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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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Bat caves in the Neotropical region harbor exceptional bat populations (> 100,000 individuals). These populations play a wider role in ecological interactions, are vulnerable due to their restriction to caves, and have a disproportionate conservation value. Current knowledge of bat caves in northeastern Brazil is still small. However, systematic monitoring of some bat caves in northeastern Brazil shows that they experience strong population fluctuations over short periods of time, suggesting large-scale movements between roosts and a much broader use of the landscape than previously considered. Spatio-temporal reproductive connectivity between distant populations would change our understanding of the use of roosts among bat species in Brazil, and important gaps in knowledge of long-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 over 700 km. Our results indicate the lack of a clear geographic structure with gene flow among all the caves analyzed, suggesting that *P. gymnonotus* uses a network of bat caves geographically segregated hundreds of kilometers apart. Facing strong anthropogenic impacts and an underrepresentation of caves in conservation action plans worldwide, the genetic connectivity demonstrated here confirms that bat caves are priority sites for bat and speleological conservation in Brazil and elsewhere. Moreover, our results demonstrate a warning call: the applied aspects of the environmental licensing process of the mining sector and its impact must be reviewed, not only in Brazil, but wherever this licensing process affects caves having exceptional bat populations.

KEYWORDS

Chiroptera (bats), ddRADseq, population structure, roost network, karstic habitats

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). The locations that provide 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 South American countries (e.g., Corso et al., 2010; Ladle et al., 2012; Medellín et al., 2017; Otálora-Ardila et al., 2019).

In Brazil, large colonies found in bat caves are predominantly made up of *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, 2019). Systematic monitoring of some of these caves indicates that while some of them appear to maintain resident populations throughout the year, in others, populations may experience large fluctuations in short periods of time (Otálora-Ardila et al., 2019; Leal and Bernard, 2021). Such variations suggest the existence of a massive displacement of bats between different shelters. Thus, bats in 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 proven displacements of up to 15 km between bat caves in the state of Pernambuco, Brazil (Leal and Bernard, 2021). However, could those bats be moving further away? Data for *Leptonycteris yerbabuenae* and *Tadarida brasiliensis* have indicated movements between summer bat caves in the 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 a dynamic use of roost site, some bat caves should be treated as a single management unit for both genetic, conservation, and environmental service purposes (e.g., Wiederholt et al., 2015, 2017; López-Hoffman et al., 2017a,b; 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. For instance, in Mexico, bat caves are key sites for the conservation of nectarivorous species such as *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 in bat caves in the USA provide an ecosystem service of pest control worth \$3.42 billion/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), the roadmap for the conservation of the subterranean biome highlights reliable scientific information that can help identify priority areas for cave conservation (Mammola et al., 2020; Wynne et al., 2021). This is especially true in Brazil, where caves are at high risk due to relaxation of protection laws (Bernard et al., 2021). Moreover, studies on caves—and especially bat caves—are still scarce in Brazil despite the richness of species and the 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 highly 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, 2021). For instance, the protection of caves has a central role in the licensing process 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 Brazilian cave heritage (Bernard et al., 2021; Ferreira et al., 2022). This is especially true for northeastern Brazil, a region that simultaneously harbors a high richness of bat species (potentially 100 species—Silva et al., 2018; Delgado-Jaramillo et al., 2020), a great potential for the occurrence of caves (Jansen et al., 2012), but where the main biome (Caatinga) has already lost ~50% of its original natural cover, and is suspected of 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 the population structure 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 be significantly correlated with genetic distance, thus the 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 can assist in conservation studies (Dool, 2020) and shed some insights on the genetic structure and diversity of a species, as well as the large-scale movements of

