Strong Population Genetic Structure and Cryptic Diversity in the Florida Bonneted Bat (*Eumops Floridanus*)

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Abstract

Knowledge of genetic structure is essential for the long-term management and conservation of endangered species. We report the results from a genetic examination of the federally endangered Florida bonneted bat (*Eumops floridanus*) sampled from its range in southern Florida, USA. Bonneted bats are primarily found in four regions separated by approximately 100 to 250 kms, including three western natural areas (BW, PC, and CC) and one urban population on the east coast [Miami-Dade County (MD)]. We used 22 microsatellite loci and cytochrome *b* sequences to assess the extent of connectivity and levels of genetic diversity. Regional populations were highly differentiated (F_{ST} = 0.178) and model-based and multivariate analyses showed that MD was the most distinct among pairwise comparisons. Regional populations are small (i.e., N_e< 100) but demographically stable. Estimates of contemporary migration and historic gene flow suggest that regional populations do not frequently exchange migrants, but simulations suggest that the divergence among western regions is likely a result of recent genetic drift rather than long-term isolation. Significantly, mitochondrial DNA revealed that haplotypes from MD were similar or shared with those recognized as *Eumops ferox* from Cuba and Jamaica, and divergent (1.5%) from the remainder of bonneted bats in Florida. Our data support the management of each of the four populations as distinct population segments, and that BW, PC and CC combined are on an independent evolutionary trajectory from bats in MD. Critically, bonneted bats in Florida appear to harbor cryptic diversity that will require a reassessment of their taxonomy.

Introduction

A recognized early step in formulating management plans for endangered species is to quantify genetic diversity (Coates et al. 2018). This information allows managers to prioritize populations, such as those with high diversity, unique polymorphisms, or that are demographically uncoupled (Taylor et al. 2020). Additionally, measuring genetic connectivity between populations has direct conservation and management implications (Lowe and Allendorf 2010) including modeling how species will respond to environmental change. For example, immigration into small populations can increase demographic stability and by extension population persistence (Gonzalez et al. 1998; Hufbauer et al. 2015). In contrast, small, disconnected populations may lose genetic variability via drift, lessening the impact of selection and reducing the ability to adapt (Abbott 2011; Hanski and Mononen 2011). Overall, isolated populations are expected to display greater levels of genetic differentiation and population genetic structure.

Vertebrates capable of flight are subject to fewer barriers to dispersal, and by extension typically show less genetic structure, than species that only walk or swim (Medina et al. 2018). However, there is considerable variation among bat species (order Chiroptera) with respect to philopatry and the resulting population genetic structure, despite their ability to sustain long flight times. Dispersal in bats, defined here as the one-way movement from one location to another, is linked to differences in social structure, mating systems, and individual behavioral responses to geographic barriers (reviewed in Moussy et al. 2013). Difficulty in obtaining direct estimates of inter-population dispersal in bats has meant that population genetic studies have been the predominant means of estimating their spatial dynamics

(Moussy et al. 2013). In non-migratory species, genetic structure has been explained as an outcome of the extent of gene flow resulting from either contemporary processes such as natal dispersal, mating dispersal or recent colonization, or from historical isolation (i.e., phylogeographic processes Avise 2000). Distinguishing historical processes (i.e., predating anthropogenic impacts) from more recent processes (i.e., contemporary colonization and founder events) can be difficult because the latter may be strongly influenced by the former (Keyghobadi 2007). However, genotypic arrays of microsatellites are expected to reflect the shortest and finest-scale contemporary processes whereas mitochondrial sequence data provides a better picture of deeper historical processes (Sunnucks 2000). In geographically restricted species having no obvious historical barriers to dispersal, contemporary processes are generally predicted to predominate the genetic signature of these marker types.

The Florida bonneted bat (*Eumops floridanus*, family Molossidae) is a federally endangered bat that is endemic to southern Florida, USA. Despite having one of the most limited geographic distributions of any bat in the United States, and recent efforts to examine occupancy across its range (Bailey et al. 2017a), its distribution within Florida is still poorly understood. Initially known only from Miami-Dade County in southeastern Florida (Barbour 1936), Florida bonneted bats were first observed on the west coast of Florida in 1979 and are currently known to roost in Charlotte, Lee, Collier, and Polk counties (Belwood 1981; Angell and Thompson 2015; Braun de Torrez et al. 2016; Braun de Torrez et al., 2020). The Florida bonneted bats in Miami-Dade County roost primarily in buildings (Gore et al. 2015; Webb et al. 2021), and artificial roost boxes. Florida bonneted bats from the other counties typically roost in groups of <25 in tree cavities or artificial roost boxes in natural areas, but there has been limited evaluation of urban areas (Braun de Torrez et al. 2016). The largest known population is currently in Babcock-Webb Wildlife Management Area (Charlotte County) where >300 unique individuals have been PIT tagged since 2014 (Braun de Torrez et al. 2020); however, there is little information about the abundance of Florida bonneted bats outside of Babcock-Webb WMA.

