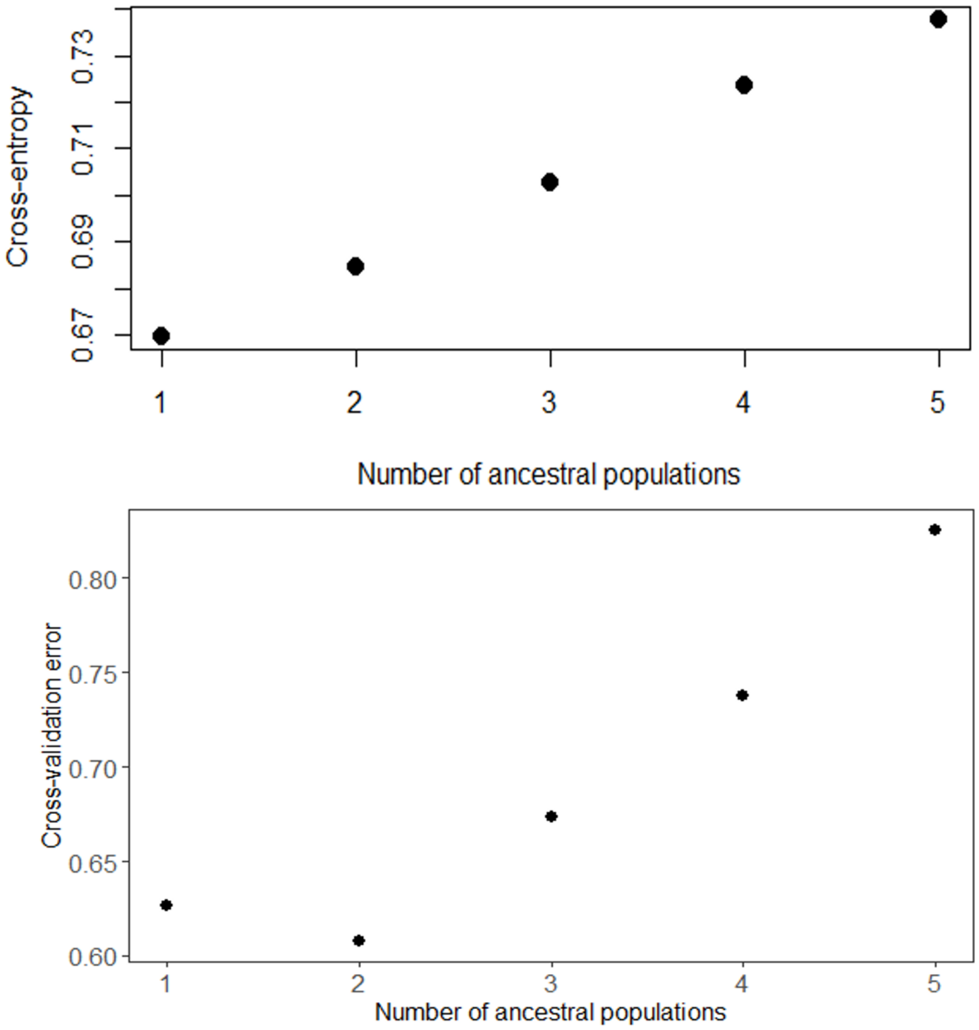

Figure 3. Cross-entropy values obtained with sNMF (top) and Cross-validation error values obtained with ADMIXTURE (bottom) for each K (number of ancestral populations) calculated for *Pteronotus personatus* from four bat caves in Northeastern Brazil. K = 1 has the lowest cross-entropy value and K = 2 has the lowest cross-validation value.

