

# A preliminary assessment of population genetic structure of the common vampire bat (*Desmodus rotundus*) in Colombia

Paige Van de Vuurst<sup>1,2,3</sup>, Analorena Cifuentes-Rincon<sup>1</sup>, Andrea S. Bertke<sup>3,4</sup>, Diego Soler-Tovar<sup>1,5</sup>, Nicolás Reyes-Amaya<sup>6</sup>, Fabiola Rodriguez Arévalo<sup>7</sup>, Julieth Stella Cárdenas Hincapié<sup>8</sup>, Jhon Rivera-Monroy<sup>9</sup>, Luis E. Escobar<sup>1,3,10,11</sup> and Eric Hallerman<sup>1</sup>

<sup>1</sup> Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA, United States of America

<sup>2</sup> Translational Biology, Medicine, and Health Program, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA, United States of America

<sup>3</sup> Center for Emerging Zoonotic and Arthropod-borne Pathogens, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA, United States of America

<sup>4</sup> Population Health Sciences, Virginia Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA, United States of America

<sup>5</sup> Epidemiology and Public Health Research Group, Faculty of Agricultural Sciences, Universidad de La Salle - Bogotá, Colombia

<sup>6</sup> Colección de Mamíferos, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia

<sup>7</sup> Laboratorio Nacional de Diagnóstico Veterinario (LNDV), Instituto Colombiano Agropecuario (ICA), Bogotá, Colombia

<sup>8</sup> Museo de La Salle (MLS), Universidad de La Salle - Santafé de Bogotá, Bogotá, Colombia

<sup>9</sup> Laboratorio Instrumental de Alta Complejidad (LIAC), Universidad de La Salle - Santafé de Bogotá, Bogotá, Colombia

<sup>10</sup> Kellogg Center for Philosophy, Politics, and Economics, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA, United States of America

<sup>11</sup> Global Change Center, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA, United States of America

## ABSTRACT

Rabies virus (RABV) is a neglected tropical pathogen in Latin America predominantly transmitted to mammals by the common vampire bat (*Desmodus rotundus*). Transmission of RABV among *D. rotundus* individuals and colonies is a function of individual dispersal between colonies, patterns of which can be inferred from population genetic structure. Nevertheless, a baseline assessment of population genetic structure among *D. rotundus* individuals has been lacking for some areas of South America, including Colombia, where RABV has impacted some areas more heavily than others. To assess individual dispersal and hence population structure of *D. rotundus* across heterogeneous landscapes in Colombia, we conducted a cross-elevational assessment of population genetic variation using nuclear microsatellite DNA markers. We quantified genetic variance and geographic distribution of genetically clustered *D. rotundus* individuals across the landscape of Colombia with reference to a comparator group of individuals from Mexico. We found population-level differentiation and genetic structure within our collection of samples, and we inferred patterns of dispersal and genetically effective migration between *D. rotundus* populations. Analysis of molecular variance (AMOVA) showed considerable variation among inferred populations in Colombia (14.9% of

Submitted 23 May 2025  
Accepted 7 October 2025  
Published 10 November 2025

Corresponding author  
Luis E. Escobar, [escobar1@vt.edu](mailto:escobar1@vt.edu)

Academic editor  
David Nelson

Additional Information and  
Declarations can be found on  
page 18

DOI [10.7717/peerj.20306](https://doi.org/10.7717/peerj.20306)

© Copyright  
2025 Van de Vuurst et al.

Distributed under  
Creative Commons CC-BY 4.0

## OPEN ACCESS

genetic covariance,  $df = 2$ , Sum of Squares = 164.9, Sigma = 1.28,  $\phi = 0.15$ ,  $p = 0.01$ ), with an associated  $G'_{ST}$  of 0.34. Direct migrant identification suggested 15 likely first-generation migrants among sites. We found that there were no statistically significant differences between the landscapes occupied by the inferred populations, though our limited sampling size suggests a trend toward differences in relation to elevation ( $t = 1.91$ ,  $df = 71.72$ ,  $p = 0.06$ ). These results indicate that *D. rotundus* is mobile within the region, potentially contributing to RABV transmission among colonies. Our results support previous hypotheses ecological resistance-mediated patterns of dispersal for *D. rotundus*, and inform future research on the role of genetic connectivity in RABV transmission among bat colonies.

**Subjects** Biogeography, Ecology, Genetics, Spatial and Geographic Information Science, Population Biology

**Keywords** *Desmodus rotundus*, Genetic variation, Populations dynamics, Elevation, Phylogeography

## INTRODUCTION

Rabies virus (RABV) is a prevalent and highly impactful pathogen ([Anderson et al., 2014](#); [Velasco-Villa et al., 2017](#)) predominantly spread from bats to other mammalian species in Latin America by the common vampire bat (*Desmodus rotundus*) ([Barquez et al., 2015](#)). RABV outbreaks in humans and livestock regularly occur in Latin America ([Stoner-Duncan, Streicker & Tedeschi, 2014](#); [Soler-Tovar & Escobar, 2025](#)), where human infections regularly follow outbreaks in livestock ([Meske et al., 2021](#)). Spread of RABV among *D. rotundus* colonies is thought to be a function of individual dispersal and migration patterns between populations ([Blackwood et al., 2013](#); [Benavides, Valderrama & Streicker, 2016](#); [Megid et al., 2021](#)) patterns of which can be inferred from population genetic structure. An understanding of basic population genetics and geographic structure of *D. rotundus* is beneficial for local stakeholders to mitigate RABV's impact on humans and animals. Assessments of populations structure along landscapes is further justified in areas with pronounced landscape heterogeneity, such as that found in Latin America. Landscape heterogeneity impacts the spatial distribution and overall health of wildlife species and infectious disease dynamics ([Biek & Real, 2010](#); [García-Morales, Badano & Moreno, 2013](#); [Becker et al., 2019](#)). Furthermore, assessing population genetic structure can bypass the need for lengthy telemetry, mark-recapture, or longitudinal serology-based assessments of bat movement that would otherwise be needed to elucidate possible transmission routes between populations ([Blackwood et al., 2013](#); [Mara, Wikelski & Dechmann, 2014](#); [Schorr et al., 2014](#); [Streicker & Allgeier, 2016](#); [Megid et al., 2021](#)). To assess how landscape heterogeneity may influence population structure of *D. rotundus*, we conducted a cross-elevational assessment of population genetic structure for this species in Colombia using nuclear microsatellite DNA markers.

Colombia is a country with large environmental gradients within a relatively small area, and has endemic circulation of RABV ([Fernando et al., 2017](#); [Cediel, 2020](#); [Rojas-Sereno et al., 2022](#)), which is regularly monitored by the Epidemiological Information

and Surveillance System of the Instituto Colombiano Agropecuario (ICA) (*Instituto Colombiano Agropecuario (ICA)*, 2019; *Instituto Colombiano Agropecuario (ICA)*, 2020; *Bonilla-Aldana et al.*, 2022). Bovine rabies in Colombia shows marked regional variation and evolving outbreak dynamics. *Bonilla-Aldana et al.* (2022) reported 4,888 confirmed cases between 2005 and 2019 in Colombia, with a significant decline after 2014 and a notable concentration of cases in the Department of Sucre (Caribbean Region, North Colombia). Other research has cited low vaccination coverage and implementation difficulties as potential drivers for RABV outbreaks (*Álvarez, Buitrago & Sáenz*, 2014). Across the last five decades, 2,858 outbreaks of RABV have been reported in Colombia in cattle alone (*Soler-Tovar & Escobar*, 2025). Furthermore, Colombia has experienced an expansion of *D. rotundus*-associated RABV into novel areas, and an increase in landscape change since the cessation of armed conflict in 2016 (*Clerici et al.*, 2020; *Rojas-Sereno et al.*, 2022; *Bonilla-Aldana et al.*, 2022). As a result, there is a need for a population genetics assessment for vampire bats in this region, especially within the context of landscape heterogeneity. Elevation can be used as a valuable proxy for environmental variation, since changes in altitude often correlate with changes in numerous environmental factors, such as temperature, vegetation composition, and humidity, making elevation a useful metric for studying landscape heterogeneity impacts (*González, Willig & Waide*, 2013). We therefore evaluated genetic diversity, population structure and its spatial distribution among sampling locations across an elevational gradient in the Colombian Andes. Our objective was to assess the extent to which population genetic diversity and structure of *D. rotundus* populations varied across space and were associated with environmental factors, which could be used to infer RABV transmission dynamics. This information could be useful to target RABV surveillance and pre-exposure human and livestock vaccination efforts.

## MATERIALS AND METHODS

### Experimental design

To assess spatial patterns of genetic diversity and structure in *D. rotundus* populations, and to test their association with environmental factors relevant to rabies virus (RABV) transmission, we implemented a multi-step population genetic approach. This included: (1) broad-scale field sampling across an elevational gradient in Colombia, (2) genotyping at highly polymorphic microsatellite loci, (3) quantifying within- and among population genetic diversity and structure, and (4) testing the relationships between genetic metrics and environmental variables including geography, elevation, climate, and landscape features at the locations of capture.