An additional obstacle, which has constrained conservation and management efforts on Florida bonneted bats, is a lack of understanding of population connectivity. Bonneted bats have narrow wings suitable for fast flight in open areas and they can cover large distances when foraging (Ober et al. 2017a; Webb 2018). Despite their mobility, bonneted bats have not been detected uniformly across southern Florida by acoustic surveys or captures of individuals (USFWS 2013; Bailey et al. 2017a). The Florida bonneted bat's presumed small overall population size and apparently fragmented distribution may leave it vulnerable to extinction due to inbreeding depression, genetic drift, and sensitivity to stochastic events such as major storms (USFWS 2013). There is a need for research to fill data gaps on fundamental aspects of bonneted bat ecology including patterns of dispersal, connectivity among populations, and intraspecific patterns of genetic diversity. The latter is of importance because a species or population may be considered of conservation concern if it possesses a limited amount of adaptive potential, if small populations are isolated from one another, or if they represent distinct evolutionary units (Frankham 2010).

In addition to the limited knowledge of the spatial distribution, abundance and connectivity of the Florida bonneted bat, the evolutionary relationships between it and closely related species remains poorly resolved. Until recently, the Florida bonneted bat was considered a subspecies of *E. glaucinus*, a widespread species complex ranging across Central and South America and the Caribbean (Eger 1977; Best et al. 1997). The Florida bonneted bat was elevated to species status based on morphological differences (primarily overall size) from *Eumops* in the Caribbean and Mexico (Timm and Genoways 2004; Wilson and Reeder 2005); however, genetic evidence (mtDNA and AFLP) has not supported species distinction (i.e., monophyly) of Florida bonneted bats from closely related *Eumops* in the Caribbean and Mexico (McDonough et al. 2008; Bartlett et al. 2013).

Here we aim to develop a first approximation of Florida bonneted bat population structure in southern Florida. Using a combination of microsatellite markers and mitochondrial sequence data we sought to identify the relative roles of contemporary processes and historical isolation in shaping the genetics of the Florida bonneted bat. Our primary objectives were to: 1) assess the degree of genetic isolation among bonneted bats in southern Florida using estimates of population genetic structure and by quantifying migration and gene flow; 2) calculate the current genetic diversity and contemporary and historical effective population size (N_e) of regional bonneted bat genetic demes; and 3) test the support for alternative historical population models using an Approximate Bayesian Computational (ABC) approach.

Methods

Florida bonneted bat sampling and DNA extraction

We obtained Florida bonneted bat samples from four core regions of the species' range in Southern Florida (see Supplemental File for details). These four regions were: 1) the vicinity of Avon Park Air Force Range, Polk County (PC), 2) Fred C. Babcock/Cecil M. Webb Wildlife Management Area, Charlotte County (BW), 3) Collier County (CC), and 4) areas in Miami-Dade County (MD) (Fig. 1). The first three regions are in central and southwestern Florida (hereafter referred to as "western populations") and represent largely natural habitat types including pine flatwoods, freshwater prairies, cypress swamp, and hardwood hammock. Sampling in MD occurred in the developed environment of the Miami metropolitan area.

DNA was obtained from live bats from oral swabs (Catch-All™ Sample Collection Swab, Epicentre cat. QEC89100), wing punches (4 mm biopsy punch of wing membrane, Worthington Wilmer and Barratt 1996), or both. After optimizing PCR conditions (Supplemental File) we did parallel DNA isolations on both swab and wing punch samples collected simultaneously from 20 bonneted bats to test for genotyping consistency across sampling methods.

Primer development and marker assessment

We included one published locus (TabrD15; Russell et al. 2005) and developed microsatellite loci *de novo* from PacBio sequence reads following the methodology outlined in Saarinen and Austin (2010). Primer

information, including details on development, Genbank Accession numbers, and PCR conditions are provided in the Supplemental File.

Null alleles were estimated within each regional sample and globally using the method of Brookfield (1996) implemented in the R (vers. 3.6.1, R Core Team 2019) program PopGenReport vers. 3.0.4 (Adamack and Gruber 2014). Pairwise tests for genotypic linkage disequilibrium (LD) were estimated using Fisher's exact test implemented in Genepop R v. 1.13 (Rousset 2008). Departures from the null hypothesis of no LD was evaluated using sequential Bonferroni correction at $\alpha = 0.05$.

The power of these 22 loci to detect genetic heterogeneity among regional samples was tested using POWSIM vers. 4.0 (Ryman and Palm 2006). We simulated empirical sample sizes at effective population sizes of 500, 1000, 3000, and 5000, with F_{ST} calculated at generation 0, 20, 35, 50, 75 and 100. Tests for homogeneity were assessed using Fisher's exact tests with tests at generation 0 expected to give false significance (α) at approximately 0.05. The proportion of significant tests over 1,000 replicates at each value of F_{ST} was used to indicate power of the markers to detect differentiation due to drift. Departures from Hardy Weinberg Equilibrium (HWE) were assessed within regional samples and globally using the *hw.test* function in the R program pegas vers. 0.13 (Pradis 2010). Significance was assessed using exact tests (Guo and Thompson 1992) with 1000 permutations.

Assessing genetic structure and population genetic diversity

We visualized the level of genetic clustering among sample regions using STRUCTURE vers 2.3.4 (Pritchard et al. 2000). We applied the linkage model and evaluated K ranging from 1 to 10 with 20 replicate runs per K, 100,000 burnin iterations and 200,000 sampling iterations per run. Model (K) selection included 1) the maximal estimate of the posterior probability of the data for a given K, $\Pr(X \mid K)$, hereafter $L(K)$; 2) The adhoc ΔK method (Evanno et al. 2005); and 3) The supervised measures (MedMed$K$, MedMean$K$, MaxMed$K$, and MaxMean$K$) proposed by Puechmaille (2016). For the latter, we evaluated inclusion thresholds of 0.5, 0.6, and 0.7. (Puechmaille 2016). Finally, based on the results from $L(K)$, ΔK, and the supervised measures, we inspected the results from a range of K values (Novembre 2016) to evaluate their potential implications for the interpretation of latent genetic structure.