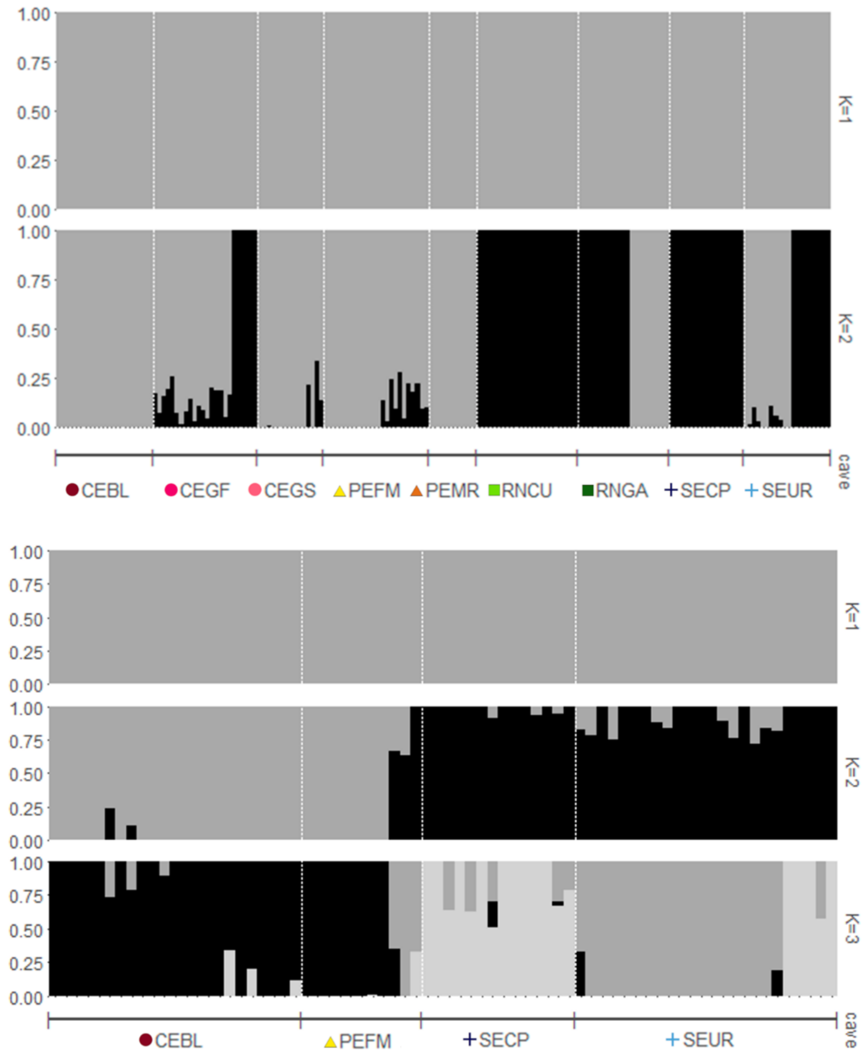

Figure 9. Results of inference of individual ancestry coefficients with ADMIXTURE for *Pteronotus gymnonotus* (top) and *Pteronotus personatus* (bottom). K values represent the number of ancestral populations. Colors do not represent the same ancestral population in each K graphic representation. For caves acronyms and symbols, please see Figure 1.

In general, the nucleotide diversity was lower, with Casa de Pedra cave (SECP) presenting the lowest value (0.25176) and Urubu cave (SEUR) the highest (0.27062; Table 2). Although heterozygosity was high for the populations of the four caves analyzed in this species, two caves (CEBL and SECP) presented higher values for the inbreeding coefficient: 0.1332 and 0.11166, respectively (Table 2). These results indicate that *P. personatus* from the bat caves in Northeastern Brazil are structured, but with some degree of gene flow between the populations analyzed.

Table 3. Genetic diversity of *Pteronotus* bats from bat caves in the Brazilian Northeast, including nucleotide diversity (π), inbreeding coefficient, and the expected (H_E) and observed (H_O) heterozygosity. Data for *P. gymnonotus* as in Ito et al. (2022).