individuals at the 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 strong population fluctuations observed in some bat caves, bats use networks of caves as roosts and thus no strong population structure would be observed. If spatio-temporal reproductive connectivity between populations hundreds of kilometers apart is identified, it will fill important gaps in knowledge of long-distance bat movements in Brazil. Moreover, the legal status of those caves and their populations will have to be revised, as they have to be considered priority areas for the conservation of the 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 2019 ([Figure 1](#), [Table 1](#)). The distance to the nearest bat cave ranged from 15 km (PEMR to PEFM) up to 196 km (RNGA to RNCU) ([Table 1](#)), and the distance from the southernmost to the northernmost cave was 700 km. Colony size estimates were made using a non-invasive thermal detection system (see [Otálora-Ardila et al., 2019](#); [Pimentel et al., 2022](#)), with two counts per cave. Following the guidelines of the American Society of Mammalogists, the bats were captured with a hand net inside the cave and euthanized, and liver tissue samples were collected from ~20 adult individuals in each cave ([Sikes and Animal Care and Use Committee of the American Society of Mammalogists, 2016](#)). Tissue samples were stored in 1.5-ml tubes with 95% EtOH and stored at -80°C until further analysis. All specimens were deposited in the Mammal Collection of UFPE as vouchers. The project activities were conducted under the SISBIO/ICMBio permit 68992-1, registered in 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 deoxyribonucleic acid (DNA) was extracted using the Biopur Mini Spin Plus kit (Biometrix) according to the manufacturers' protocols. All extractions were performed 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 from 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. \(2020\)](#).

Deoxyribonucleic acid was prepared for genotyping-by-sequencing using a double digestion RAD-seq method adapted from [Elshire et al. \(2011\)](#) and [Lemopoulos et al. \(2018\)](#) for the use of low-concentration samples. Therefore, 180 ng of each DNA extract was used in a reaction together with 20 U of each of the restriction enzymes PstI-HFTM and BamHI (New England Biolabs) and 2 μl of CutSmart Buffer. 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 runs are provided as [Supplementary Information](#), and raw data are available from the NCBI SRA archive under the bio project PRJNA824143.