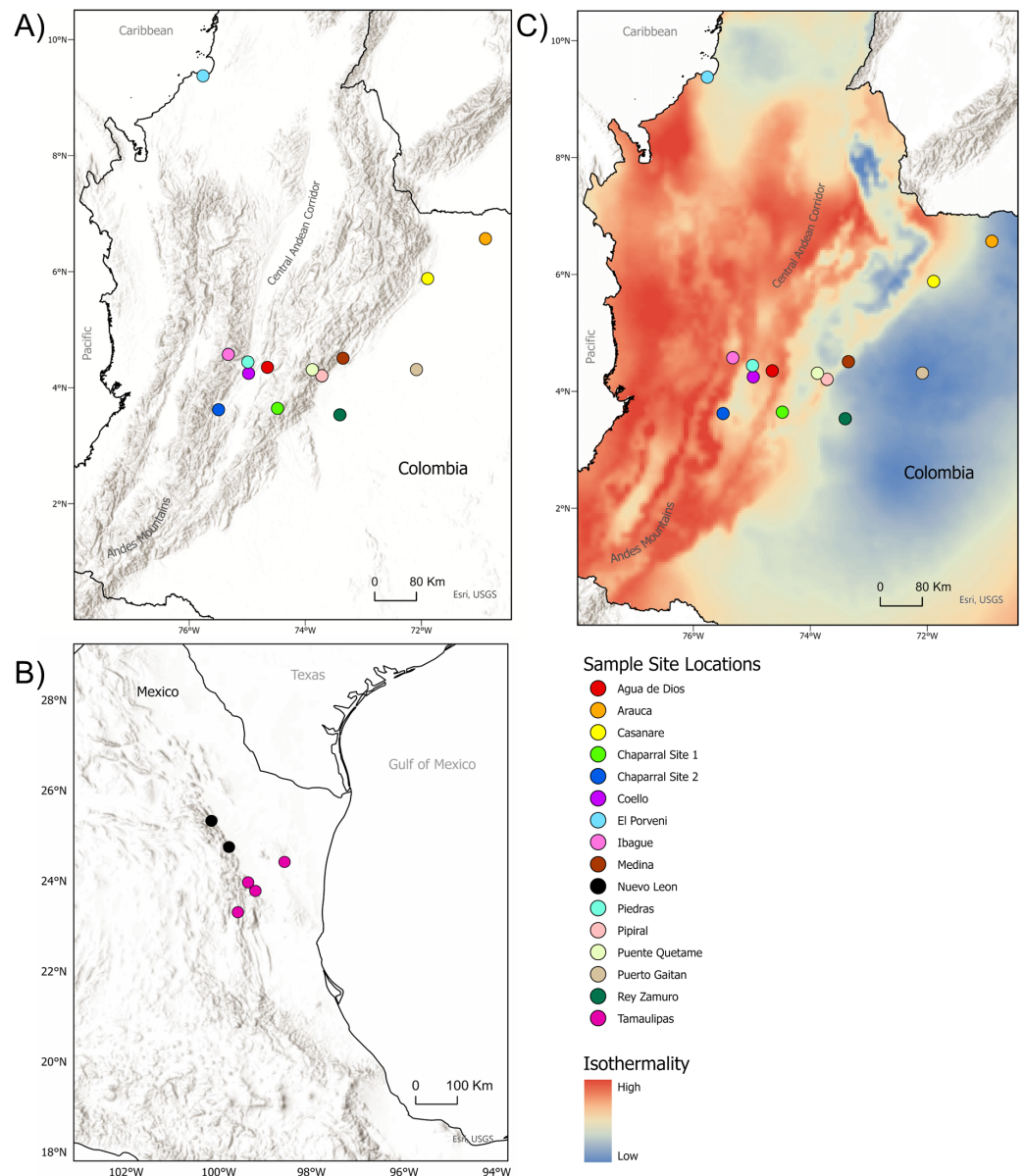
We utilized Colombia's dynamic topography and ecological diversity to select sampling locations across an elevational gradient in the Andes Mountains, under the assumption that elevation can be used as a reliable proxy for a variety of environmental factors (*González, Willig & Waide*, 2013; *Lyons & Kozak*, 2019). Thus, sample sites were selected to encompass elevations from low (<500 m), to moderate (500–1,000 m), to high (>1,000 m) to ensure that *D. rotundus* individuals were captured from a diversity of environments. Sites were also

selected to minimize potential spatial autocorrelation and to reflect home-range distances of *D. rotundus* (McNab, 1973; Trajano, 1996), and hence were spaced at least 20 km apart. Elevational categories (*i.e.*, low, moderate, and high) were utilized during site selection, but not in subsequent analyses. That is, all assessments were made independent of elevational category so as to not skew any potential relationships identified in subsequent analyses.

We obtained tissue samples from 81 *D. rotundus* individuals from Colombia in RABV-endemic areas during June and July of 2022 and 2023 (Table S1). Complementarily, five samples from a previous study collected between 2019 and 2022 were provided by the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (Bogota, Colombia) for the purposes of this study (Table S1), totaling 86 *D. rotundus* individuals from Colombia. As a genetic comparator, we included six *D. rotundus* individuals from western Mexico (Piaggio, Johnson & Perkins, 2008) (Table S1, Fig. 1) to contextualize population divergence. Environmental variables used for downstream associations with genetic structure included elevation (m), isothermality (average diurnal temperature range in °C divided by annual temperature range in °C), minimum temperature of the coldest month (°C), annual average precipitation (mm), precipitation seasonality (coefficient of variance) and landscape features such as associated land use and vegetation cover (*i.e.*, cattle density or vegetation indices present at the location of capture) for each sample site. Environmental variables were derived from the NASA Socioeconomic Data and Applications Center (SEDAC) (NASA Earth Data, 2022), Gridded Livestock of the World Database (Food and Agriculture Organization of the United Nations, 2022), and the WorldGrids Archived database (Hengl, 2018) at 1-km spatial resolution. Variables were selected based on their ecological importance to *D. rotundus* and RABV spillover risk as established in previous research (Lyman & Wimsatt, 1966; Zarza *et al.*, 2017; Mantovan *et al.*, 2022; Magalhães *et al.*, 2023; Van de Vuurst *et al.*, 2023; Jones *et al.*, 2024). Variable temporal range of the climate and landscape data were from 2016–2020.

### **Bat capture and tissue collection**

*Desmodus rotundus* individuals were collected using mist nets (12 × 2.6 m, mesh size 36 mm) for at least five consecutive hours after sunset targeting periods of peak activity during the waning moon phase (Zeppelini, Azeredo & Lopez, 2019). Animal handling followed protocols adhering to recognized bat handling guidelines of the American Society of Mammalogists (Sikes, 2016), as approved by the Institutional Animal Care and Use Committee at Virginia Tech (IACUC approval #21-138). Approval was also obtained from the Institutional Care and Use Committee of the Universidad de La Salle (IACUC approval #087). Our sampling protocol abided by legal regulations of Colombia, including the National Statute for the Protection of Animals, Resolution 8430 of the Ministry of Health: scientific, technical, and administrative standards for health research, the Code of Ethics for the professional practice of veterinary medicine, and Law 1774, amending the Civil Code, Law 84 of 1989, the Penal Code, and the Code of Criminal Procedure. Capture of bats and data collection in Colombia were carried out under environmental permit Resolución 1473 de 2014 of the Autoridad Nacional de Licencias Ambientales (ANLA) (*i.e.*, UniSalle Wild Species Collection Framework Permit). Both capture-and-release sampling and lethal



**Figure 1** Sample sites for tissue collection. (A) Locations of sample sites for *D. rotundus* individuals in Colombia. All sampling sites were at least 20 km from one another. Greatest distance between sampling sites was 669 km, originating from the sampling location of El Porvenir, Córdoba (far north Colombia), which was provided by the Humbolt Institute of Colombia. (B) Sites of collection for *D. rotundus* samples from Mexico provided by A. Piaggio. (C) Colombia sample sites overlayed over a map of Isothermality (average diurnal temperature range divided by Annual temperature range) to denote bioclimatic differences in sampling sites. Geographic coordinates for sampling sites are provided in Table S1. Maps created using ArcGIS Pro software version 2.5 with shape files from DIVA-GIS and topographic maps from USGS (Hijmans, 2017; Esri, Redlands, CA, USA, 2019). Isothermality data were sourced from the WorldGrids Archived database (Hengl, 2018).

Full-size [DOI: 10.7717/peerj.20306/fig-1](https://doi.org/10.7717/peerj.20306/fig-1)



sampling were conducted to collect the tissues analyzed in this study. Tissue samples were collected *via* pectoralis muscle dissection from individuals that were lethally sampled as part of an independent bat virome study aimed at reconstructing the virosphere of bats in the tropics (IACUC approval #21-138). For released individuals, tissue samples were collected *via* a 1-mm wing punch. All individuals that were captured during netting were sampled without discrimination based on age class, sex, or location.

To minimize stress and potential harm for captured individuals, bats were removed from nets and handled gently using clean cloth bags and restrained carefully by trained personnel. For euthanasia, bats were carefully placed inside a sealed Ziploc-type plastic bag containing a cotton pad soaked with two mL of USP-grade isoflurane, positioned to prevent direct contact with the animal. Bats were continuously observed for signs of anesthesia (*i.e.*, decreased responsiveness to stimuli, loss of righting reflex, and slowed respiration). Once deep anesthesia was confirmed *via* absence of reflexes, cardiac puncture was conducted to induce exsanguination, followed by cervical dislocation to ensure death (Sikes, 2016). Death was confirmed by the absence of heartbeat, respiration, and the presence of fixed, dilated pupils. All samples were preserved at  $-20^{\circ}\text{C}$  and stored long-term at  $-80^{\circ}\text{C}$  at the Universidad de La Salle in Bogotá, Colombia.

### **DNA extraction and microsatellite genotyping**

DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) and quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) (Qiagen, 2023) at the Universidad de La Salle in Bogotá, Colombia. All extractions had a yield of 50 ng/ $\mu\text{L}$  or greater. For each individual, we then amplified 12 nuclear microsatellite loci previously developed by Piaggio, Johnson & Perkins (2008) using Promega GoTaq Gold PCR protocols (Promega, 2019). Each 25- $\mu\text{L}$  PCR reaction consisted of 1.0  $\mu\text{L}$  25 mM  $\text{MgCl}_2$ , 5.0  $\mu\text{L}$  of 5X colorless GoTaq Flexi buffer (Promega, Madison, WI, USA), 0.5  $\mu\text{L}$  of 10 mM PCR nucleotide mix, 1.0  $\mu\text{L}$  of 10  $\mu\text{M}$  forward and reverse primers, 0.125  $\mu\text{L}$  of GoTaq DNA polymerase, 2.0  $\mu\text{L}$  of template DNA ( $<0.25\text{ }\mu\text{g}/25\text{ }\mu\text{L}$ ), and 14.375  $\mu\text{L}$  of nuclease-free water. PCR amplification was conducted using a T-100 thermocycler (BioRad, Hercules, CA, USA) with a profile consisting of initial denaturation at  $95^{\circ}\text{C}$  for 10 min; followed by 35 cycles of  $94^{\circ}\text{C}$  for 30s,  $52^{\circ}\text{C}$  for 45s, and  $72^{\circ}\text{C}$  for 45s; and a final extension at  $72^{\circ}\text{C}$  for 10 min. Samples then were held at  $12^{\circ}\text{C}$  for at least 15 min prior to being placed in storage at  $2^{\circ}\text{C}$  (Romero-Nava, León-Paniagua & Ortega, 2014). PCR products were sent to the Cornell University Institute of Biotechnology Resource Center (Ithaca, NY) for fragment analysis. We used the *Fragman* package (version 1.0.9) (Covarrubias-Pazarán *et al.*, 2016) in R statistical software version 4.1.0 (R Core Team, 2021) to automate peak calls for determining amplification fragment sizes for alleles at each of the 12 loci for each *D. rotundus* individual. Allele lengths were confirmed *via* a visual inspection of peaks for each locus in GeneMarker version 2.6.3 software (SoftGenetics, 2018). We used Brookfield (Brookfield, 1996; Campagne *et al.*, 2012) and Van Oosterhout methods (Van Oosterhout *et al.*, 2004; Van Oosterhout, Weetman & Hutchinson, 2006; The Earth and Life Systems Alliance, 2023) through the *PopGenReport* package version 3.1 (Adamack & Gruber, 2014).

in R to identify and exclude loci with high null allele frequencies ( $>0.5$ ), ensuring data reliability.