	P. gymnonotus				***P. personatus***			
Cave	**Nucleotide diversity**	**Inbreeding coefficient**	**Heterozygosity**		**Nucleotide diversity**	**Inbreeding coefficient**	**Heterozygosity**	
			Observed	**Expected**			**Observed**	**Expected**
RNGA - Gruta do Arnold	0.81868	0.06561	0.2485 (± 0.028)	0.26195 (± 0.021)	-	-	-	-
RNCU - Caverna do Urubu	0.82472	0.05572	0.2459 (± 0.028)	0.2553 (± 0.021)	-	-	-	-
CEGS - Gruta do Sobradinho	0.8242	0.02273	0.25887 (± 0.036)	0.25372 (± 0.022)	-	-	-	-
CEBL – Boqueirão de Lavras	0.81783	0.04596	0.25741 (± 0.030)	0.2631 (± 0.020)	0.26868	0.1332	0.23601 (± 0.042)	0.26208 (± 0.021)
CEGF - Gruta do Farias	0.82269	0.00379	0.26737 (± 0.033)	0.25792 (± 0.020)	-	-	-	-
PEMR - Meu Rei	0.82652	0.02045	0.2586 (± 0.040)	0.24954 (± 0.024)	-	-	-	-
PEFM - Furna do Morcego	0.81807	0.05209	0.2558 (± 0.029)	0.26337 (± 0.020)	0.26658	0.08995	0.23991 (± 0.046)	0.25313 (± 0.025)
SEUR - Urubu	0.82049	0.07602	0.24473 (± 0.029)	0.25951 (± 0.021)	0.27062	0.07896	0.24499 (± 0.042)	0.26427 (± 0.021)
SECP - Casa de Pedra	0.88359	0.04088	0.17825 (± 0.025)	0.18131 (± 0.018)	0.25176	0.11166	0.23018 (± 0.040)	0.24096 (± 0.023)

Although *P. personatus* also presented overall low values of pairwise F_{ST}, the same pattern of structuration obtained by the PCA and ADMIXTURE can be observed (Table 3). The lowest pairwise value was obtained for Boqueirão de Lavras (CEBL) and Furna do Morcego (PEFM) caves, which are clustered together in the PCA (Figure 2) and in the ADMIXTURE (Figure 4). The highest value of pairwise F_{ST} was obtained between Furna do Morcego (PEFM) and Casa de Pedra (SECP) caves (Table 3). Even though Furna do Morcego (PEFM) is at an intermediate latitude within the sampling area (Table 3 and Figure 1), the bats from this cave did not group together with the individuals from Casa de Pedra cave (SECP; Figure 1) and belong to different ancestral population (Figure 4).

Table 3. Pairwise F_{ST} (above diagonal) and geographic distance in kilometers (below diagonal) for *Pteronotus personatus* bats sampled in four different bat caves in Northeastern Brazil.

	CEBL	**PEFM**	**SEUR**	**SECP**
CEBL	-	0.01713	0.0192	0.02133
PEFM	270	-	0.01976	0.02893
SEUR	489	241	-	0.01745
SECP	487	252	33	-

No strong correlation between genetic and geographical distance was observed for *P. personatus*, as estimated by the Mantel test: r = 0.0036 (p = 0.55). ConStruct results indicated that the genetic variation in the species was influenced by discrete process, but the geographic information was also important to determine the clusters, since the spatial model had greater predictive accuracy over the non-spatial in the cross-validation analysis (Figure 5). Also, the analyses indicated the existence of two clusters (K = 2), supporting the two ancestral populations suggested by ADMIXTURE.