Bioinformatic processing

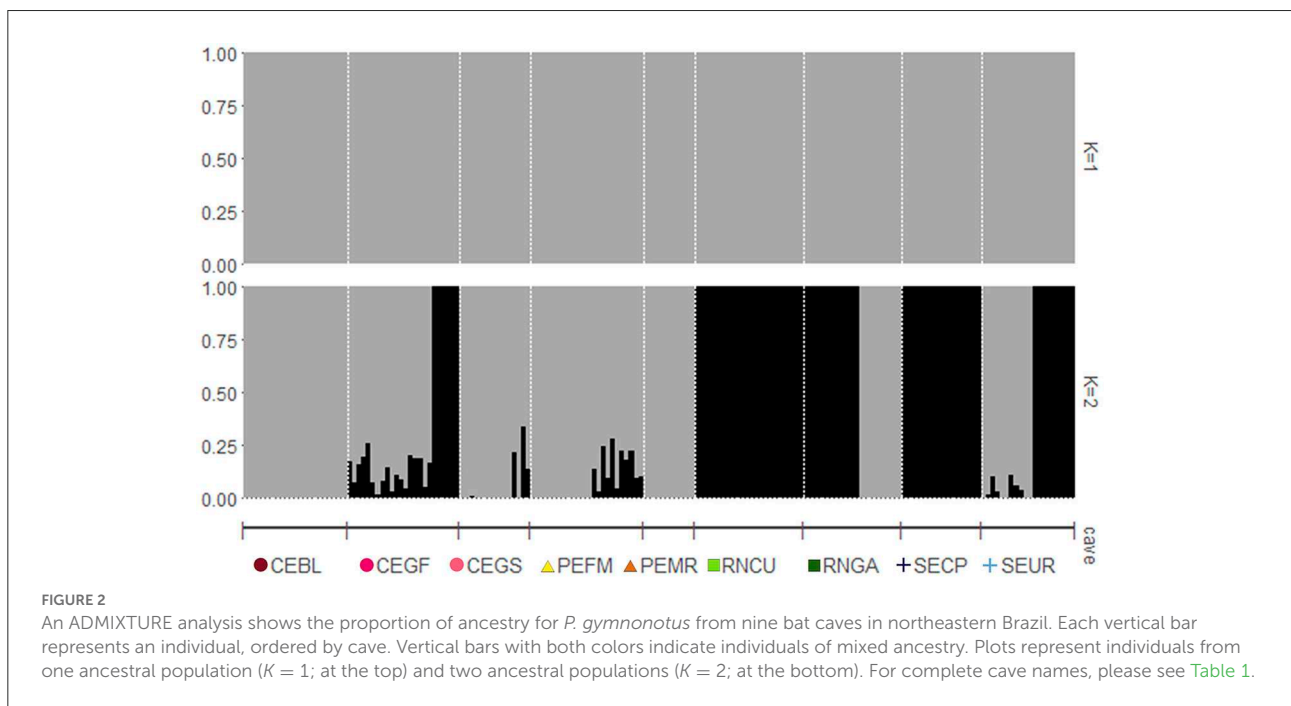
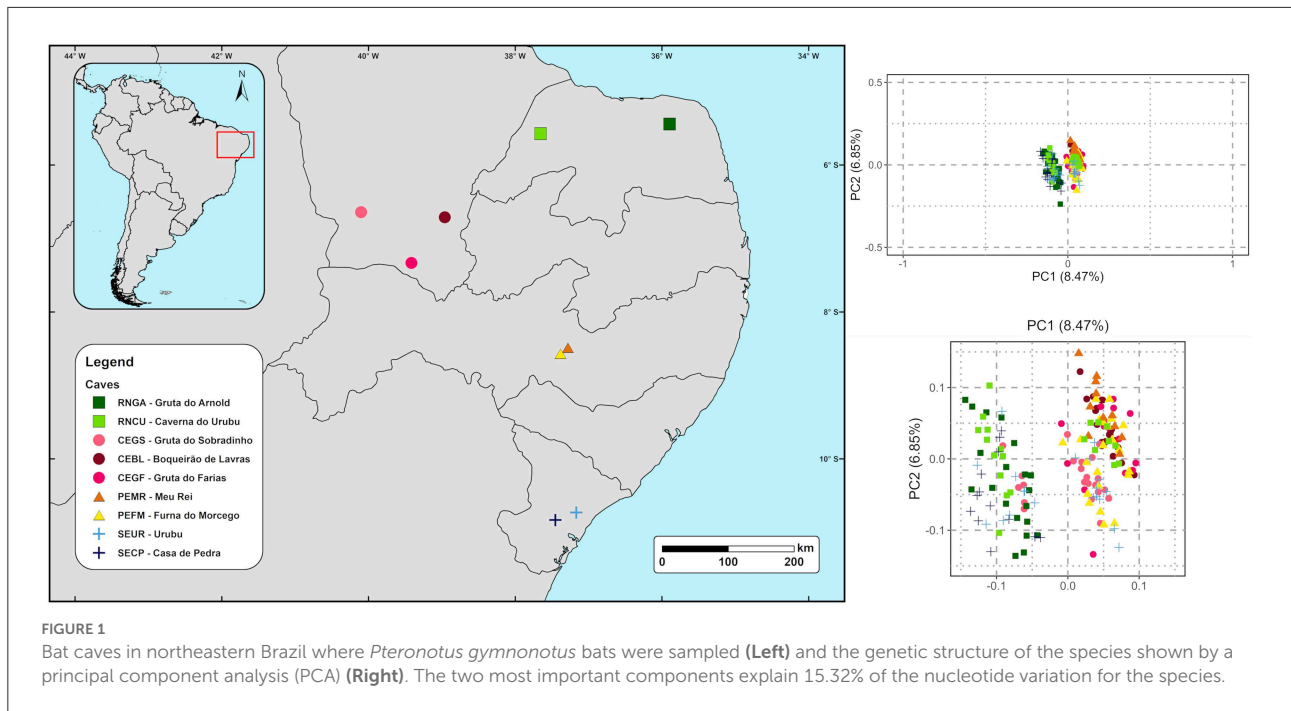
The resulting reads were processed according to [Lilley et al. \(2020\)](#), and the complete scripts and pipelines are available at https://github.com/itofs/Pgymno_radseq. Demultiplexing and quality filtering were carried out using Stacks 1.48 ([Catchen et al., 2013](#)) "process_radtags." Trimming of barcodes, low-quality regions, and uncalled bases was performed, followed by a minimum read length filter of 30 bp. After quality control, reads were mapped against the *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 single nucleotide polymorphism (SNP) calls and filtering. Several stack depths were tested to optimize analyses ([Catchen et al., 2013](#)), with a value of 3 being used for a downstream analysis. The samples were assigned to nine different populations, representing the bat caves where they were collected. SNPs with a low (≤ 5) or high (≥ 125) coverage were excluded, and only biallelic SNPs were used in subsequent analyses ([Lilley et al., 2020](#)).