### **Population genetics analysis**

To assess how genetic variation was structured across space and potentially linked to environmental factors, we conducted the following sequential analyses:

*Population structure and clustering.* To infer genetic clusters without prior assumption, we applied two complementary approaches: Discriminant Analysis of Principal Components (DAPC) ([Jombart, Devillard & Balloux, 2010](#)) using sequential  $K$ -means of the number of populations or conformance of genotype frequencies to Hardy-Weinberg equilibrium (HWE) expectations ([Jombart, 2015](#); [Desvars-Larrive et al., 2019](#)), optimized using Bayesian information criterion (BIC) for each increasing number of  $K$ . We also applied Bayesian Clustering via STRUCTURE software ([Pritchard, Stephens & Donnelly, 2000](#)) applying the log-likelihood approach of [Pritchard, Stephens & Donnelly \(2000\)](#) and the delta  $K$  ( $\delta K$ ) method of [Evanno, Regnaut & Goudet \(2005\)](#) to identify the best-supported value of  $K$ . We applied the *poppr* package version 2.9.6 ([Kamvar, Tabima & Grünwald, 2014](#)) to conduct analysis of molecular variance (AMOVA) within individuals, among individuals within clusters, and between clusters. These methods allowed us to identify putative populations and assess admixture, forming the basis for all population-specific analyses.

*Genetic diversity and differentiation.* For each cluster identified by DAPC, we used *hierfstat* version 0.5-11 ([Jerome et al., 2022](#)) and *dartRverse* version 2.0 ([Mijangos et al., 2022](#)) to calculate:

1. Allelic richness, observed and expected heterozygosity, and inbreeding coefficient ( $F_{IS}$ , ([Weir & Cockerham, 1984](#)).
2. Pairwise  $F_{ST}$  and  $G'_{ST}$  ([Yang, 1998](#); [Hedrick, 2005](#)) as measures of genetic differentiation using *dartRverse*. Statistical significance was evaluated using 1,000 permutations, with  $p$ -values  $< 0.05$  indicating significant genetic differentiation.
3. Deviations from Hardy-Weinberg Equilibrium (HWE) using the *poppr* package ([Kamvar, Tabima & Grünwald, 2014](#))

Effective population size ( $N_e$ ) was estimated using NeEstimator ([Do et al., 2014](#)) with the Waples correction ([Waples & Do, 2010](#); [Waples, Larson & Waples, 2016](#)) to infer local demographic stability.

*Isolation-by-distance and sex-based dispersal.* To evaluate whether genetic differentiation was driven by distance or environmental factors, we conducted a [Mantel \(1967\)](#) test using Euclidean distance,  $F_{ST}$ , and  $G'_{ST}$  in the *PopGenReport* and *dartRverse* packages ([Adamack & Gruber, 2014](#); [Mijangos et al., 2022](#)). A Mantel test also was used to assess isolation-by-distance among our Colombia samples, and the relationship between the elevation of sample sites and genetic distance. Statistical significance was assessed using 10,000 permutations, with significance thresholds set at  $p < 0.05$ .

Additionally, sex-specific spatial autocorrelation analyses was conducted to test for fine-scale structure and sex-biased dispersal using  $F_{ST}$  of females *vs* males, and by

comparing genetic distance and geographic distance of each sex ([Banks & Peakall, 2012](#)). Autocorrelation values were calculated using Euclidean distance matrices of genetic and geographic distances and visualized using the *dartR* package ([Mijangos et al., 2022](#)). We then estimated 95% bootstrap confidence intervals (100 bootstrap replications) around the *r* values for each sex at different distance-classes based on our sample sites. Assessment of dispersal by sex for each independent population was not possible due to limited power for Population Three, where only five females were captured. As a result, sex-biased dispersal was conducted using all pooled samples from Colombia.

*Inference of migration.* To distinguish between historical migration and contemporary movement, we estimated equilibrium migration rates (*m*) using the  $F_{ST}$  formula ([Wright, 1951](#); [Zhivotovsky, 2015](#)). Values were interpreted based on conventional thresholds (e.g.,  $Nm > 1$  indicates historic migration between populations). Also, we identified putative recent first-generation migrants using the Bayesian criterion in GeneClass2.0 ([Piry et al., 2004](#)) with 10,000 simulated individuals and a significance threshold of  $p < 0.01$ . Identifying individuals likely to have moved among populations ([Piry et al., 2004](#)). This dual approach allowed us to infer both long-term and recent connectivity among *D. rotundus* populations in Colombia.

*Linking genetics and environment.* Finally, we tested for associations between genetic structure and environmental characteristics, both independently and simultaneously, using multiple *t*-tests and a multivariate analysis of variance (MANOVA) test. For the purpose of this comparison, the comparator group from Mexico was not considered. This allowed us to assess whether environmental variables differed between populations within Colombia without confounding the comparison with data from Mexico. Populations were compared for differences in elevation (m), isothermality (average diurnal temperature range in °C divided by annual temperature range in °C), minimum temperature of the coldest month (°C), annual average precipitation (mm), precipitation seasonality (coefficient of variance), cattle density (individuals per km<sup>2</sup>), human population density, and annual average enhanced vegetation index (EVI) (factors with potential ecological relevance to RABV exposure and transmission risk). We confirmed that the selected environmental characteristics varied across the elevational gradient used for sample site selection using a multiple linear regression model. A *p*-value  $< 0.05$  was considered statistically significant. We utilized the *rstatix* package (version 0.7.2) and base R to complete these analyses in R statistical software version 4.1.1 ([R Core Team, 2021](#); [Kassambra, 2023](#)).

## RESULTS

### Sample demographics and genetic characteristics

Genetic variation within a total of 92 *D. rotundus* individual samples was assessed, 86 from Colombia and six from Mexico. Of the samples collected in Colombia, 79 had known sex, comprising 22 females and 57 males. Forty-two of the sampled individuals were adults, two were sub-adults, six were juveniles, and the remaining 11 were of undetermined age. No individuals sampled were visibly diseased, but pathogen testing on these individuals has yet



to be completed. All microsatellite loci proved polymorphic, with numbers of alleles per locus ranging from three to 16. Private alleles, (*i.e.*, alleles found only in specific populations or specific sampling sites) (Petit, Mousadik & Pons, 1998; Szpiech & Rosenberg, 2011), were observed in all populations (Table 1). Evidence of divergence of genotype frequencies from HWE expectations was present for sampling sites Agua de Dios (Cundinamarca), Puente Quetame (Cundinamarca), and Pipiral (Meta) (Fig. 1, Table 1, Table S1) ( $p = 0.001$ , 0.03, and 0.01 respectively). All microsatellite loci had null allele frequencies below 0.35, except for locus *Dero\_H02F\_C03R* (Fig. S1), data for which was excluded from subsequent analysis. While usual criterion for null allele exclusion is 0.2, this stringent of a threshold would have eliminated over half the loci included in this analysis (Fig. S1). We chose to utilize 0.35 as our threshold in order to retain an optimum number of loci, and based on the understanding that microsatellite loci affected by null alleles are not thought to affect the overall outcome of assignment testing (Carlsson, 2008).

### Populations cluster analysis

Analysis of Bayesian Information Criterion (*BIC*) for increasing numbers  $K$  of discriminate analysis of principal components (DAPC) clusters indicated greatest support for three clusters within the dataset (Fig. S2). This inference was supported by the results of STRUCTURE analysis, with delta  $K$  most strongly supporting presence of three clusters among the sampling sites (Fig. S3). The clusters were partitioned geographically (Fig. 2). Population One encompassed the Mexico samples provided by A. Piaggio (United States Department of Agriculture) used as a comparator. Two populations were distributed across Colombia, with most of Population Two occurring in high-elevation portions of Colombia, east of the central Andes Mountain passage (Figs. 2 & 3) (807.5 m average elevation of capture). Population Three, in contrast, was distributed across central and western portions of the study area, slightly lower in elevation (Fig. 2) (575.2 m average elevation of capture). One sampling site (Agua de Dios) with the largest sampling effort (40 individuals) was split between populations two and three. We used the three clusters identified from the DAPC analysis as hierarchical strata (*i.e.*, populations), while the respective sampling sites were considered subpopulations to calculate descriptive genotypic metrics (*e.g.*, allelic variation, heterozygosity, and genetic distances) across both sampling sites and inferred populations. Observed heterozygosity ( $H_o$ ) was less than expected heterozygosity ( $H_e$ ) for all sampling sites and populations (Table 1).

### Genetic variation and migration between and among populations

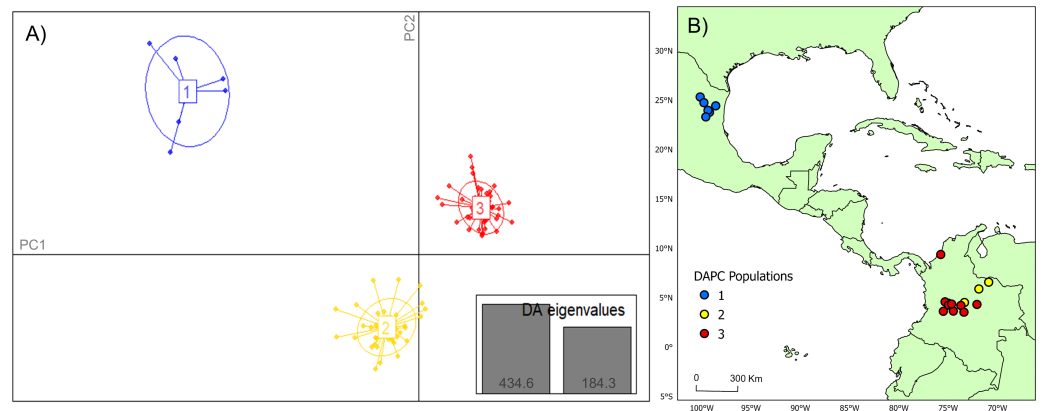
Results of AMOVA analysis showed significant genetic variance (27.7%,  $p = 0.01$ ) within the respective sampling sites (Table 2). We also identified significant variance between sampling sites within populations (5%,  $p = 0.01$ ), and between populations in Colombia (14.9%,  $p = 0.01$ ) (Table 2). Populations Two and Three from Colombia had similar mean numbers of alleles per locus (7.4 and 7.1, respectively) and allelic richness (4.9 and 4.5) (Table 1). The inbreeding coefficient  $F_{IS}$  for all populations was between 0.31 and 0.33, indicating an excess of homozygotes above Hardy-Weinberg equilibrium expectations (Campagne *et al.*, 2012), suggesting inbreeding or family structure. Estimated effective

**Table 1** Descriptive metrics of populations and sampling sites. Populations (Pops) represent clusters identified by DAPC analysis. Sampling sites represent locations of collection.  $N$  indicates the number of individuals collected at each site. We utilized multiple R packages to quantify the mean number of alleles per locus, private alleles per locus (alleles only found in specific populations or subpopulations), allelic richness, observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, significance of divergence from Hardy-Weinberg equilibrium (HWE), and effective population size ( $N_e$ ) of each cluster identified by DAPC (right column under each metric) and for each sampling site (left column under each metric).  $N_e$  was estimated using the Waples correction (Waples & Do, 2010; Waples, Larson & Waples, 2016) to mitigate downward bias. Inf indicates that  $N_e$  was estimated as infinite, likely owing to small sample size and related lack of heterozygotes. There was evidence of divergence from HWE for sampling sites Agua de Dios, Pipiral, and Puente Quetame.