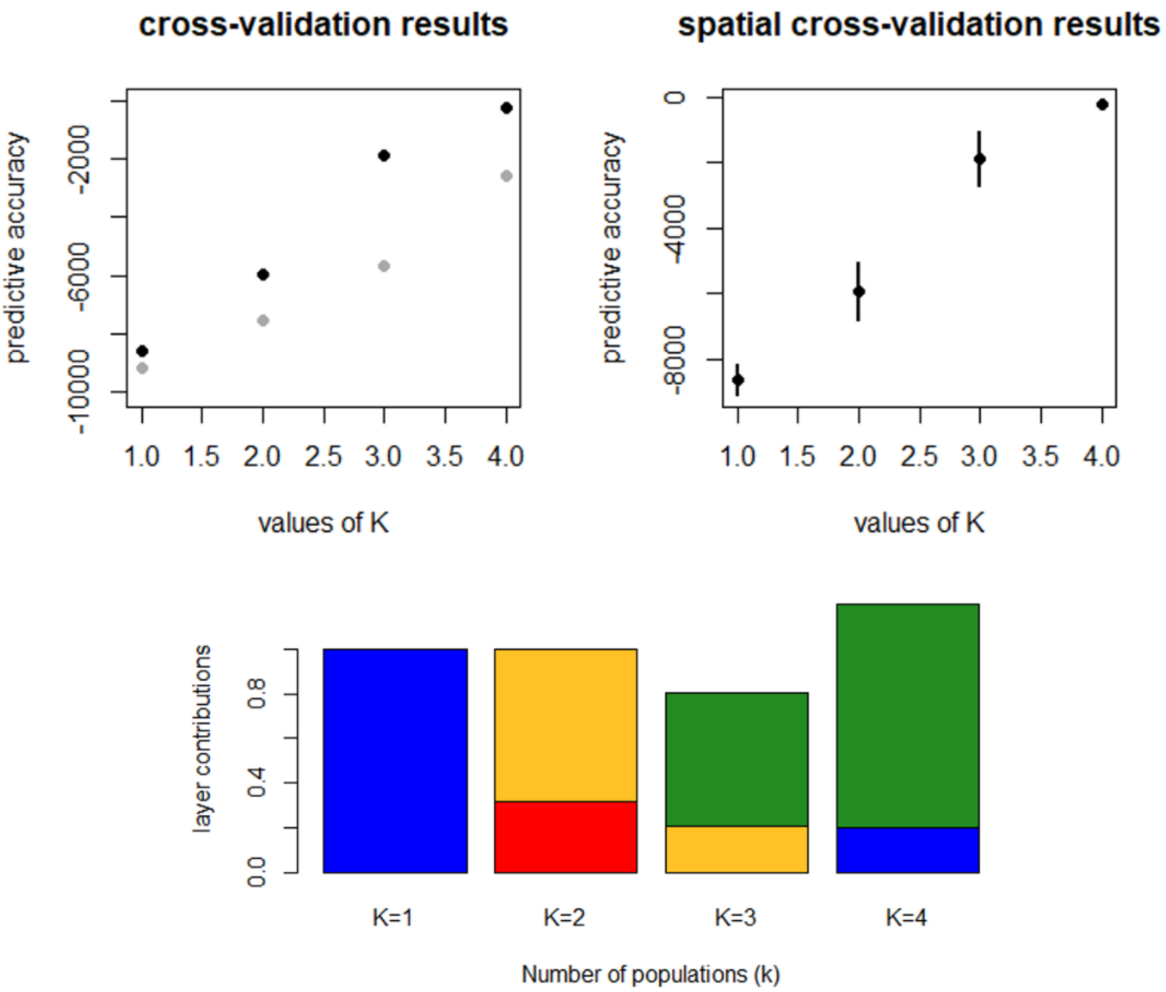

Figure 5. Results from conStruct analysis for *Pteronotus personatus* from the bat caves in Northeastern Brazil. Cross-validation results (top) comparing spatial (black) and non-spatial (gray) models for one to four layers tested in the conStruct analysis. Layers contributions results (bottom) from one to four to determine the best K value for the species. The model support K=2 for *P. personatus*.