Data analysis

After sequencing data processing and SNP filtering, 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 non-negative matrix factorization (NMF) and least-squares optimization, implemented in the sNMF software ([Frichot et al., 2014](#)); and the second with ADMIXTURE, which is based on likelihood models with the quasi-Newton convergence acceleration method ([Alexander et al., 2009](#)). Considering each of the nine caves as a population, sNMF runs were performed for values of the number of clusters fixed at $K = 1-10$ and the regularization parameter (α) varying between 0 and 1,000. The cross-entropy criterion was used to select the best K . For ADMIXTURE, possible K values ranging from 1 up

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

Cave	Locality	Geographical coordinates	Distance to the nearest bat cave (km)	<i>N</i>	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	85	22	17/5	86,089	54,122	0.81783	89	0.04596	0.25741 (± 0.03004)	0.2631 (± 0.02095)
CEGF—Gruta do Farias	Arajara Park/CE	07°19'59.0"S 39°24'45.9"W	85	24	13/11	11,540	51,382	0.82269	36	0.00379	0.26737 (± 0.03312)	0.25792 (± 0.02072)
CEGS—Gruta do Sobradinho	Aiuaba/CE	6°38'35.6"S 40°5'57.1"W	108	15	10/5	57,200	49,167	0.8242	59	0.02273	0.25887 (± 0.03673)	0.25372 (± 0.0229)
RNGA—Gruta do Arnold	João Câmara/RN	05°26'36.2"S 35°53'37.1"W	196	21	11/10	5,365	49,130	0.81868	68	0.06561	0.2485 (± 0.02825)	0.26195 (± 0.02116)
RNCU—Caverna do Urubu	Felipe Guerra/RN	05°34'22.8"S 37°39'08.8"W	192	23	13/10	22,743	46,296	0.82472	14	0.05572	0.2459 (± 0.02807)	0.2553 (± 0.02102)
PEMR—Meu Rei	PARNA Catimbau/PE	08°29'14.1"S 37°16'48.8"W	15	11	8/3	13,828	47,989	0.82652	49	0.02045	0.2586 (± 0.04052)	0.24954 (± 0.02432)
PEFM—Furna do Morcego	PARNA Catimbau/PE	08°34'14.1"S 37°22'55.4"W	15	24	16/8	37,789	55,286	0.81807	111	0.05209	0.2558 (± 0.02900)	0.26337 (± 0.02052)
SECP—Casa de Pedra	Campo do Brito/SE	10°50'03.0"S 37°27'03.6"W	33	17	2/15	98,986	18,510	0.88359	8	0.04088	0.17825 (± 0.02521)	0.18131 (± 0.01858)
SEUR—Urubu	Divina Pastora/SE	10°43'58.1"S 37°09'56.0"W	33	20	3/17	62,149	51,679	0.82049	70	0.07602	0.24473 (± 0.02943)	0.25951 (± 0.02162)



to 9 were evaluated, representing the bat caves analyzed, and 10 runs were performed for each K number.

Nei's pairwise F_{ST} , the 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, which represents the

portion of the total genetic variance that is due to differences between subpopulations (Hartl and Clark, 1997). F_{ST} values of 0 to 0.05 were considered poorly differentiated, and 0.05 to 0.15 moderately differentiated, respectively, whereas F_{ST} values > 0.15 were considered distinctly differentiated (Hartl and Clark, 1997). The latitude and longitude coordinates of the sampling locations were used to calculate the pairwise

geographic distances between the caves in kilometers using the Haversine method, assuming a spherical earth, implemented in the 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 of genetic distances and distances between the caves as geographic coordinates for populations and considering $\alpha = 0.05$.

Results

Colony size estimates 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 calls with Stacks, we obtained 62,644 biallelic genotypes and 26,091 SNPs were retained after pruning the data sets of variants that were linked (Purcell et al., 2007).