Pops	Sampling sites (Department)	$N$	Elevation (m)	Mean alleles per locus	Private alleles per locus	Allele richness	$H_o$	$H_e$	Departure from HWE	$N_e$
1	Tamaulipas	4	623.0	2.91	10	1.56	0.55	0.56	0.55	0.3
	Nuevo Leon	2	1200.0	2.36	7	1.68	0.55	0.81	0.36	Inf
2	Agua de Dios (Cundinamarca)	18	407.8	6.45	4	1.65	0.40	0.66	<b>0.001</b>	20.8
	Los Araguatos (Arauca)	1	127.0	1.09	1	NA	0.20	NA	0.86	Inf
	Yopal (Casanare)	1	151.0	1.27	0	1.27	0.27	NA	0.81	In.
	Chaparral Sites 1 & 2 (Tolima)	9	615.0 & 654.0	4.45	1	1.64	0.47	0.65	0.24	Inf
	Coello (Tolima)	7	439.2	3.09	0	1.67	0.48	0.72	0.49	Inf
	Medina (Cundinamarca)	1	760.0	1.36	0	1.36	0.36	NA	0.75	Inf
	Pipiral (Meta)	5	965.3	4.18	5	1.73	0.35	0.79	<b>0.01</b>	Inf
	Agua de Dios (Cundinamarca)	22	407.8	6.50	4	1.65	0.40	0.66	<b>0.01</b>	20.8
	El Porvenir (Córdoba)	1	36.2	1.36	0	1.36	0.36	NA	0.75	Inf
3	Ibagué (Tolima)	5	2198.9	3.55	0	1.64	0.35	0.68	0.36	Inf
	Piedras (Tolima)	6	619.1	3.45	1	1.55	0.33	0.58	0.29	0.8
	Puente Quetame (Cundinamarca)	7	1890.0	3.82	2	1.63	0.39	0.65	<b>0.03</b>	42.6
	Puerto Gaitán (Meta)	1	207.0	1.09	1	NA	0.20	NA	0.86	Inf
	San Martín (Meta)	2	297.3	2.36	0	1.67	0.41	0.78	0.42	Inf
				7.09	11	4.49	0.4	0.64	0.23	41.6

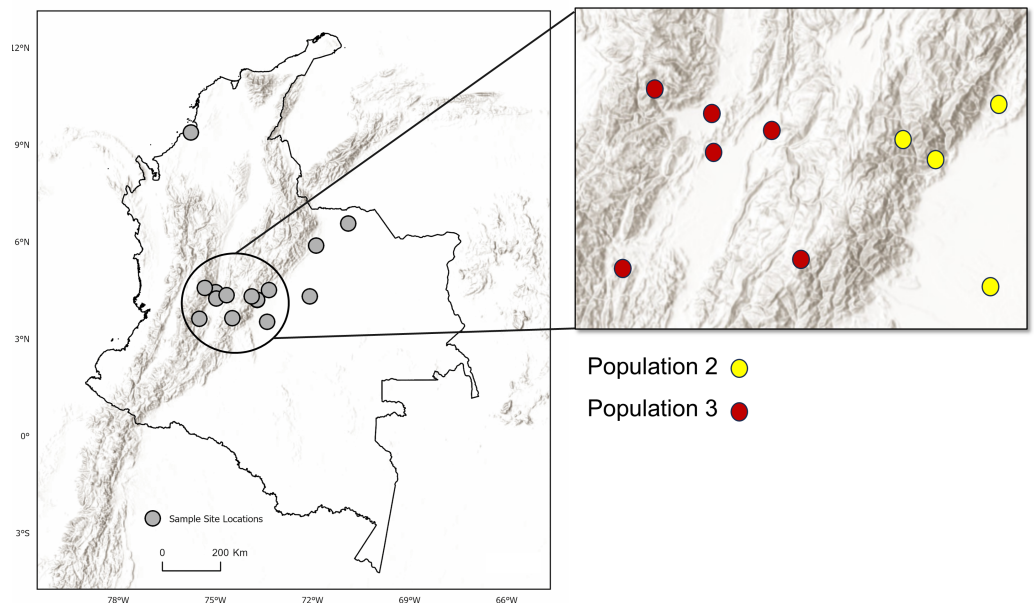
**Notes.**  
Bolded values indicate significance.

population sizes for the populations in Colombia were 16.9 for Population Two and 41.6 for Population Three, but were unbounded (Table 1). High genetic distances ( $F_{ST} \sim 0.30$ ,  $G'_{ST} \sim 0.80$ ) were observed between Population One in Mexico and Populations Two and Three in Colombia (Table 3), as was expected since Population One was at a greater distance from the others, had a small sample size, and was utilized as a comparator. Genetic distances between Populations Two and Three in Colombia were comparatively low ( $F_{ST} = 0.12$ ,  $G'_{ST} = 0.34$ ). The estimated equilibrium rate of migration across an ecological time-scale (*i.e.*, genetically effective migration) between Populations Two and Three was 1.83 individuals per generation. Estimates of genetically effective migration between sites indicated that there was historic migration ( $>1$  individuals per generation) between sites that were closer together ( $<120$  km) and located on either side of the Río Magdalena valley, Llanos Orientales, and Orinoquia of the central Andean corridor (*i.e.*, Ibaguè and Chaparral,



**Figure 2** DAPC cluster distribution of genotype data by collection. (A) Visual representation of each population cluster identified by our discriminant analysis of principal components (DAPC). The axes represent the first two principal components with two discriminant analysis eigenvalues (DA eigenvalues, inset) summarizing 75.0% of the data. (B) Geographic distribution of DAPC populations. The colors of the respective DAPC clusters and geographic locations are the same. The first population consists of only six individuals, which was utilized as a comparator for this study. Maps created using ArcGIS Pro software version 2.5 with shape files from DIVA-GIS (Hijmans, 2017; Esri, Redlands, CA, USA, 2019).

Full-size [DOI: 10.7717/peerj.20306/fig-2](https://doi.org/10.7717/peerj.20306/fig-2)



**Figure 3** Topographic relief and population distribution. Location of sampling sites across the Andes Mountain range. Inset map shows the central Andean corridor, where higher rates of migration between sampling sites were detected. Partition of DAPC populations Two and Three (inset map on right) are shown as well. Observe the topographic pattern in the partitioning of populations from Population Three to Population Two. Maps created using ArcGIS Pro software version 2.5 with shape files from DIVA-GIS (Hijmans, 2017; Esri, Redlands, CA, USA, 2019).

Full-size [DOI: 10.7717/peerj.20306/fig-3](https://doi.org/10.7717/peerj.20306/fig-3)

**Table 2** Analysis of molecular variance (AMOVA) between and among collection of *D. rotundus* populations and sampling sites analyzed. Variance and significance values within and between both Colombia populations delineated by DAPC and subpopulation (i.e., sample site locations). Significant variance was present across all strata tested (i.e.,  $p$  values  $< 0.05$ ). Most covariance originated from within the samples.

Source	Degrees of freedom	Sum of squares	Mean square	Sigma	% Covariance	$\phi$	$p$ value
Between populations	2	164.93	82.47	1.28	14.89	0.15	0.01
Between sample sites within populations	20	239.34	11.97	0.43	5.01	0.06	0.01
Between individuals within each sample site	69	640.05	9.28	2.38	27.70	0.35	0.01
Within individuals	92	414.81	4.51	4.51	52.40	0.48	0.01
<b>Total</b>	183	1,459.14	7.97	8.61	100	–	–

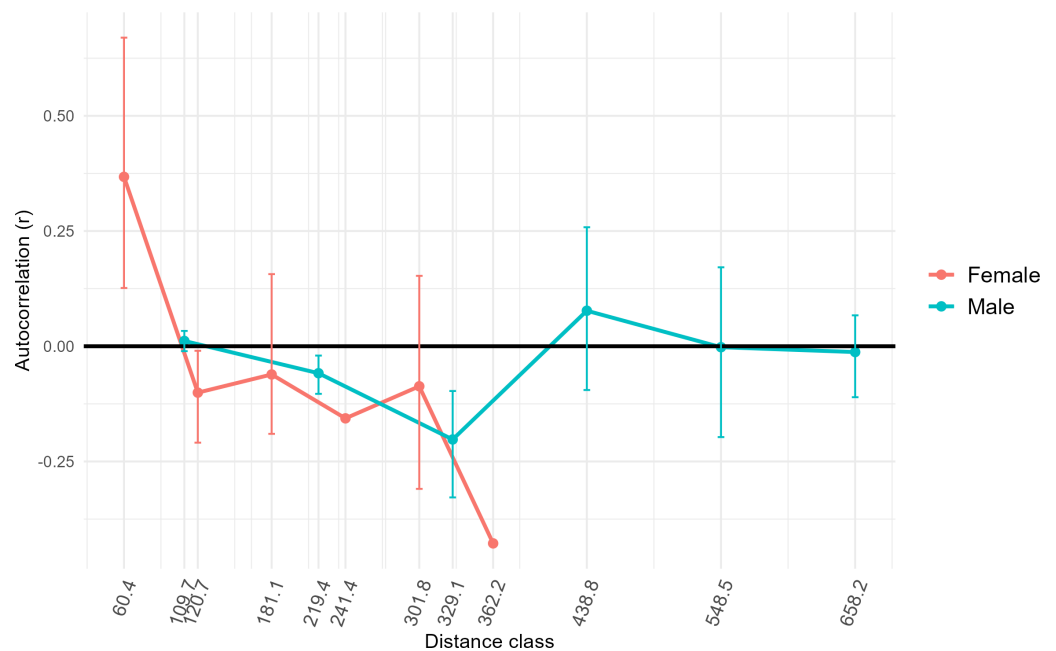
**Table 3** Metrics of genetic distance between populations.  $F_{ST}$  (below diagonal) and  $G'_{ST}$  (above diagonal) metrics of genetic distance between populations identified by DAPC. Pairwise  $F_{ST}$  (Yang, 1998; Hedrick, 2005) was assessed using dartRverse. Greater genetic distance is signified by large numbers. The largest genetic distance was present between populations one and three. Less genetic distance was present between Colombian populations two and three, indicating greater gene flow between the two populations.

	Population 1	Population 2	Population 3
Population 1	–	0.78	0.83
Population 2	0.28	–	0.34
Population 3	0.31	0.12	–

Tolima, Yopal, Casanare, Agua de Dios, Cundinamarca, and San Martín, Meta) (Table S2). GeneClass2 direct migrant assessment revealed 15 likely first-generation migrants. Ten of these likely migrants were captured in Population Three (Table S3) and the remaining five in Population Two. Migrants were captured at sites Agua de Dios and Puente Quetame in Cundinamarca, Coello and Piedras in Tolima, Yopal in Casanare, and at San Martín and Pipiral in Meta. Identifying first-generation migrants between local collection sites was not possible due to limited sample size from some such sites.  $F_{ST}$  across all females sampled for this study was higher (0.24) than for all males sampled (0.14). Only females at the lowest distance class (60.4) calculated for our samples demonstrated significant positive spatial autocorrelation ( $r = 0.31$ ,  $p = 0.001$ ) (Fig. 4). Males had positive spatial autocorrelation at larger distance classes (438.8,  $r = 0.06$ ), but this was not significant per our 95% confidence interval ( $p = 0.08$ ). Females had stronger fine-scale genetic structure than males at short geographic distances. Assessment of differential dispersal by sex for each independent population was not possible due to limited power for Population Three, where only five females were captured.