Principal component analysis (PCA) was conducted using the function *dudi.pca* in the R package ade4 vers. 12.7-16 (Dray and Dufour 2007) applying weighted vectors and mean centering. We transformed absolute variance of principal component axes to percentage of total variance represented in the genotype data.

Finally, we performed a discriminant analysis of principal components (DAPC) in the R package adegenet vers. 2.1.3 (Jombart 2008; Jombart and Ahmed 2011).DAPC reduces intra-group principal component variation in order to maximally explain between group variation (Jombart et al. 2010). We first performed cross-validation for discriminant analysis using the *xvalDapc* function. We tested up to a maximum of 200 PCA components, retaining three axes for each DA (one less than the number of regional groups). DA

clusters were plotted in adegenet with an overlayed minimal spanning network, implemented in the R package ade4, and we examined posterior assignment values for individual bats to assess structure.

We calculated summary statistics on global and latent genetic clusters using the R program diveRsity vers. 1.9.90 (Keenan et al. 2013). These included observed (H_o) and expected heterozygosity (H_e), unbiased heterozygosity (uH_e), inbreeding coefficient (F_{IS}), number of alleles per locus (A) and the 95% confidence interval (CI) based on 999 bootstrap intervals. Allelic richness was calculated using rarefaction to the smallest sample size (A_{r15}).

We evaluated the proportion of total genetic variation that was contained within and among regional genetic clusters using analysis of molecular variance (AMOVA) implemented in GENODIVE vers 3.04 (Meirmans and Van Tienderen 2004). We performed a standard AMOVA to assess the proportion of variance among regions and a second AMOVA in which western populations (BW, CC, PC) were grouped together to evaluate the variance among MD and western locations (assuming hierarchical genetic structure, see results). F_{ST} (Nei 1987) and its 95% CI was calculated using 9,999 bootstraps replicates. We calculated pairwise *F*-statistics and D_{Jost} using the function *fastDivPart* in PopGenReport (Adamack and Gruber 2014).

Non-spatial estimates of structure (i.e., STRUCTURE and AMOVA) assume an island model (Wright 1943) which includes equal migration regardless of the spatial proximity of populations. We tested for isolation by distance among regional populations by regression of genetic differentiation ($F_{ST}/(1-F_{ST})$) to log geographic distance (centroid of sampling location within each region) using 1000 bootstrap permutations to estimate the 95% CI of the regression slope in Genepop. We calculated a standard Mantel test which tests the correlation of genetic (Euclidian) and geographic distance matrices, and we ran a stratified Mantel test permuting individuals within BW+CC+PC to allow for the potential influence of hierarchical structure as inferred from PCA, DAPC and DIYABC results. Both Mantel tests utilized 9,999 permutations and were conducted in GENODIVE.

Estimates of migration and gene flow

Estimates of genetic differentiation may be biased when subpopulations have high levels of unique allelic variation (i.e., display negative dependence; Jost 2008). We used the *corPlot* function in diveRsity to plot F_{ST} (Θ)/D_{Jost} calculated for individual loci against the number of alleles per locus. Contrasting slopes between estimators (e.g., negative F_{ST} and positive D_{Jost}) was taken as evidence of bias stemming from elevated mutation rates (Keenan et al. 2013).

We used the GENSBACK option in STRUCTURE to evaluate the probability of recent migration or mixed ancestry. This analysis used USEPOPINFO = 1 to identify which individuals were unlikely to be residents of their sampled population or were a descendant of a recent migrant. STRUCTURE was run for K=4 based on outputs from previous STRUCTURE and multivariate analyses (see results). We set GENBACK=2 to assign migrants (0) and recent migrant ancestry (parental=1, grandparent=2). We evaluated different

migration rate priors (MIGPRIOR) to assess sensitivity to this variable as an initial condition (Pritchard and Wen 2004), using a range of values (0.01, 0.05, and 0.1) and ran 200,000 and 500,000 iterations for burnin and run length, respectively.

We further calculated contemporary migration using BayesAss ver. 3.0 (Wilson and Rannala 2003). BayesAss estimates the posterior mean migration rates based on posterior individual ancestry. We used a total of 1.0×10^7 iterations, discarding the first 1.0×10^6 as burnin and a sampling frequency of 100 to avoid autocorrelation between samples. Final parameters were dF = 0.3, dM = 0.1, dA = 0.3. Migration rate estimates were considered non-zero where the 95% credibility set (mean ± 1.96 × stdev) did not overlap zero.

While the previous approaches can identify contemporary (0-2 generations) migration, we also calculated the directional relative migration among populations and the symmetry of gene flow using the *divMigrate* function in diveRsity (Alcala et al. 2014; Sundqvist et al. 2016). We calculated effective number of migrants (*Nm*) to quantify gene flow and assessed the asymmetry of gene flow among pairs of populations by looking for overlap of 95% CI around estimates of migration generated using 1,000 bootstrap values (Sundqvist et al. 2016). Overlapping confidence intervals were interpreted as evidence for symmetrical gene flow.