In the PCA, the first two axes explained 15.32% of the data variation (Figure 1), and based on the two axes there is no clear geographic structure among *P. gymnonotus* sampled in the nine bat caves, along 700 km. Although at a fine scale PC1 generated two clusters (Figure 1, bottom panel at right), the variation 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 (Supplementary Figure S1). Additionally, individuals from the northernmost cave (RNGA) are grouping together with bats from the southernmost bat cave (SECP). Also, the individuals from the caves located at intermediate points of the sampling area (PEFM and PEMR) are clustering with bats from the north (RNCU, west (CEGS, CEBL, and CEGF), and south caves (SEUR; Supplementary Figure S1), which indicates that the genetic distance observed here for *P. gymnonotus* has no correlation with the geographical distance between the caves. Moreover, individuals from the RNCU, CEGS, and SEUR caves are found in both clusters, despite the geographical distance between them. Therefore, bats in these three caves are connected to bats in all of the caves analyzed. Genetic connectivity between cave populations is also observed in both sNMF and ADMIXTURE (Figure 2), where $K = 1$ shows the lowest cross-entropy (Figure 3). 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), with a range from 0.012 (SEUR~CEGF) to 0.029 (PEMR~SECP). As F_{ST} represents the portion of

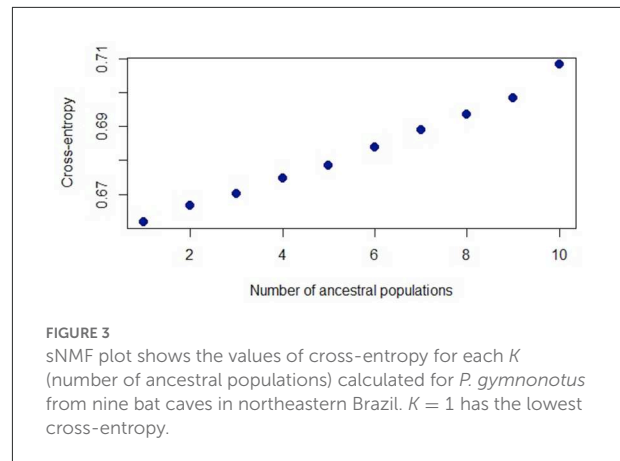


FIGURE 3
sNMF plot shows the values of cross-entropy for each K (number of ancestral populations) calculated for *P. gymnonotus* from nine bat caves in northeastern Brazil. $K = 1$ has the lowest cross-entropy.

the total genetic variance that is due to differences between subpopulations (Weir, 1996), the low values observed here indicate no genetic differentiation between colonies. This also suggests the existence of gene flow between *P. gymnonotus* colonies, which is supported by the low values of the inbreeding coefficient in all populations analyzed, which ranged from 0.003 to 0.076 (Table 1). Furthermore, the observed (H_O) and expected (H_E) heterozygosity ranged from 0.178 to 0.267 and from 0.181 to 0.263, respectively. We did not find a 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 geographical structure. Although some caves are separated by 700 km, the large bat populations analyzed show a poor pattern of isolation by distance and present high gene flow between bat caves. Our results help better understand the large population fluctuations reported in some bat caves (Otálora-Ardila et al., 2019) and confirmed our initial hypothesis that *P. gymnonotus* move between distant bat caves, using them as a network of reproductive roosts. As shown in the PCA, individuals from the SEUR, CEGS, and RNCU caves had close genetic proximity to individuals from all sampled caves, while samples from the PEMR and PEFM caves were genetically similar to samples from the six other caves. Although this did not happen in all caves, the bats sampled in one cave are similar to populations from at least four different caves, suggesting that these animals move between the analyzed roosts. 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 in distant

TABLE 2 Pairwise F_{ST} (above the diagonal) and pairwise geographical distance (below the diagonal) between subpopulations of *P. gymnonotus* from nine bat caves in northeastern Brazil.

	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	–

No strong correlation was observed between the variables (Mantel test, $r = 0.1372$; $p = 0.01$). Cave name acronym is presented in Table 1.

caves indicates a much broader use of the landscape than previously understood.

Pteronotus gymnonotus is a relatively mobile species (Pavan and Tavares, 2019), with the broadest latitudinal range among mormoopid bats, and is found in several different habitats (Pavan and Tavares, 2019). The wide distribution range could contribute to the genetic population structure (Burland and Wilmer, 2001), 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, such as in the genera *Chiroderma*, *Myotis*, and *Pteronotus* (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 understood, but some available data indicate a 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 indicate that *P. gymnonotus* occurs as a strongly connected population in northeastern Brazil, with a poor correlation between geographic and genetic distances.