### Environmental differences between and among populations

We confirmed that the environmental variables we chose to assess at the locations of capture for our samples varied with the elevational gradient used for sample site selection *via* a multiple linear regression model ( $F = 2267$ ,  $p < 0.001$ ). Multiple  $t$ -tests of environmental characteristics at the respective sampling locations showed no significant differences



**Figure 4** Genetic structure across distance by sex. To assess whether fine-scale genetic structure was evident based on sex, we conducted a one-tailed permutation test to identify autocorrelation coefficients ( $r$ ). We then estimated 95% bootstrap confidence intervals (whiskers around points) around the value for  $r$  for each sex at different distances classes based on our samples. X axis distance classes represent geographic distance classes (Euclidean distance rather than simple distance) calculated across standardized across our samples. In this case, if the bootstrap confidence intervals do not overlap zero, then fine-scale genetic structure is present. Only female individuals at the lowest distance class showed significant positive spatial autocorrelation ( $r = 0.37$ ,  $p = 0.001$ ).

Full-size [DOI: 10.7717/peerj.20306/fig-4](https://doi.org/10.7717/peerj.20306/fig-4)

between the environments of each population in Colombia (all  $p > 0.05$ ). The MANOVA of environmental variables did not elucidate any significant difference between populations (Pillai's Trace = 0.08,  $F(9,70) = 0.65$ ,  $p = 0.75$ ). Population Three occurred at an overall lower average elevation (mean elevation 575.2 m) than Population Two (mean of 807.5 m), a difference which was not significant statistically ( $t = 1.91$ ,  $df = 71.72$ ,  $p = 0.06$ ). We identified a positive but non-significant relationship between genetic distance ( $F_{ST}$ ,  $G'_{ST}$ , or Euclidean distance) and geographic distance (km) (Mantel statistic = 0.05,  $p = 0.27$ , Mantel statistic = 0.07,  $p = 0.18$ , and Mantel statistic = 0.05,  $p = 0.23$ , respectively). No significant relationship was present between the elevation of sampling sites and genetic distance (Mantel statistic = 0.05,  $p = 0.15$ ).

## DISCUSSION

This study's identification of two genetically distinct but interconnected *D. rotundus* populations in Colombia provides a significant insight into the potential mechanisms of RABV spread. The relatively low genetic differentiation between these populations based on both the  $F_{ST}$  (0.12) and  $G'_{ST}$  (0.34) metrics (Table 3), despite considerable geographic distances (21.6–696.7 km) supports the inference of both long-term migration and recent



dispersal, potentially along low-elevation corridors such as the Rio Magdalena valley. The presence of first-generation migrants, especially individuals originating in the east but captured in the west, implies that current movement patterns may mirror historical migration, sustaining connectivity across the region. The presence of two populations at one sampling location (Agua de Dios), which rests at the center of the Central Andean corridor, supports this interpretation.

Given that *D. rotundus* dispersal is known to be limited to relatively short range (10–20 km) (Trajano, 1996; Streicker et al., 2012b; Rocha & Dias, 2020), and short-range dispersal has been related to slow wavefronts of outbreaks of *D. rotundus*- associated RABV (Benavides, Valderrama & Streicker, 2016), the observed genetic connectivity suggests that even moderate dispersal rates may enable RABV to cross regional ecological and geographical barriers. This dispersal is particularly concerning in areas where livestock densities are high, as infected bats could transmit RABV across populations despite physical separation by elevation or landscape. Overall, the consistency of this dispersal with previous findings and the importance of low-resistance corridors strengthen the ecological inference that migration and, potentially, viral flow in *D. rotundus* is governed more by geographic permeability than strict distance or habitat type (Pinto, 2009; Das et al., 2019; Seetahal et al., 2024).

Allelic richness, a metric of genetic diversity (Foulley & Ollivier, 2006), also showed similar genetic diversity within the two Colombian populations (4.91 and 4.49) (Table 1). The small number of populations identified, coupled with the limited genetic distance between these two populations indicates that *D. rotundus* colonies in Colombia are interconnected by migration (Martins et al., 2009; Streicker et al., 2012a; Streicker et al., 2016). Other research on genetic structure of *D. rotundus* in French Guiana using microsatellites and mitochondrial DNA found a greater degree of historic migration at a similar geographic scale to that in our study (i.e., regional) when compared to larger-scale comparisons between North and South America (Huguin et al., 2018). The spatial scale and geographic locations of previous studies may account for any differences between our results and those for studies done in different locations.

Our estimate of the equilibrium rate of migration ( $m = 1.83$ ) showed long-term connection between Colombian populations, and our direct migrant identification assessment identified many first-generation migrants within the sampled generation ( $n = 15$  of 86 individuals assessed). Most first-generation migrants ( $n = 10$ ) originated in the eastern population in Colombia (Population Two), but were captured in the western population (Population Three) (Fig. 4). All migrants were captured at lower elevations (<1,000 m). The demonstration of interconnected bat populations across wide regions despite presumed physical barriers (e.g., the Andes Mountains) implies that regional rabies control cannot be confined to localized hotspots. Surveillance systems should be designed to track RABV not just where outbreaks occur, but in regions of potential bat movement, especially along valleys and lowland corridors.

No significant differences in environment or climate of occupancy were apparent between the two inferred populations, nor were there differences in anthropogenic factors such as agricultural activity (i.e., cattle density) or human population density ( $p > 0.05$ ).

Greater genetically effective migration rates were inferred between sites surrounding the Río Magdalena valley of the central Andean corridor, with sites to the east (Population 2) having the most apparent migration (Fig. 4, Table S2). The two inferred populations were geographically separated by the Andes Mountains to the east of the valley (Fig. 4). Other studies of *D. rotundus*-associated RABV have found that this bat may disperse through low-resistance areas such as valleys (Benavides, Valderrama & Streicker, 2016; Streicker & Allgeier, 2016), a finding convergent with our results. As such, our results support the potential role of elevation or topography in shaping *D. rotundus* dispersal, and hence we suggest that livestock vaccination campaigns prioritize areas adjacent to high-migration zones, such as the Río Magdalena valley.

Previous research using mitochondrial DNA also has found low genetic divergence among *D. rotundus* populations over large distances in some regions (*i.e.*, Central America and the Pantanal region of Bolivia), while other regions in the same study had large genetic divergence over short distances (*i.e.*, Atlantic forests in southern Brazil) (Martins *et al.*, 2009). The authors hypothesized that greater genetic divergence may be driven by differences in ecoregion, rather than by distance per se for this species due to its volant nature. These results are consistent with those of our study, as distance among sites did not relate significantly to genetic differentiation. Nevertheless, other studies using the same microsatellite loci as this study have found relatively low genetic differentiation across different ecoregions for *D. rotundus* at the leading edge of the species' broader geographic range in Mexico (Piaggio *et al.*, 2017). In contrast, another study in Mexico using methods similar to those of our study found a higher degree of genetic diversity across a larger sample size and geographic area (Romero-Nava, León-Paniagua & Ortega, 2014). The lack of consensus among previous studies, and the lack of significant geographic or landscape patterns between populations in this study, reflects variation of geographic population genetic patterning in *D. rotundus* across its range. Results from this study may support previous hypotheses of male-biased dispersal for *D. rotundus* (Martins *et al.*, 2009; Streicker & Allgeier, 2016). Females showed positive spatial autocorrelation as lower distance-classes than males, and positive spatial autocorrelation for males was not significant even at greater distances (Fig. 4). Our finding of related males potentially being collected at locations much farther apart than females echoes results from previous studies (Streicker & Allgeier, 2016). Nevertheless, previous studies have assessed inbreeding coefficients ( $F_{IS}$ ) by sex and by population to further assess male-biased dispersal (Streicker & Allgeier, 2016). Assessment of  $F_{IS}$  by sex and by population was not possible here, however, due to the limited number of females—just five—collected in Population Three. Dispersal of infected *D. rotundus* individuals could facilitate RABV transmission between subpopulations (Streicker *et al.*, 2012b; Bakker *et al.*, 2019; Becker *et al.*, 2021; Horta *et al.*, 2022).

A limitation of this study is the lack of RABV prevalence data for the individuals collected. Hence, we are unable to directly assess the effect of genetic structure of *D. rotundus* in Colombia upon RABV spillover. RABV spillover occurrence data are available from Colombia, and are collected *via* the Epidemiological Information and Surveillance System of ICA (Instituto Colombiano Agropecuario (ICA), 2019). This organization performs surveillance for RABV outbreaks in livestock and reports suspected cases from local-level

stakeholders (Rojas-Sereno et al., 2022; Instituto Colombiano Agropecuario (ICA), 2023). Recent reports have identified a persistent concentration of cases in the Northern Caribbean region of the country, mostly in cattle, and an expansion of RABV cases into previously naive areas (Rojas-Sereno et al., 2022). Between 1982 and 2023, Instituto Colombiano Agropecuario (ICA) (2024) recorded 3878 rabies outbreaks in animals (89% in cattle), distributed across 31 of Colombia's 32 departments. Cases peaked in 2014 (542 cases), but have declined to as few 43 cases reported in 2019 (Bonilla-Aldana et al., 2022). The Caribbean region remains at high risk, however, with constant impact in the departments Cesar, Magdalena, Bolívar, and Sucre (Bonilla-Aldana et al., 2022). In the Orinoquia region (east of the Andes Mountains), 13 spatial clusters were identified in Vichada, in addition to cases in Casanare and Meta (Bonilla-Aldana et al., 2022). During the 2005–2019 period, the departments with the most reported cases of RABV were Cesar (18.6%), Magdalena (11.1%), Arauca (9.3%), Casanare (8.5%), and Sucre (6.6%) (Bonilla-Aldana et al., 2022). In 2020, most bovine rabies outbreaks were concentrated in Sucre, indicating focalized viral circulation that requires continuous epidemiological monitoring (Instituto Nacional de Salud, INS). Our findings of broader interconnection between *D. rotundus* populations in Colombia across many of these regions could help explain how RABV is spread across the country, particularly from eastern locations with higher cattle density to other, less agriculturally dense areas (Rojas-Sereno et al., 2022). To build upon the assessment conducted in this study, future assessments with a broader sampling base, potentially in the Caribbean region where cases are concentrated, could connect *D. rotundus* populations genetics information with RABV spillover data and coupled antibody tests for field samples.