Demographic analyses

To inform conservation and planning efforts we conducted multiple N_e analyses, namely: 1) we estimated contemporary (i.e., previous 1-2 generations) N_e based on patterns of linkage disequilibrium (LD); and 2) we tested for evidence of historical (i.e., over the past 500 generations) changes in N_e based on microsatellite repeat motif frequencies and differences between alleles.

Contemporary N_e was estimated using the LD method (Waples and Do 2008) implemented in NeEstimator vers. 2 (Do et al. 2014). We calculated N_e under both a random mating and monogamy models as mating system can have a large impact of inferences of N_e (Weir and Hill 1980).

We used VarEff vers. 1.0 (Nikolic and Chevalet 2014) to test for changes in N_e over the past 500 generations, encompassing the period of major anthropogenic change in south Florida. For the final analyses we applied a generic mutation rate prior of 0.001 and used a two-phase mutation model ('T 0.15'). Additional population-specific parameters are in Supplemental File, Table S2). Following 10,000 burnin iterations we ran 10,000 steps with 10 steps per batch and 100 steps between sampled steps to avoid autocorrelation for a total of 10,000,000 MCMC iterations.

To assess the relative support for competing demographic models of population history we used approximate Bayesian computation implemented in DIYABC (Cornuet et al. 2014). We developed four models based on outputs from genetic clustering and tests of genetic differentiation, with competing models differing in patterns and times since divergence from a common ancestral population (Fig. 2). The four models were: (M1) Simultaneous radiation of four populations from an ancestral population at

time t_A in the past; (M2) Ancestral divergence leading to MD and an unsampled ancestral population N_W, with the latter subsequently radiating into three populations (BW, PC, CC); (M3) an ancestral population diverged into MD and BW populations with subsequent colonization of the CC population from BW at t_2, followed by the colonization of PC from BW at t_1; (M4) an ancestral population diverged forming MD and BW followed by the serial colonization of CC at t_2 and PC at t_1. Prior selection, summary statistics and assessment of confidence in model choice are provided in the Supplemental File.

Mitochondrial sequencing and analysis

Given the relatively high microsatellite differentiation between MD and western populations, we generated sequence data for the cytochrome b (cyt *b*) gene to improve the resolution of deeper historical patterns of divergence. Cyt *b* has been demonstrated to perform well at species level delimitation in bats including *Eumops* (Caraballo et al. 2020). We sequenced a subset of 49 samples including (13 MD, 11 BW, 17 CC, and 8 PC). Details on PCR and sequencing methods are provided in the Supplemental File. Sequences were aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) then imported into Mesquite vers. 3.7 (Maddison and Maddison 2021) for manual error correction and trimming.

Due to the extent of haplotype divergence detected within Florida bonneted bats, and to improve our phylogeographic inference, we added available cyt *b* sequences of closely related *Eumops* lineages representing Cuba (N = 14), Jamaica (N = 3), and Mexico (N = 13) that have been shown to form a well-supported clade that includes *E. floridanus* (McDonough et al. 2008; Bartlett et al. 2013). We also included three previously sequenced Florida bonneted bats from near Fort Myers, Lee County FL, and from Miami-Dade County, and *E. glaucinus* (N = 6) from South America (See Supplemental File Table S4).

The number of unique haplotypes (*h*), polymorphic nucleotide sites (*S*), haplotype diversity (the probability that two randomly selected haplotypes will be identical, *Hd*; Nei 1987), and nucleotide diversity (the average number of nucleotide differences per site, π; Nei 1987) were calculated for each location and overall with DnaSP vers. 6.12.03 (Rozas et al. 2017). Mesquite was used to visually examine codon polymorphisms. Pairwise differentiation was calculated as G_{ST} (Nei 1973) and the pairwise average number of nucleotide differences among populations. Evolutionary relationships among haplotypes were visualized using the statistical parsimony network (Templeton et al. 1992; Clement et al. 2000) implemented in PopART vers. 1.7 (Leigh and Bryant 2015) which estimates the most parsimonious pairwise connections among haplotypes at ≥ 95% probability (Templeton et al. 1987). In addition, distance networks were created using the NeighborNet algorithm (Bryant and Moulton 2004) in SplitsTree vers. 4.11.3 (Huson and Bryant 2006). We used an uncorrected p-distance as input to identify similar haplotypes. The algorithm generates clusters of haplotypes and illustrates ambiguity of connections ('nets') where necessary.

We used DIYABC to test support for two competing models that contrast the matrilineal history of MD bats relative to the western populations of *E. floridanus* and that of the Caribbean bats. The first model (Mt1) assumed that the diversity of haplotypes and differentiation between MD and the other Florida

populations reflected the retention of ancestral polymorphism from a Pleistocene bonneted bat population in Florida. The second model (Mt2) assumed that western *E. floridanus* had an independent population history from MD, and that the latter derived separately and more recently from a Caribbean ancestor (Fig. 2; see Supplemental File for modeling details).

Results

Sampling and marker assessment

We genotyped 125 Florida bonneted bats for our final dataset (BW = 60, PC = 15, CC = 31, MD = 19). Sample sizes were skewed primarily due to the ready access to breeding colonies in artificial roosts at BW, whereas samples from the other locations were from free-flying bats captured in nets or otherwise opportunistically sampled.