Estimates of the inbreeding coefficient and genetic differentiation of *P. gymnonotus* presented here suggest strong gene flow between caves on a broad geographic scale. Thus, together with population fluctuations (Otálora-Ardila et al., 2019) and movements previously observed for *Pteronotus* in some of the caves that contribute to our study (Leal and Bernard, 2021), these results suggest a very dynamic use of roosts. These observed fluctuations had no correlation with the amount of rainfall or the environmental conditions around the caves (Otálora-Ardila et al., 2019) and nursery colonies have been observed in some, but not all, of the bat caves analyzed (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, suggests that reproduction-related displacements can be a driver for better understanding extreme population fluctuations already observed.

Movements associated with sexual segregation are known to occur in bat species in the family Mormoopidae, with female philopatry and male-biased dispersal (Pavan et al., 2019). Similar patterns are also observed in other species that form bat caves, such as *T. brasiliensis* (Llaven-Macías et al., 2021) and *L. curasoeae* (Galindo et al., 2004). These species can be found in bat caves in Mexico, and their movements between roosts are also related to reproductive periods (Galindo et al., 2004; Llaven-Macías et al., 2021). The free-tailed bat (*T. brasiliensis*) forms the largest colonies ever reported for mammals, up to ~2 million individuals (Betke et al., 2008), and uses a roost network along the southwestern USA and the south of Mexico (Llaven-Macías et al., 2021). The species migration hypothesis proposes that northward migration is mostly undertaken by female individuals, while males only migrate short distances with mating occurring in transitional roosts (Llaven-Macías et al., 2021). Migrations between the southern USA and Mexico were also evidenced by genetic analysis, proving that the bats in these caves are genetically similar to each other (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 northeastern Brazil 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 national bat conservation. In a scenario of strong anthropogenic impacts, maintaining 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., 2013; Lino et al., 2019, 2021). These bat caves are essential for the preservation of *Pteronotus* bats, as the species of this genus are mostly cave-dwelling (de la Torre and Medellín, 2010; Pavan and Tavares, 2019) and the large colonies they form are essential to control both 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 from an ecological point of view due to a wide range of ecological interactions in which large *Pteronotus* aggregations participate (Mittermeier et al., 2003; Furey and Racey, 2016; Pimentel et al., 2022). For instance, recent studies show that energy input into bat caves is strongly related to their presence in the caves, with guano deposition on the ground in some bat caves reaching 738 g/m²/96 h (Pimentel et al., 2022). Thus, the presence of bats is essential for the maintenance of the cave ecosystem as 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). In addition, 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 analyzed bat caves, 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, 2020; Otálora-Ardila et al., 2019; Leal and Bernard, 2021). Therefore, in addition to 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 700 km distance apart also has legal implications in Brazil. Currently, Brazil's national legislation on the environmental licensing process of mining activities (Burland and Wilmer, 2001; Brasil, 2022) establishes that caves within those areas have to pass a classification process and only those with "maximum relevance" will be under strict protection. Bat caves are identified as caves of maximum relevance (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 found within mineral exploration areas in Brazil, and this concept of an area "under the influence" of a cave is crucial, as mining activities must stop at its limits. However, bats have remarkable mobility and the 250 m established by law is not sufficient to represent such an 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, emphasizes that the 250-m buffer around the caves adopted by Brazilian mining companies is an underestimated measure and clearly insufficient to maintain all ecological interactions those animals have with the caves and their surroundings. Therefore, our data provides a warning call that the current practices adopted in the licensing of mining activities in Brazil must be reviewed in the face of the new existing evidence.

Data availability statement

The datasets presented in this study can be found in the NCBI SRA repository, under SRA BioProject PRJNA824143, <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA824143>, and the complete scripts for the analyses performed are available in the GitHub repository, https://github.com/itofs/Pgymno_radseq.

Ethics statement

The animal study was reviewed and approved by Comissão de Ética no Uso de Animais (CEUA) Universidade Federal de Pernambuco (UFPE).

Author contributions

FI contributed to conception and design, data collection, analysis, and interpretation, and manuscript drafting. TL contributed to data analysis and interpretation, funding acquisition, and manuscript drafting. VT contributed to data analysis and interpretation and manuscript drafting. EB contributed to conception and design, funding acquisition, data analysis and interpretation, and manuscript drafting and revision for publication. All authors contributed to the article and approved the submitted version.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.934633/full#supplementary-material>

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