Our sample size was smaller than those of previous assessments (Romero-Nava, León-Paniagua & Ortega, 2014; Piaggio et al., 2017), though our samples were collected over a narrower time-range. Assessment of a greater sample size across a broader geographic area or more diverse environments may further elucidate the impacts of environmental variation upon population genetic patterning of *D. rotundus* in Colombia. Utilizing only amplified fragment-size analysis for scoring microsatellite markers may have limited our observation of genetic variation as well. Other methods, such as microsatellite genotyping by sequencing (SSR-GBS) or whole-genome sequencing for single nucleotide polymorphisms (SNPs), could have shown more detailed patterns of genetic structure (Tibihika et al., 2019). Future research in this system should work to increase the geographic scope of sampling to capture more ecoregions or fine-scale landscape factors that may shape genetic differentiation for *D. rotundus* populations. Future research should also couple genetic assessments with RABV seroprevalence assessment to more directly test hypotheses of RABV transmission driven by dispersal of infected *D. rotundus* individuals.

## CONCLUSIONS

This study aimed to establish a baseline assessment of population genetic structure of *D. rotundus* individuals across an elevational gradient in Colombia, with the broader goal of evaluating how genetic connectivity may influence RABV transmission dynamics. Screening 11 microsatellite loci developed by Piaggio, Johnson & Perkins (2008) and applying standard

population genetic analyses, we quantified genetic variation and assessed the geographic distribution of genetic clusters. Comparative analysis with a reference population from Mexico allowed us to contextualize our findings within a broader regional framework. Our results revealed substantial genetic connectivity among Colombian *D. rotundus* populations, even across geographically complex terrains. This finding supports the hypothesis that interpopulation bat movement contributes to the dissemination of RABV. While environmental and anthropogenic barriers showed limited impact, natural geographical features such as valleys appeared to provide important movement corridors. These results indicate that it is difficult to predict vampire bats' gene flow and dispersal, especially within the context of predicting RABV transmission between populations, as has been noted in previous research ([Streicker et al., 2012b](#); [Blackwood et al., 2013](#)).

Our results contribute to current knowledge of the regional distribution of genetic variation for *D. rotundus*. Our results demonstrate that *D. rotundus* populations in Colombia exhibit significant genetic connectivity, even across complex landscapes, supporting the hypothesis that interpopulation bat movement can facilitate RABV spread. These findings advance our understanding of the spatial dynamics of vampire bat populations and underscore the relevance of incorporating ecological and genetic data into public health planning. Effective rabies control strategies should consider the role of landscape connectivity in shaping bat movement and virus spread. A more detailed assessment of the role of elevation is warranted to fill remaining knowledge gaps; specifically, future studies should incorporate denser sampling along elevational gradients, finer-scale genotyping (*e.g.*, SNP-based approaches), and integration of environmental niche modeling to better understand how elevation and landscape features influence population structure and viral transmission risk. While environmental and anthropogenic differences between sites occupied by the respective populations were minimal, geographical corridors such as valleys play a key role in movement. Such integrative approaches can support more ecologically informed public health policies and practices, including targeted vaccination, strategic land-use management, and interdisciplinary surveillance.

## ACKNOWLEDGEMENTS

The authors thank our collaborators from Colombia, including Natalia Cediél, Gelys Igrèth Mestre Carrillo, Fernando Sarmiento Parra, Catalina Cárdenas González, Catherine Mora, Hugo Fernando López Arévalo, Andrew Jackson Crawford, Sebastián García Restrepo, Sandra Patricia Galeano Muñoz, Andrés Julián Lozano Flórez, Elizabeth Aya Baquero, Francisco Alejandro Sánchez Barrera, Giovany Guevara Cardona, Gladys Reinoso Flórez, Leidy Azucena Ramírez Francel, Leidy Viviana García Herrera, Hector Ramírez Chaves, Andrea Bustamante Cadavid, Victor Hugo Serrano Cardozo, Alexander Velásquez Valencia, Oscar Marín Ducuara, and Alejandro Rocha. Special thanks to the students and technicians who collaborated in the data collection, including Mariana Castaneda Guzman, Quan Dong, Shariful Islam, Julia Alexander, Laura Valentina Ávila Vargas, Nicolas David Sanabria Rivera, Karen Sarmiento, Juan Camilo Quintero Navarro, Carlos Dubon Hinojosa, Kaitlyn Enstice, Carlos Bravo, and Alirio Rey (Don Ali). They also acknowledge Antoinette Piaggio

and Annie Tibbels for the provision of bat samples from Mexico for the completion of this study.

## ADDITIONAL INFORMATION AND DECLARATIONS

### Funding

This study was supported by the National Science Foundation under CAREER (2235295) and HEGS (2116748) awards, by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number K01AI168452, and by seed grants from Virginia Tech's Institute for Critical Technology and Applied Science, Pandemic Prediction and Prevention Destination Area, and the Center for Emerging, Zoonotic, and Arthropod-borne Pathogens. Luis Escobar was supported by the Chinese Academy of Sciences PIFI project 2024PVC0085. The participation of Eric Hallerman was supported through the Virginia Agricultural Experiment Station with support from the U.S. Department of Agriculture National Institute for Food and Agriculture. There was no additional external funding received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors:

National Science Foundation under CAREER (2235295) and HEGS (2116748) awards.

National Institute of Allergy and Infectious Diseases of the National Institutes of Health: K01AI168452.

Virginia Tech's Institute for Critical Technology and Applied Science, Pandemic Prediction and Prevention Destination Area, and the Center for Emerging, Zoonotic, and Arthropod-borne Pathogens.

Chinese Academy of Sciences PIFI project: 2024PVC0085.

Virginia Agricultural Experiment Station with support from the U.S. Department of Agriculture National Institute for Food and Agriculture.

### Competing Interests

The authors declare there are no competing interests.

### Author Contributions

- Paige Van de Vuurst conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Analorena Cifuentes-Rincon conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Andrea S. Bertke conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Diego Soler-Tovar conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.



- Nicolás Reyes-Amaya performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Fabiola Rodriguez Arévalo performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Julieth Stella Cárdenas Hincapié performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Jhon Rivera-Monroy performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Luis E. Escobar conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Eric Hallerman conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

### Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Capture and handling protocols followed recognized bat handling guidelines of the American Society of Mammalogists Sikes (2016), as approved by the Institutional Animal Care and Use Committee at Virginia Tech (IACUC approval #21-138). Approval was also obtained from the Institutional Care and Use Committee of the Universidad de La Salle (IACUC approval #087).

### Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Capture of bats and data collection in Colombia were carried out under environmental permit Resolución 1473 de 2014 of the Autoridad Nacional de Licencias Ambientales (ANLA) (i.e., UniSalle Wild Species Collection Framework Permit).

### Data Availability

The following information was supplied regarding data availability:

The data is available in the [Supplemental Files](#).

The raw data and code are available at Figshare:

- [https://figshare.com/projects/Raw\\_Data\\_and\\_Code\\_for\\_Assessing\\_the\\_effects\\_of\\_environmental\\_variation\\_on\\_population\\_genetic\\_structure\\_of\\_the\\_common\\_vampire\\_bat\\_in\\_Colombia\\_/248969](https://figshare.com/projects/Raw_Data_and_Code_for_Assessing_the_effects_of_environmental_variation_on_population_genetic_structure_of_the_common_vampire_bat_in_Colombia_/248969). - Van de Vuurst, Paige (2025). Sample data and meta data for analyses. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078804.v1>

The data for locus: Dero\_B10F\_E01R. is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_B10F\_E01R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078033.v1>

The data for locus: Dero\_B03F\_B03R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_B03F\_B03R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29077775.v1>

The data for locus: Dero\_B11F\_B11R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_B11F\_B11R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078144.v1>

The data for locus: Dero\_C12F\_B02R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_C12F\_B02R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078294.v1>

The data for locus: Dero\_D06F\_D06R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_D06F\_D06R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078336.v1>

The data for locus: Dero\_C07F\_A02R F is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_C07F\_A02R F. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078345.v1>

The data for locus: Dero\_D12F\_D12R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_D12F\_D12R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078441.v1>

The data for locus: Dero\_G10F\_B03R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_G10F\_B03R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078492.v1>

The data for locus: Dero\_H02F\_C03R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_H02F\_C03R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078504.v1>

The data for locus: Dero\_A08F\_B01R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_A08F\_B01R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078651.v1>

The data for locus: Dero\_C11F\_C11R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_C11F\_C11R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078699.v1>

The data for locus: Dero\_D02F\_D02R is available at Figshare: Van de Vuurst, Paige (2025). Raw data for locus: Dero\_D02F\_D02R. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078738.v1>

The data for Environmental Values at location of capture is available at Figshare: Van de Vuurst, Paige (2025). Environmental Values at location of capture for *D. rotundus*] in Colombia. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29583239.v1>

The sample data and meta data for analyses is available at Figshare: Van de Vuurst, Paige (2025). Sample data and meta data for analyses. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.29078804.v1>

The code for Population Genetics Assessments is available at Figshare: Van de Vuurst, Paige (2025). Code for Population Genetics Assessments. figshare. Online resource. <https://doi.org/10.6084/m9.figshare.29077340.v1>.

## Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.20306#supplemental-information>.

## REFERENCES

- Adamack AT, Gruber B. 2014. PopGenReport: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution* 5:384–387 DOI 10.1111/2041-210X.12158.
- Álvarez LMM, Buitrago JDR, Sáenz JR. 2014. Analysis of a program for the prevention and control of wild rabies and its role in the number of cattle outbreaks during years 2001 to 2011. *Revista CES Medicina Veterinaria y Zootecnia* 9:203–217.
- Anderson A, Shwiff S, Gebhardt K, Ramírez AJ, Shwiff S, Kohler D, Lecuona L. 2014. Economic evaluation of vampire bat (*Desmodus rotundus*) rabies prevention in Mexico. *Transboundary and Emerging Diseases* 61:140–146 DOI 10.1111/tbed.12007.
- Bakker KM, Rocke TE, Osorio JE, Abbott RC, Tello C, Carrera JE, Valderrama W, Shiva C, Falcon N, Streicker DG. 2019. Fluorescent biomarkers demonstrate prospects for spreadable vaccines to control disease transmission in wild bats. *Nature Ecology and Evolution* 3:1697–1704 DOI 10.1038/s41559-019-1032-x.
- Banks SC, Peakall R. 2012. Genetic spatial autocorrelation can readily detect sex-biased dispersal. *Molecular Ecology* 21:2092–2105 DOI 10.1111/j.1365-294X.2012.05485.x.
- Barquez R, Perez S, Miller B, Diaz M. 2015. *Desmodus rotundus*. Available at <https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T6510A21979045.en> (accessed on 25 January 2021).
- Becker DJ, Nachtmann C, Argibay HD, Botto G, Escalera-Zamudio M, Carrera JE, Tello C, Winiarski E, Greenwood AD, Méndez-Ojeda ML, Loza-Rubio E, Lavergne A, De Thoisy B, Czirják G, Plowright RK, Altizer S, Streicker DG. 2019. Leukocyte profiles reflect geographic range limits in a widespread Neotropical bat. *Integrative and Comparative Biology* 59:1176–1189 DOI 10.1093/icb/icz007.
- Becker DJ, Simmons NB, Broos A, Bergner LM, Meza DK, Fenton MB, Altizer S, Streicker DG. 2021. Temporal patterns of vampire bat rabies and host connectivity in Belize. *Transboundary and Emerging Disease* 68:870–879 DOI 10.1111/tbed.13754.
- Benavides JA, Valderrama W, Streicker DG. 2016. Spatial expansions and travelling waves of rabies in vampire bats. *Proceedings of the Royal Society B: Biological Sciences* 283:20160328 DOI 10.1098/rspb.2016.0328.
- Biek R, Real LA. 2010. The landscape genetics of infectious disease emergence and spread. *Molecular Ecology* 19:3515–3531 DOI 10.1111/j.1365-294X.2010.04679.x.
- Blackwood JC, Streicker DG, Altizer S, Rohani P. 2013. Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. *Proceedings of the National Academy of Sciences of the United States of America* 110:20837–20842 DOI 10.1073/PNAS.1308817110.
- Bonilla-Aldana DK, Jimenez-Diaz SD, Barboza JJ, Rodriguez-Morales AJ. 2022. Mapping the spatiotemporal distribution of bovine rabies in Colombia, 2005–2019. *Tropical Medicine and Infectious Disease* 7(12):406 DOI 10.3390/TROPICALMED7120406.
- Brookfield JFY. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* 5:453–455.
- Campagne P, Smouse PE, Varouchas G, Silvain JF, Leru B. 2012. Comparing the van Oosterhout and Chybicki-Burczyk methods of estimating null allele