There was no evidence of null alleles present within any of the four regional samples. Nine of 22 loci were significant when tested with pooled genotypes (L5989, L55690, TabrD15, L40155, L8400, L109865, L30125, L18018, L32421). Because individual regional samples did not possess null alleles, and results of genetic structuring (see below) supported the independence of regional gene pools, we did not correct for null alleles. The number of alleles per locus ranged from 2 to 10.

Fisher's method for testing the significance of LD between locus pairs across all regional samples combined identified four pairs of loci that were significant following Bonferroni correction (all $p_{adj} <$ 0.0002; L55690 and L30125; L5989 and L117077; L38728 and L8400; L11764 and L39543). However, LD tests within individual regional samples revealed that the previously mentioned significant values were each driven by a single significant LD test (i.e., 1 of 4 tests for each pair were less than $p < 0.05$); three significant tests were from PC and one from BW. Because LD was not present between loci in more than one regional sample, we treated loci as independent.

We identified high power for our marker set to detect differentiation due to drift (i.e., in the absence of gene flow) after only a small number of generations. For even very large populations (N_e = 5,000) power was above 0.8 after 50 generations. At N_e = 500 power was near 1.0 after 10 generations of drift (see Supplemental File). These results suggested that the 22 loci had sufficient power to detect recent genetic divergence due to drift alone.

Two of 88 tests for departures from HWE were significant at α = 0.05 (PC, L91027, p = 0.043; CC, L11764, p = 0.037), but neither remained significant after Bonferroni correction of alpha (adjusted α value = 0.0005). Global departures from HWE were significant (≤ 0.05) at 9 of 22 loci, with five loci remaining significant after Bonferroni correction (not shown). Evidence of population substructure (see below) and limited support for null alleles in conjunction with HWE test results suggested global departures from HWE were likely a byproduct of population substructure (Crow and Kimura 1970).

Population structure and diversity

The number of genetic clusters detected via STRUCTURE varied as a function of which post hoc metric was evaluated. For example, the highest $L(K)$ was observed for $K = 8$, whereas ΔK identified the greatest rate of change moving from $K = 5$ to $K = 6$ (Supplemental File, Fig. S1). However, ΔK values were small overall (all < 20; Supplemental File, Fig. S1). The Puechmaille (2016) metrics supported $K = 4$ across inclusion thresholds of 0.5, 0.6 and 0.7. At $K = 4$ each regional sample was clustered independently from one another (Fig. 3). Examining models where $K = 5$ to $K = 10$, MD, CC, and PC represented largely uniform, discrete clusters, whereas the remaining additional diagnosed clusters were admixed to various degrees among BW bats (Fig. 3).

Results from PCA mirrored the STRUCTURE results in that each regional sample represented largely non-overlapping scatter, with the 1st axis (12.34% of total variation) separating MD (east) from the western (BW + CC+PC) regions, and the 2nd axis (7.14%) differentiating CC from northern sites (Fig. 4A). This trend was similar when comparing the 1st and 3rd (6.54%) principal components (Fig. 4B).

The results for DAPC resembled results from STRUCTURE in that increases in K beyond 4 clusters added structure to bats from BW only (i.e., BW bats were assigned to >1 cluster), whereas the group membership of bats from PC, CC and MD remained the same (not shown). The lowest BIC score (203.02) was at $K = 6$, followed by $K = 5$ (203.20), and $K = 4$ (204.44) (Supplemental File, Fig. S2). We present results from $K = 4$ for simplicity (Fig. 4C). At $K = 4$ (and $K = 5$ to $K = 7$), one bat genotyped from CC had a higher posterior probability of assignment to the BW cluster ($p = 0.98$), and all other bats were assigned to their regional population of origin (all $p > 0.99$). Bats from PC and BW were least dissimilar, while PC+BW were most dissimilar to MD, with CC intermediate (Fig. 4C).

Genetic diversity (Table 1) measured as mean number of alleles ranged from 3.0 to 4.2 among regional populations; corrected for sample size this ranged from 3 to 3.8. Private alleles were found in all four populations: 3 in PC, 9 in CC, 10 in MD, and 12 in BW. Fixation index (F_{IS}) was generally low across loci within each population. Locus-specific heterozygosity varied by marker (low: H_e 0-0.10 to high: $H_e > 0.7$) but markers were largely consistent across populations. Locus-specific statistics are provided in Supplemental File, Table S6.

Results from the standard AMOVA found that most of the variation in microsatellite allele frequencies was detected within individuals. However, variation among individuals within populations was substantially lower (and non-significant at 20 of 22 loci) than was variation partitioned among the four populations (significant at 20 of 22 loci; see Supplemental File, Table S4). Over all loci, 82% of variation in allele frequencies was observed within individuals ($F_{IT} = 0.18$), 1.7% among individuals within regional populations ($F_{IS} = 0.02$), and 16.3% among regional populations ($F_{ST} = 0.16$). With western populations grouped to reflect potential hierarchical structure, 76% of variation in allele frequencies was within individuals ($F_{IT} = 0.239$), 1.5% among individuals within regional populations ($F_{IS} = 0.02$), 10.8% among regional populations nested within western and eastern (MD) groups ($F_{SC} = 0.12$), and 11.6% representing western vs. eastern groups ($F_{CT} = 0.116$). Regional genetic clusters were dissimilar based on pairwise F_{ST} (range: 0.095 to 0.226, and differentiation based on D_{Jost} (range: 0.08 to 0.218) (Table 2). The highest

levels of differentiation were all pairwise comparisons involving MD. Global values were significantly greater than zero for both F_{ST} (0.178; 95% 0.166–0.192) and D_{Jost} (0.270; 95% CI 0.253–0.288). Twenty-one loci had bootstrap values indicating 95% CI greater than zero for F_{ST}, and 22 loci were significant for D_{Jost} (Supplemental File, Table S7).