DISCUSSION

Pteronotus personatus' population genetics

The analyses of the population genetic structure of *P. personatus* inhabiting four bat caves in Northeastern Brazil reveal a clear structure in the species. However, there was no correlation with the geographical distance among the subpopulations sampled, indicating that other environmental factors might influence the structuration in *P. personatus*. Although geographical information is an important factor influencing the population structure in *P. personatus*, it does not represent a pattern of Isolation by

Distance (IBD), where the genetic differentiation increases with the geographic distance (Wright 1943; Wang and Bradburd 2014). Since geography is only one of the key components that can influence population connectivity (Lee and Mitchell-Olds 2011; Wang and Bradburd 2014), environmental features could be shaping the distribution of genetic variation in this bat species (Wang and Bradburd 2014). In cases like that, ecological differences would influence in the patterns of gene flow among the bat caves' subpopulations, irrespective of geographical distance (Wang and Bradburd 2014). Besides distance, a variety of factors could shape the population structure in nonmigratory bats (Bradburd et al. 2018). Such as the dispersal ability (*e.g.* Burland et al. 1999; Newton et al. 2003) and sex-biased dispersal (*e.g.* Rivers et al. 2005; Hua et al. 2013), evolutionary and demographic history (*e.g.* Carstens et al. 2004; Martins et al. 2007; 2009; Lilley et al. 2020), social behavior (*e.g.* Kerth and Van Schaik 2012), roosting habits (*e.g.* Wilmer et al. 1999; Papadatou et al. 2008), and habitat and foraging preferences (*e.g.* Campbell et al. 2006; Cruz-Salazar et al. 2018). Also, a combination of these factors could be driving the population genetic structure in the species (*e.g.* Newton et al. 2003; Campbell et al. 2006), which seems to be the case of *P. personatus*.

In terms of morphology, for example, this species is the smallest among the mustached bats (de la Torre and Medellín 2010) which can affect its dispersal capability, since a positive correlation between flight speed and length of the forearm has already been suggested for the Mormoopidae family (Hopkins et al. 2003). Furthermore, species with more restricted distribution ranges and exhibiting divergent lineages tend to present strong population structure (*e.g.* Carstens et al. 2004; Campbell et al. 2006). The evidences that *P. personatus* is a species complex with at least two species with restricted distributions (Pavan and Marroig 2016; Zárate-Martínez et al. 2018) is in accordance with the results here presented. Also, previous studies on the demographic history influencing the genetic divergence predict that demographic expansion events can be related with more accentuated population structure (*e.g.* Campbell et al. 2006; Martins et al. 2009) and molecular data points to a recent diversification for the genus, during the Pleistocene (Pavan and Marroig 2017). *P. personatus* lineage diversification occurred early in the genus history (~13.9Ma) and the Pleistocenic climatic oscillations, as well as the sea-level variations, are suggested as factors shaping its demographic and evolutionary histories (Pavan and Marroig 2017; Zárate-Martínez et al. 2018). Although a north-south pattern of diversification is proposed for *P. personatus* during several extreme vegetation and climate changes over time (Pavan and Marroig 2017; Zárate-Martínez et al. 2018), the analysis of its demographic history is still needed.

P. personatus is sensitive to cold environments and roosts in bat cave systems, which allows individuals to maintain a relatively high and constant body temperature (de la Torre and Medellín 2010), as the ones observed in these caves due to the presence of the large number of bats (Ladle et al. 2012). Moreover, this species is more associated with dense vegetation, presenting adaptations even in its echolocation that allow the species to forage and navigate through dense foliage (Smotherman and Guillén-Servent 2008). *P. personatus* is reported foraging in areas approximately 3.5 km from the roost (de la Torre and Medellín 2010). Such habitat preferences can influence its dispersal ability and lead to isolation of the subpopulations and strong population structure, as already reported to species of plants (*e.g.* Katayama et al. 2016), invertebrates (*e.g.* Finn and Adler 2006), birds (Luna et al. 2021), lizards (*e.g.* Branch et al. 2003) and other bats (*e.g.* Campbell et al. 2006). In a fine-scale, though, these studies suggest that gene flow can still be observed among similar areas and the populations are not completely isolated (*e.g.* Luna et al. 2021; Yamazaki and Chiba 2022), which is consistent with the results presented here, that are pointing to existent gene flow among the subpopulations analyzed, albeit reduced.