- frequencies for inbred populations. *Molecular Ecology Resources* **12**:975–982 DOI [10.1111/1755-0998.12015](https://doi.org/10.1111/1755-0998.12015).
- Carlsson J. 2008. Effects of microsatellite null alleles on assignment testing. *Journal of Heredity* **99**:616–623 DOI [10.1093/jhered/esn048](https://doi.org/10.1093/jhered/esn048).
- Cediel N. 2020. *History of rabies in Colombia*. Cham: Springer Nature.
- Clerici N, Armenteras D, Kareiva P, Botero R. 2020. Deforestation in Colombian protected areas increased during post-conflict periods. *Scientific Reports* **10**:e4971 DOI [10.1038/s41598-020-61861-y](https://doi.org/10.1038/s41598-020-61861-y).
- Covarrubias-Pazaran G, Diaz-Garcia L, Schlautman B, Salazar W, Zalapa J. 2016. *Fragman*: an R package for fragment analysis. *BMC Genetics* **17**:1–8 DOI [10.1186/s12863-016-0365-6](https://doi.org/10.1186/s12863-016-0365-6).
- Das S, Smith K, Sarker S, Peters A, Adriaanse K, Eden P, Ghorashi SA, Forwood JK, Raidal SR. 2019. Assessing circovirus gene flow in multiple spill-over events. *Virus Genes* **55**:802–814 DOI [10.1007/s11262-019-01702-x](https://doi.org/10.1007/s11262-019-01702-x).
- Desvars-Larrive A, Hamed A, Hodroge A, Berny P, Benoît E, Lattard V, Cosson JF. 2019. Population genetics and genotyping as tools for planning rat management programmes. *Journal of Pest Science* **92**:691–705 DOI [10.1007/s10340-018-1043-4](https://doi.org/10.1007/s10340-018-1043-4).
- Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size ( $N_e$ ) from genetic data. *Molecular Ecology Resources* **14**:209–214 DOI [10.1111/1755-0998.12157](https://doi.org/10.1111/1755-0998.12157).
- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**(8):2611–2620 DOI [10.1111/j.1365-294X.2005.02553.x](https://doi.org/10.1111/j.1365-294X.2005.02553.x).
- Fernando J, Jiménez C, Mvz E, Darío R, López P, Garcia NV. 2017. Bat reservoirs for rabies virus and epidemiology of rabies in Colombia: a review. *CES Medicina Veterinaria y Zootecnia* **12**:134–150 DOI [10.21615/cesmvz.12.2.5](https://doi.org/10.21615/cesmvz.12.2.5).
- Food and Agriculture Organization of the United Nations. 2022. Gridded livestock of the world database. Available at <https://data.apps.fao.org/catalog/dataset/glw> (accessed on 1 June 2023).
- Foulley JL, Ollivier L. 2006. Estimating allelic richness and its diversity. *Livestock Science* **101**:150–158 DOI [10.1016/j.livprodsci.2005.10.021](https://doi.org/10.1016/j.livprodsci.2005.10.021).
- García-Morales R, Badano EI, Moreno CE. 2013. Response of Neotropical bat assemblages to human land use. *Conservation Biology* **27**:1096–1106 DOI [10.1111/cobi.12099](https://doi.org/10.1111/cobi.12099).
- González G, Willig M, Waide R. 2013. Ecological gradient analyses in a tropical landscape: multiple perspectives and emerging themes. *Ecological Bulletins* **54**:13–20.
- Hedrick PW. 2005. A standardized genetic differentiation measure. *Evolution* **59**:1633–1638 DOI [10.1111/j.0014-3820.2005.tb01814.x](https://doi.org/10.1111/j.0014-3820.2005.tb01814.x).
- Hengl T. 2018. WorldGrids archived layers at 1 km to 20 km spatial resolution. Zenodo. DOI [10.5281/ZENODO.1637816](https://doi.org/10.5281/ZENODO.1637816).
- Hijmans RJ. 2017. DIVA-GIS (2017) Free Spatial Data by Country. Available at <http://www.diva-gis.org/gdata> (accessed on October 11, 2019).

- Horta MA, Ledesma LA, Moura WC, Lemos ERS. 2022. From dogs to bats: concerns regarding vampire bat-borne rabies in Brazil. *PLOS Neglected Tropical Diseases* 16:6–10 DOI 10.1371/journal.pntd.0010160.
- Huguin M, Arechiga-Ceballos N, Delaval M, Guidez A, de Castro IJ, Lacoste V, Salmier A, Setién AA, Silva CR, Lavergne A, de Thoisy B. 2018. How social structure drives the population dynamics of the common vampire bat (*Desmodus rotundus*, Phyllostomidae). *Journal of Heredity* 109(4):393–404 DOI 10.1093/jhered/esx111.
- Instituto Colombiano Agropecuario (ICA). 2019. Sanidad animal 2016. Dirección Técnica de Vigilancia Epidemiológica. Subgerencia de Protección Animal. Instituto Colombiano Agropecuario. In: *Boletines Epidemiológicos. Dirección Técnica de Vigilancia Epidemiológica. Subgerencia de Protección Animal*. Bogotá, Colombia: Instituto Colombiano Agropecuario, Produmedios, 5–100.
- Instituto Colombiano Agropecuario (ICA). 2020. Censos Bovinos. Available at <https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018> (accessed on 1 December 2023).
- Instituto Colombiano Agropecuario (ICA). 2023. *Programa Nacional de Prevención y control de la rabia de origen silvestre*. Bogotá, Colombia: Dirección Técnica de Protección Animal. Subgerencia de Protección Animal.
- Instituto Colombiano Agropecuario (ICA). 2024. *Resolución No. 00009028: por la cual se establecen las medidas sanitarias para la prevención y el control de la rabia de origen silvestre en Colombia y se dictan otras disposiciones*. Bogota, Colombia: Instituto Colombiano Agropecuario.
- Instituto Nacional de Salud (INS). 2020. *Informe de Evento Rabia Animal Colombia 2020*. Bogota, Colombia: Instituto Nacional de Salud.
- Jerome A, Archer E, Hardy O, Goudet MJ. 2022. Package ‘hierfstat’. Available at <https://cran.r-project.org/package=hierfstat>.
- Jombart T. 2015. A tutorial for discriminant analysis of principal components (DAPC) using adegenet. Available at <https://adegenet.r-forge.r-project.org/files/tutorial-dapc.pdf>.
- Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11:94 DOI 10.1186/1471-2156-11-94.
- Jones C, Vicente-Santos A, Clennon JA, Gillespie TR. 2024. Deforestation and bovine rabies outbreaks in Costa Rica, 1985–2020. *Emerging Infectious Diseases* 30:1039–1042 DOI 10.3201/eid3005.230927.
- Kamvar ZN, Tabima JF, Grünwald NJ. 2014. *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281 DOI 10.7717/peerj.281.
- Kassambra A. 2023. Package ‘rstatix’: pipe-friendly framework for basic statistical tests. Available at <https://cran.r-project.org/web/packages/rstatix/index.html>.
- Lyman CP, Wimsatt WA. 1966. Temperature regulation in the vampire bat, *Desmodus rotundus*. *Physiological Zoology* 39:101–109.



- Lyons MP, Kozak KH. 2019.** Vanishing islands in the sky? A comparison of correlation- and mechanism-based forecasts of range dynamics for montane salamanders under climate change. *Ecography* **43**(4):481–493 DOI [10.1111/ecog.04282](https://doi.org/10.1111/ecog.04282).
- Magalhães AR, Codeço CT, Svenning JC, Escobar LE, Van de Vuurst P, Guncalves-Souza T. 2023.** Neglected tropical diseases risk correlates with poverty and early ecosystem destruction. *Infectious Diseases of Poverty* **12**:1–15 DOI [10.1186/s40249-023-01084-1](https://doi.org/10.1186/s40249-023-01084-1).
- Mantel N. 1967.** The detection of disease clustering and generalized regression approach. *Cancer Research* **27**:209–220 DOI [10.1021/j150334a020](https://doi.org/10.1021/j150334a020).
- Mantovan KB, Menozzi BD, Paiz LM, Sevá P, Brandão PE, Langoni H. 2022.** Geographic distribution of common vampire bat *Desmodus rotundus* (Chiroptera: Phyllostomidae) shelters: Implications for the spread of rabies virus to cattle in southeastern Brazil. *Pathogens* **11**(8):942 DOI [10.3390/pathogens11080942](https://doi.org/10.3390/pathogens11080942).
- Mara MTO, Wikelski M, Dechmann DKN. 2014.** 50 years of bat tracking: device attachment and future directions. *Methods in Ecology and Evolution* **5**:311–319 DOI [10.1111/2041-210X.12172](https://doi.org/10.1111/2041-210X.12172).
- Martins FM, Templeton AR, Pavan AC, Kohlbach BC, Morgante JS. 2009.** Phylogeography of the common vampire bat (*Desmodus rotundus*): marked population structure, Neotropical Pleistocene vicariance and incongruence between nuclear and mtDNA markers. *BMC Evolutionary Biology* **9**:294 DOI [10.1186/1471-2148-9-294](https://doi.org/10.1186/1471-2148-9-294).
- McNab BK. 1973.** Energetics and the distribution of vampires. *Journal of Mammalogy* **54**:131–144 DOI [10.2307/1378876](https://doi.org/10.2307/1378876).
- Megid J, Benavides Tala JA, Silva LDB, Castro Castro FF, Ribeiro BLD, Appolinário CM, Katz IS, Scheffer KC, Ramos Silva S, Ruckert Rosa A, Almeida GTB, Baron GT. 2021.** Serological surveillance of rabies in free-range and captive common vampire bats *Desmodus rotundus*. *Frontiers in Veterinary Science* **8**:681423 DOI [10.3389/fvets.2021.681423](https://doi.org/10.3389/fvets.2021.681423).
- Meske M, Fanelli A, Rocha F, Awada L, Soto PC, Mapitse N, Tizzani P. 2021.** Evolution of rabies in South America and inter-species dynamics (2009–2018). *Tropical Medicine and Infectious Disease* **6**:2–18 DOI [10.3390/tropicalmed6020098](https://doi.org/10.3390/tropicalmed6020098).
- Mijangos JL, Gruber B, Berry O, Pacioni C, Georges A. 2022.** dartR v2: an accessible genetic analysis platform for conservation, ecology and agriculture. *Methods in Ecology and Evolution* **13**:2150–2158 DOI [10.1111/2041-210X.13918](https://doi.org/10.1111/2041-210X.13918).
- NASA Earth Data. 2022.** United States Socioeconomic Data and Applications Center (SEDAC). Available at <https://www.earthdata.nasa.gov/data/catalog> (accessed on 17 August 2023).
- Petit RJ, Mousadik AEL, Pons O. 1998.** Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* **12**:844–855 DOI [10.1111/j.1523-1739.1998.96489.x](https://doi.org/10.1111/j.1523-1739.1998.96489.x).
- Piaggio AJ, Johnson JJ, Perkins SL. 2008.** Development of polymorphic microsatellite loci for the common vampire bat, *Desmodus rotundus* (Chiroptera: Phyllostomidae). *Molecular Ecology Resources* **8**:440–442 DOI [10.1111/j.1471-8286.2007.01986.x](https://doi.org/10.1111/j.1471-8286.2007.01986.x).