Results from regression between linearized genetic distance and log geographic distance rejected the null hypothesis of no effect of distance in explaining differentiation among identified regional clusters (b = 0.143, 95% CI 0.085–0.281). Individual-level Mantel test also suggested that inter-individual Euclidean distances are positively correlated with geographic distance (r = 0.623). Results from regular permutations and stratified (MD vs western regional clusters) were both highly significant ($p < 0.001$).

Migration and gene flow

From the ad hoc tests of polymorphism bias, we found that both estimators (F_{ST} and D_{Jost}) were positively correlated with the number of alleles at individual loci (Supplemental File, Fig. S3), suggesting that the mutation rate at these loci should not be too high as to obscure their utility at inferring past demographic processes such as gene flow or population size.

Tests to detect recent migrant ancestry using the GENSBACK option in STRUCTURE varied depending on migration prior, but overall, there was minimal evidence of recent migration. Regardless of migration rate prior, all but two individuals were assigned to their sampling location with a probability > 0.9. One individual from BW had a probability of being from that population which decreased (0.759) as migration rate priors increased. Another individual assigned to CC had a probability of being from that population which increased as migration rate prior increased. All recent migration rates estimated from BayesAss overlapped zero suggesting the recent migration has been insignificant (Supplemental File, Table S8). Longer-term gene flow calculations were lower than 1 *Nm* per generation and symmetric for all estimates other than for the estimate from BW into CC (*Nm* = 1.0) which was significantly greater than *Nm* from CC into BW (*Nm* = 0.747; Table 2).

Demographic analyses

Assuming random mating, mean estimates of contemporary N_e within regional samples were less than 100, with the exception of CC, which was above 100 only when rare alleles were considered (Table 4). Overall, N_e estimates were higher when monogamy was assumed (Table 4), and under this scenario MD had an effective population size >100. Confidence intervals on the N_e estimates were narrow and similar between parametric and jackknife methods for BW and PC. For the parametric and jackknife CIs of MD, and for the jackknife CIs for CC, the upper 95% were infinity, suggesting a lack of power (i.e. small sample sizes) to estimate some CI with confidence.

Tests for temporal changes in historic N_e (i.e., over the last 500 generations) reconstructed using VarEff identified declines in each of the four populations over the past 100-200 generations (Supplemental File,

Fig. S4). Declines were most pronounced in CC which showed a dramatic and consistent decline in N_e over the last 500 generations, whereas MD and BW displayed an initial increase over the same period before declining.

Model choice and demographic estimates (ancestral N_e and t) were consistent between models based on equal sexes and female bias ratios (median estimates had overlapping 95% CI for all parameters, so we report on only the equal sex ratios here). The ABC modeling of alternative demographic scenarios supported M2 (hierarchical structure with western populations sharing a most recent common ancestor, Fig. 2) based on the weighted logistic regression (P = 0.913, 95% CI 0.907–0.919). The second most supported model (M1, simultaneous divergence from an ancestral population) had posterior support of 0.075 (95% CI 0.069–0.081). The remaining two models had posterior probabilities and 95% CI below 0.01 (Supplemental File, Fig S5). Posterior-based error rate was 0.283. Prior Type I error (probability of rejection assuming it is true) for M2 was 0.079, and Type II error for M2 was 0.046, suggesting small likelihood for selection of M2 over M1 assuming M2 is not the true best scenario. Although our primary objective for DIYABC was to assess the support for alternative demographic models, rather than to infer ancestral N_e and t, we include the latter values in Supplemental File, Table S9.

Mitochondrial sequencing results

We aligned 934 bp of the cyt *b* gene (Genbank accession # OK165510-OK165558) for the 49 Florida sequenced samples in this study. There was a total of 16 polymorphic sites representing 7 haplotypes. Haplotype diversity was 0.740 overall and nucleotide diversity was 0.0057. Among all 49 sequences the mean number of nucleotide differences was 5.363, primarily reflecting comparisons between MD and the other sequences. Average nucleotide differences and pairwise G_{ST} were highest between MD and the other three Florida populations (Table 3).

The inclusion of the 39 published cyt *b* sequences reduced the alignment (n = 75) to 705 bp with 72 polymorphic sites for 31 total unique haplotypes. One haplotype from Cuba was shared with MD (haplotype 6), and all MD haplotypes were nested among Cuba and Jamaica haplotypes (Fig. 5). With the addition of previously published sequences there were a total of 19 private missense mutations amongst 14 haplotypes (Supplemental File, Table S8). The remaining missense mutations represented two codon changes (autapomorphies) that distinguished the 38 Florida bonneted bats collected in BW (and Lee Co.), PC, and CC (haplotypes 1–4, and 23–25) from all remaining haplotypes including all from MD (Supplemental File, Table S10).