As gene flow can be a reflection of mating strategies of the species (Sugg et al. 1996), and although female philopatry and male-biased dispersal are common in bats (*e.g.* Martins et al. 2009; Hua et al. 2013; Laine et al. 2013), the movements for mating are very complex and often show little correspondence to daily home ranges (*e.g.* Wilkinson and Fleming 1996; Rivers et al. 2005; Galindo et al. 2006). For many species, gene flow is not a direct result of migration of individuals to a new population, but it can occur through mating events, when individuals disperse temporarily to mate and then return to their original populations (Sugg et al. 1996; Burland et al. 1999; Clark et al. 2007; Hua et al. 2013). In these cases, bats congregate for mating at swarming sites promoting the genetic mixing among populations (*e.g.* Kerth et al. 2003; Veith et al. 2004; Rivers et al. 2005), and these temporary movements can be driven either by female or male dispersal and may have or not a clear seasonal pattern (Rivers et al. 2005; Rossiter et al. 2005; Hua et al. 2013). For *P. personatus*, the PCA results showed no clear pattern of population genetic structuration related to sex, which together with the existence of gene flow disrespect the geographic distance, suggest that the structuration observed might be a result of reproductive-related movements of individuals among the subpopulations segregated across the bat caves. Also, previous studies in some of the bat caves we sampled suggest that the fluctuations in the colony size had no correlation with the amount of rain or environmental conditions around the caves (Otálora-Ardila et al. 2019). But nursery colonies and reproductive individuals were reported (Otálora-Ardila et al. 2019; Leal and Bernard 2021; Pimentel et al. 2022).

Comparative population genetic structure

The genetic diversity and structure of a species may rise from processes that influence the levels of gene flow and usually result in a spatial heterogeneity. For lizards of the Tropiduridae family, for example, an array of factors is suggest to be influencing the patterns of population structure (Werneck et al. 2015; Fazolato et al. 2017; Ito et al. 2020). *Tropidurus hispidus* is a habitat generalist species widely distributed in the South America, being found in most of the Caatinga region and along the Brazilian coast and in the Amazon region (Carvalho 2013). *T. semitaeniatus* and *T. hygomi*, on the other hand, are habitat specialists and have more restricted distribution, being found in association to rock outcrops and sand soils, respectively (Carvalho 2013). The species different life traits affect directly in their dispersal abilities and are pointed to be shaping their genetic distribution over time and space, as *T. hispidus* presents a highly connected population along its distribution in Brazil (Ito et al. 2020), and *T. semitaeniatus* and *T. hygomi* have highly structured population genetic correlated with geomorphological events (Werneck et al. 2015; Fazolato et al. 2017). For the genus *Elaenia*, the two sympatric species *E. chiriquensis* and *E. cristata* have similar plumage, breeding phenology and habitat preferences, however they present different patterns of population genetic structure, shaped by the differences in their migratory behavior (Freitas et al. 2022). *E. chiriquensis* is an intratropical migratory species that helps to maintain high gene flow among its subpopulations, while *E. cristata* is a sedentary species with stronger genetic structuring with geographic correspondence throughout its distribution (Freitas et al. 2022).

In Northeastern Brazil, *P. personatus* is often reported roosting in association with *P. gymnonotus*, forming large colonies in the bat caves (*e.g.* Rocha et al. 2011; Feijó and Rocha 2017; Pavan and Tavares 2020; Leal and Bernard 2021; Pimentel et al. 2022). A recent study in the population genetics of *P. gymnonotus* in the same area has shown a strong connectivity among the caves' subpopulations, despite the geographical distance

(Ito et al. 2022). Although both species of *Pteronotus* bats seem to be under the same environmental conditions, the contrasting results in their population genetics point to the possible influence of species´ natural history and ecology when shaping population structure.