- Piaggio AJ, Russell AL, Osorio IA, Jiménez Ramírez A, Fischer JW, Neuwald JL, Tibbels AE, Lecuona L, McCracken GF. 2017. Genetic demography at the leading edge of the distribution of a rabies virus vector. *Ecology and Evolution* 7:5343–5351 DOI 10.1002/ece3.3087.
- Pinto CM. 2009. Genetic diversity of the common vampire bat *Desmodus rotundus* in Ecuador: testing cross-Andean gene flow. M.S. Thesis, Texas Tech University, Lubbock, TX, USA. Available at <https://ttu-ir.tdl.org/server/api/core/bitstreams/b2556bd4-1ac2-4723-bc11-d5c1b4b2d962/content> (accessed on 1 August 2025).
- Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity* 95:536–539 DOI 10.1093/jhered/esh074.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959 DOI 10.1093/genetics/155.2.945.
- Promega. 2019. GoTaq PCR core system protocol. Madison: Promega Corporation.
- Qiagen. 2023. DNeasy Blood & Tissue Handbook. Qiagen, 6–37. Available at <https://www.qiagen.com/us/resources/download.aspx> (accessed on February 2, 2024).
- R Core Team. 2021. R: A language and environment for statistical computing. Version 4.1.0. Vienna: R Foundation for Statistical Computing. Available at <https://www.r-project.org> (accessed on 21 May 2022).
- Rocha F, Dias RA. 2020. The common vampire bat *Desmodus rotundus* (Chiroptera: Phyllostomidae) and the transmission of the rabies virus to livestock: A contact network approach and recommendations for surveillance and control. *Preventive Veterinary Medicine* 174:104809 DOI 10.1016/j.prevetmed.2019.104809.
- Rojas-Sereno ZE, Streicker DG, Medina-Rodriguez AT, Benavides JA. 2022. Drivers of spatial expansions of vampire bat rabies in Colombia. *Viruses* 14(11):2318 DOI 10.3390/v14112318.
- Romero-Nava C, León-Paniagua L, Ortega J. 2014. Microsatellites loci reveal heterozygosity and population structure in vampire bats (*Desmodus rotundus*) (Chiroptera: Phyllostomidae) of Mexico. *Revista de Biología Tropical* 62:659–669 DOI 10.15517/rbt.v62i2.9793.
- Schorr A, Robert A, Laura E, Paul M. 2014. Estimating sample size for landscape-scale mark-recapture studies of North American migratory tree bats. *Acta Chiropterologica* 16:231–239 DOI 10.3161/150811014X683426.
- Seetahal JFR, Streicker DG, Beerli P, Sahadeo N, Lemey P, Sanchez-Vazquez MJ, Broos A, Bergner L, Ramkissoon V, Mahabir R, Tihul P, Hartley D, Van Sauers A, Karg G, Mohammed RS, Biek R, Oura CAL, Carrington CVF. 2024. Population genetics, phylogeography and gene flow of mainland and island vampire bat (*Desmodus rotundus*) populations: an investigation into mainland-island bat movement. *BioRxiv*. DOI 10.1101/2024.01.29.577751.
- Sikes RS. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97:663–688 DOI 10.1093/jmammal/gyw078.

- SoftGenetics. 2018. GeneMarker. Available at <https://softgenetics.com/products/genemarker/> (accessed on 12 April 2024).
- Soler-Tovar D, Escobar LE. 2025. Rabies transmitted from vampires to cattle: an overview. *PLOS ONE* 20:e01317214 DOI 10.1371/journal.pone.0317214.
- Stoner-Duncan B, Streicker DG, Tedeschi CM. 2014. Vampire bats and rabies: toward an ecological solution to a public health problem. *PLOS Neglected Tropical Disease* 8:e2867 DOI 10.1371/journal.pntd.0002867.
- Streicker DG, Allgeier JE. 2016. Foraging choices of vampire bats in diverse landscapes: potential implications for land-use change and disease transmission. *Journal of Applied Ecology* 53(4):1280–1288 DOI 10.1111/1365-2664.12690.
- Streicker DG, Lemey P, Velasco-Villa AA, Rupprecht CE. 2012a. Rates of viral evolution are linked to host geography in bat rabies. *PLOS Pathogens* 8:e1002720 DOI 10.1371/journal.ppat.1002720.
- Streicker DG, Recuenco S, Valderrama W, Benavides JG, Vargas I, Pacheco V, Condori Condori RE, Montgomery J, Rupprecht CE, Rohani P, Altizer S. 2012b. Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. *Proceedings of the Royal Society B: Biological Sciences* 279:3384–3392 DOI 10.1098/rspb.2012.0538.
- Streicker DG, Winternitz JC, Satterfield DA, Condori-Condori RE, Broos A, Tello C, Recuenco S, Velasco-Villa A, Altizer S, Valderrama W. 2016. Host-pathogen evolutionary signatures reveal dynamics and future invasions of vampire bat rabies. *Proceedings of the National Academy of Sciences of the United States of America* 113:10926–10931 DOI 10.1073/pnas.1606587113.
- Szpiech ZA, Rosenberg NA. 2011. On the size distribution of private microsatellite alleles. *Theoretical Population Biology* 80:100–113 DOI 10.1016/j.tpb.2011.03.006.
- The Earth and Life Systems Alliance. 2023. Micro-checker version 2.2.3. *Molecular Ecology Notes* 4:535–538 DOI 10.1111/j.1471-8286.2004.00684.x.
- Tibihika PD, Curto M, Dornstaeder E, Silvia S, Alemayehu E, Waidbacher H, Meimberg H. 2019. Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. *Conservation Genetics* 20:357–372 DOI 10.1007/s10592-018-1136-x.
- Trajano E. 1996. Movements of cave bats in southeastern Brazil, with emphasis on the population ecology of the common vampire bat, *Desmodus rotundus* (Chiroptera). *Biotropica* 28:121 DOI 10.2307/2388777.
- Van de Vuurst P, Qiao H, Soler-Tovar D, Escobar LE. 2023. Climate change linked to vampire bat expansion and rabies virus spillover. *Ecography* e06714 DOI 10.1111/ecog.06714.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4:535–538 DOI 10.1111/j.1471-8286.2004.00684.x.
- Van Oosterhout C, Weetman D, Hutchinson WF. 2006. Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. *Molecular Ecology Notes* 6:255–256 DOI 10.1111/j.1471-8286.2005.01082.x.

- Velasco-Villa A, Mauldin MR, Shi M, Escobar LE, Gallardo-Romero NF, Damon I, Olson VA, Streicker DG, Emerson G. 2017.** The history of rabies in the Western Hemisphere. *Antiviral Research* **146**:221–232 DOI [10.1016/j.antiviral.2017.03.013](https://doi.org/10.1016/j.antiviral.2017.03.013).
- Wang J, Whitlock MC. 2003.** Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **163**(1):429–446 DOI [10.1093/genetics/163.1.429](https://doi.org/10.1093/genetics/163.1.429).
- Waples RS, Do C. 2010.** Linkage disequilibrium estimates of contemporary  $N_e$  using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* **3**:244–262 DOI [10.1111/j.1752-4571.2009.00104.x](https://doi.org/10.1111/j.1752-4571.2009.00104.x).
- Waples RK, Larson WA, Waples RS. 2016.** Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity* **117**:233–240 DOI [10.1038/hdy.2016.60](https://doi.org/10.1038/hdy.2016.60).
- Weir BS, Cockerham CC. 1984.** Estimating  $F$ -statistics for the analysis of population structure. *Evolution* **38**:1358 DOI [10.2307/2408641](https://doi.org/10.2307/2408641).
- Wright S. 1951.** The genetical structure of populations. *Annals of Eugenics* **15**:323–354.
- Yamamichi M, Innan H. 2012.** Estimating the migration rate from genetic variation data. *Heredity* **108**(4):362 DOI [10.1038/hdy.2011.83](https://doi.org/10.1038/hdy.2011.83).
- Yang RC. 1998.** Estimating hierarchical  $F$ -statistics. *Evolution* **52**:950–956 DOI [10.1111/j.1558-5646.1998.tb01824.x](https://doi.org/10.1111/j.1558-5646.1998.tb01824.x).
- Zarza H, Martínez-Meyer E, Suzán G, Ceballos G. 2017.** Geographic distribution of *Desmodus rotundus* in Mexico under current and future climate change scenarios: implications for bovine paralytic rabies infection. *Veterinaria México OA* **4**:1–16 DOI [10.21753/vmoa.4.3.390](https://doi.org/10.21753/vmoa.4.3.390).
- Zeppelini CG, Azeredo LMM, Lopez LCS. 2019.** Bats like dimmer lights: lunar phobia as a luminosity threshold phenomenon on Neotropical bats (Mammalia: Chiroptera). *Acta Ethologica* **22**:125–128 DOI [10.1007/s10211-019-00314-w](https://doi.org/10.1007/s10211-019-00314-w).
- Zhivotovsky LA. 2015.** Relationships between Wright’s  $F_{ST}$  and  $F_{IS}$  statistics in a context of Wahlund effect. *Genetics* **2**:306–309 DOI [10.1093/jhered/esv019](https://doi.org/10.1093/jhered/esv019).