For the combined Florida bonneted bat and Caribbean/South American data set *Hd* was 0.911 overall and nucleotide diversity was 0.0154. MD was more similar (G_{ST}) to samples from Cuba (0.145), Jamaica (0.177), and Mexico (0.110) than to other Florida populations (BW = 0.247, PC = 0.295, CC = 0.292) (Table 3).

Statistical parsimony analyses revealed that the MD haplotypes were closely related to the haplotypes from the Caribbean differing by 1 to 4 mutations and were nested within a cluster of Caribbean haplotypes (Fig. 5). In contrast, the haplotypes from BW, PC, and CC were relatively divergent from MD, differing by at least 8 mutational steps (Fig. 5) or 11 steps for the 934 bp alignment (not shown). The two previously published haplotypes from near BW (Lee Co.) were also divergent from MD (a minimum of 8 mutational steps) (Fig. 5).

The ABC modeling of the two scenarios, retention of ancestral polymorphism within Florida (Mt1) vs. secondary colonization of MD from an ancestral population shared with the Caribbean (Mt2), showed strong support for secondary colonization (Mt1, P = 0.017, 95% CI 0.015–0.019 vs. Mt2, P = 0.983, 95% CI 0.981–0.985). Posterior-based error rate was 0.026 and both prior type I and type II error were 0.

Discussion

We present the first assessment of population genetic structure in the federally endangered Florida bonneted bat. We found strong population genetic structure among all regional sample locations with little evidence of recent connectivity despite being separated by modest distances (100 to 250 kms). Measures of genetic divergence (F_{ST} and D_{Jost}) and results from DAPC, PCA, and ABC simulations supported a hierarchical model of structure with the earliest differentiation occurring between the eastern (MD) and western (CC, PC, BW) populations with subsequent divergence among the latter locations. The strong differentiation between MD and the other populations is further emphasized by the deep divergence among cyt *b* mtDNA gene sequences observed within Florida. Bats from MD were closely related to Caribbean bats, while bonneted bats from the western region of Florida are evolutionarily distinct from MD, the Caribbean population, and Mexico.

Population structure and connectivity within Florida

Non-migratory bats typically display genetic structuring at smaller geographic scales relative to migratory species (Moussy et al. 2013), although patterns of structure are inconsistent across non-migratory types (Ruedi and McCracken 2009). We found significant and strong genetic fixation overall (i.e., global F_{ST} = 0.166) and significant pairwise genetic differentiation across < 250 km, which was surprising given the seeming absence of major biogeographic barriers. Tests of genetic differentiation among regional populations implied that bonneted bats in Florida display strong fidelity despite long-distance (>15 kms) foraging behavior and large average home range sizes (50 – 200 kms^2) (Webb 2018). The observed levels of divergence support the conclusion that these four regions represent individual demographic units and should be treated as ‘distinct population segments’ or ‘management units’ (Funk et al. 2012). Therefore, it will be important to account for the status and stability of all the regional populations when considering habitat conservation decisions and not just the overall status of the species throughout Florida. In addition, maintaining habitat connectivity across large scales will be important to ensure new colony formation is not impeded over time due to a lack of available habitat.

The observed levels of genetic structure could be explained by a combination of the polygynous social system and demographic patterns (Ober et al. 2017b; Braun de Torrez et al. 2020). Within a harem-based social structure, females are unlikely to disperse long distances from their natal colony and dispersing juveniles are less likely to secure mates if they travel far from existing colonies. Both scenarios would limit gene flow and genetic connectivity between populations. In Florida bonneted bats, the apparent survival of juveniles is low (Bailey et al. 2017b) implying that juveniles either have low survival or tend to disperse. Juveniles that disperse may either fail to establish new breeding colonies or, when successful, do so over small distances relative to the total range of the species.

Small estimates of N_e, high pairwise differentiation, POWSIM results, and short geographic distances separating populations support the interpretation that genetic drift has played a large role in shaping the dynamics of Florida bonneted bats. That we observed evidence of genetic drift and failed to detect evidence of dispersal supports the idea that western populations of Florida bonneted bats may represent small, recently (e.g., past few decades) established populations, as opposed to a scenario of long-term (e.g. > 100s of years) allopatric isolation. Furthermore, in systems where genetic drift is prominent, spurious relationships between measures of differentiation and geographic distance can be erroneously attributed to processes such as isolation by distance (Prunier et al. 2017).Why regional populations are separated by significant gaps is unknown and requires further investigation but could in part be related to habitat availability/suitability (lack of roosting opportunities), the general spatial stochasticity of species with female defense or resource defense polygynous mating systems (Storz 1999; Parreira and Chikhi 2015) or some combination thereof. Importantly, our results suggest that genetic drift over short time periods (10s of generations) are likely driving the level of differentiation observed among the western populations (BW, CC, PC), whereas historical factors must be accounted for in explaining the divergence between MD and western populations (see below).