In addition to being found in a larger number of caves compared to *P. personatus* (Ito et al. 2022), *P. gymnonotus* exhibits other important ecomorphological differences: it is the largest species among the naked-backed bats and has the greatest degree of reduction of the wing musculature, which allows it a more rapid flight and enhanced performance (Pavan and Tavares 2020). In addition to having the widest latitudinal range of the Mormoopidae family (Pavan and Tavares 2020), phylogenetic analyses including both molecular and morphological data indicated that *P. gymnonotus* is a monophyletic group with no subspecies described (Pavan and Marroig 2016; 2017).

Although *P. gymnonotus* also roosts in bat caves, it is highly adapted in warm and arid environments (Pavan and Tavares 2020). This species is reported frequently exploring edge space habitats and equally occupying habitats with distinct levels of disturbance (Kalko et al. 2008; Bader et al. 2015; Pavan and Tavares 2020). Such characteristics facilitate the maintenance of gene flow across open areas and anthropogenic habitats (*e.g* Campbell et al. 2006; Bader et al. 2015; Lino et al. 2021). Also, reports on *P. gymnonotus* reproductive status suggest a wider breeding season when compared with *P. personatus* (de la Torre and Medellín 2010; Pavan and Tavares 2020). A longer breeding season can grant more mating opportunities with individuals from different subpopulations and may increase the gene flow among them (Sugg et al. 1996; Hua et al. 2013). In fact, pregnant females of *P. gymnonotus* have been captured in the Northeastern Brazil mostly from September to January (Feijó and Rocha 2017; Pavan and Tavares 2020; Leal and Bernard 2021) and reproductive males have been observed during the entire year (Leal and Bernard 2021). Also, previous studies reported nursery colonies and the movement of adult male individuals among bat caves for *P. gymnonotus* (Otálora-Ardila et al. 2019; Barros et al. 2020; Leal and Bernard 2021; Pimentel et al. 2022).

Therefore, our data for two close-related species, which may share roosts and habitat areas, suggest that when interpreting their population genetic structure, population studies in bats should consider a range of factors that are species-specific. Moreover, our data highlights the need for in deep ecological studies of cave-dwelling bats in Brazil. In the case of *P. personatus*, for example, a taxonomic review is imperative. Many studies have pointed it as a species complex, including molecular and morphological data (Pavan and Marroig 2016; 2017; Zárate-Martínez et al. 2018), but the description of the possible species is still in need. In addition to that, updated data on its distribution range and reproductive patterns are also important for the interpretation of its current population structure.

Bat caves and their conservation implications

Our results refute the initial hypothesis of high connectivity for *P. personatus*, as observed for *P. gymnonotus* (Ito et al. 2022), indicating a much more complex use of caves as roosts and providing important information about movement ecology of cave-dwelling species in Brazil. Many studies have reported the use of swarming sites for several bat species, such as *Plecotus auritus* (Burland et al. 2001; Veith et al. 2004), *Myotis nattereri* (Rivers et al. 2005), *Rhinolophus ferrumequinum* (Rossiter et al. 2002), and the cave-dwelling species *Tadarida brasiliensis* (Llaven-Macías et al. 2021) and *Leptonycteris curasoae* (Galindo et al. 2006). For all those examples, bats were reported travelling over several kilometers to temporary roosts for the explicit purpose of mating, which involved individuals from different natal populations and allowed gene flow among them, despite the geographical distance. As in *P. gymnonotus* (Ito et al. 2022), the results obtained for *P. personatus* suggest that these bats undergo long-distance movements for reproductive purpose, which also explains the fluctuations in the colony size observed in some bat caves (Otálora-Ardilla et al. 2019). Such evidence of long-distance mating movements shed light on the need of a landscape approach for bat and cave protection, especially in highly impacted regions, like the Brazilian Caatinga (Antongiovanni et al. 2020).

Current evidence indicate that fragmented habitats cause a decrease in both species and genetic diversity of bats (Ripperger et al. 2012; Lino et al. 2021), while highly connected habitat patches allow the maintenance of high levels of genetic diversity (Lamy et al. 2013; Lino et al. 2021). The region we sampled is dominated by the Caatinga, the largest seasonally dry tropical forest in South America (Pennington et al. 2006; Silva et al. 2017). Despite its ecological importance (de Araújo et al. 2022), the Caatinga is the third most degraded region in Brazil, with more than 63% of its area altered by anthropic impacts (Antongiovanni et al. 2020), and one of the least protected regions in the

Neotropics, with only 1.13% of its area under protection (Leal et al. 2005; Silva et al. 2017). Based on the macroecological theory (Brown 1995), we could expect that the impacts experienced by the Caatinga would affect the two *Pteronotus* species in different ways: *P. personatus* is more susceptible to habitat loss and fragmentation due to its smaller distribution range and habitat preferences (de la Torre and Medellín 2010), while *P. gymnonotus* is more likely to better adapt to anthropogenic ecosystems due to its wider distribution range and occurrence in different habitats (Pavan and Tavares 2020). However, current evidence has shown that the Caatinga is a very dynamic and heterogeneous system, shaped by multiple ecological process in different spatial and temporal scales along its area (de Araújo et al. 2022) and that anthropogenic disturbance is unevenly distributed across the landscape (Antongiovanni et al. 2020). Thus, the bat caves could be under different degrees of anthropic pression and, due to the complex use of the bat caves, both species would be negatively impacted.

Previous studies suggest that sites where bats congregate for mating should be considered as hot spots for gene flow in nonmigratory species (Kerth et al. 2003; Veith et al. 2004; Hua et al. 2013) and managed as a single conservation unit (Medellín et al. 2017). Such a view highlights the importance of the bat caves in Northeast Brazil for *Pteronotus* bats, since one of the species found at these caves is more abundant and features a highly connected population (Ito et al. 2022), and the other is more rare, with structured populations and reduced gene flow. More importantly, setting the bat caves as priority areas for conservation would benefit both bat species, and all other organisms inhabiting those caves, and their speleological heritage in Brazil.

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5 CONSIDERAÇÕES FINAIS

Morcegos do gênero *Pteronotus* constituem as grandes colônias das bat caves no Nordeste brasileiro, sendo *P. gymnonotus* e *P. personatus* as duas espécies principais. Os dados obtidos mostram que apesar de serem espécies proximamente relacionadas e de serem encontradas em condições ecológicas similares, as duas espécies de morcegos analisadas apresentam padrões de estruturação genética populacional distintos. *Pteronotus gymnonotus*, espécie encontrada nas nove bat caves analisadas, apresentou uma população com alta conectividade, apesar da distância de quase 700 km entre algumas das cavernas. Já *P. personatus* apresentou um grau de estruturação mais acentuado, contudo, também sem correlação com a distância geográfica entre as quatro subpopulações analisadas. Como em ambos os casos não foi observada uma correlação forte entre a distância geográfica entre as populações analisadas e a variação genética, isto indica que mesmo pequenas diferenças nas características das espécies podem influenciar e gerar padrões espaciais divergentes da distribuição da variação genética. Entre os fatores influenciando a genética populacional, a capacidade de dispersão, a especificidade de habitat e a história evolutiva das espécies ganham importância devido as diferenças que apresentam entre os dois taxa.

Mais além, estas diferenças destacam a complexidade das interações ecológicas que envolvem as bat caves brasileiras. Pois além de proverem abrigo e proteção para diversas espécies de morcego, estas cavernas desempenham um papel chave na reprodução de ambas as espécies de *Pteronotus*. Neste cenário, as cavernas podem ser consideradas como hot spots de fluxo gênico, já que indivíduos de diferentes subpopulações se encontram e se reproduzem, garantindo a mistura genética e a manutenção do fluxo gênico. Em termos práticos, os resultados apontam as bat caves do Nordeste brasileiro como áreas prioritárias para a conservação de morcegos e do patrimônio espeleológico no Brasil. E chamam a atenção para a necessidade de revisão dos critérios legais do processo de licenciamento ambiental e proteção das cavernas no país. Meus resultados apontam também para a necessidade de se desenvolverem mais estudos com morcegos cavernícolas no país, especialmente na Caatinga.

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