Diversity did not vary strongly among regional populations (Table 3) and overall, each region appeared to fit closely to an equilibrium model of genetic variation. These observations suggest that regional populations are demographically stable, and despite their small effective population sizes, have not undergone significant inbreeding. Polygynous mating systems are expected to reduce N_e relative to monogamy as fewer males contribute genes to subsequent generations (Storz et al. 2001). Consequently, we might expect our estimates of N_e based on a random mating model to be artificially inflated given that Florida bonneted bats display a polygynous mating system. However, the effect of a polygynous mating system may be counteracted by the suspected long-life span of this species (~5 years, unpublished data), which would allow for more reproductive opportunities over multiple breeding seasons and would increase N_e (Clutton-Brock 1988). This is in line with predictions that in highly social species, genotypic diversity can be maintained even in small populations (Parreira and Chikhi 2015). Data from BW suggests that turnover in dominant males within individual roosts and competition from outside males may be common (Ober et al. 2017b; Braun de Torrez et al. 2020), and the potential for cuckolding would further counter the downward impact of polygyny on N_e. Taken together, our numbers suggest that low N_e is not simply a result of the mating system but may reflect true small census population sizes.

Phylogeographic relationships within Florida and the Caribbean

Eumops in Florida have been proposed to be derived from a Caribbean origin (Baker and Genoways 1978; Morgan 1985) perhaps during one of the Pleistocene glacial stages when Florida's peninsular land mass would have increased by 25% to over 100% (Emslie 1998). Even at its peak gain in land mass, this still would have required colonization across the Florida Strait. Alternatively, range expansion from Mexico along the northern Gulf of Mexico, which facilitated the expansion of some other Neotropical species (Emslie 1998) has been proposed (Morgan 1991). The pattern of variation found in cyt *b* suggests a Caribbean origin, as western Florida haplotypes are more similar to Caribbean (and MD) haplotypes than they are to those identified from Mexico. (Fig. 5).

Florida bonneted bats from MD and from western Florida have been demographically isolated for a substantial period. The presence of deep mtDNA divergence within Florida bonneted bats was initially suspected after close examination of the results presented in McDonough et al. (2008), where two bonneted bats from the west coast (Lee Co. < 20 kms south of BW) were represented by a relatively long branch with high posterior support within a larger clade representing *Eumops* haplotypes from Cuba, Jamaica, Mexico, and one sample from Miami-Dade County (see Fig. 1 of McDonough et al. 2008). The level of divergence between western and MD + Caribbean haplotypes (~1.5%) suggests that western bonneted bats may be descendants of the Rancholabrean fossils identified from eastern Florida (Nabholz et al. 2008). The similarity in cyt *b* between MD and Caribbean bats does not support the notion that the current MD population has had a long-term (i.e., early Holocene or older) presence in Florida. The latter scenario (long-term presence of MD haplotypes in Florida) would require incomplete lineage sorting, assuming all Florida bonneted bats share a most recent common ancestor, or alternatively, would suggest periodic maternal gene flow from the Caribbean to MD (mitochondrial introgression). Our ABC model comparison strongly supports the secondary colonization hypothesis over the retention of ancestral polymorphism in Florida (although we did not assess the possibility of gene flow). The addition of genomic data will likely improve our ability to resolve whether MD bats were recently introduced (Barbour 1936) or were more distantly established (Allen 1932; Koopman 1971).

Management implications

Given that MD and the western Florida bonneted bats likely have had mutually exclusive histories over the Holocene/Pleistocene, they should be recognized as two Evolutionary Significant Units (ESUs). ESUs are defined here as populations that demonstrate independent evolutionary trajectories and restricted gene flow (Fraser and Bernatchez 2001). Ideally, diagnosis of ESUs would include both neutral and adaptive genetic loci (Funk et al. 2012), however in the absence of genomic data, combined mtDNA and nuclear microsatellites have been used to support the delineation of ESUs in various taxa (e.g., King et al. 2006; Austin et al. 2011; Carvalho et al. 2012; Puckett et al. 2021). While it is unknown whether the identified missense mutations have fitness effects, they do represent rare intraspecific variants that add support to the argument for managing as separate evolutionary units (Crandall et al. 2000). Separate ESUs, for example, will each require sufficient critical habitat to ensure long term demographic resilience

and will likely require ESU-specific conservation actions. Management strategies, such as translocations of bats (i.e. remediation translocations) between ESUs would not be appropriate due to their genetic differentiation and potential ecological or adaptive differences. Additional research into the roosting and foraging requirements of bats within each ESU would provide insight into the degree of adaptive divergence between the two ESUs. Similarly, the three western distinct population segments may have different habitat preferences and responses to management actions. Additional research is needed to make appropriate population-level conservation recommendations across the species range and to facilitate movement among populations.

Although examining the taxonomic status of the bonneted bat was not an objective of this study, the cryptic variation uncovered in Florida requires a brief discussion. *Eumops* in Florida was first described from Pleistocene fossils (*Molossides floridanus*, Allen 1932). Live specimens from the Miami area were subsequently recognized as being conspecific with *E. glaucinus* (Ray et al. 1963) and considered a subspecies (*E. g. floridanus*; Koopman 1971). The bat was elevated to species status by Timm and Genoways (2004) based on overall larger size and several significantly larger cranial measurements. They examined 47 *E. floridanus* specimens, however only 5 were from outside Miami Dade County. Their morphological analysis included 28 of the 47 specimens (see Table 2 in Timm and Genoways 2004). Thus, the most recent morphological description of *E. floridanus* is heavily reliant on the MD lineage. Further systematic study of the Florida bonneted bat should include a reevaluation of the morphology from across the range and include genome re-sequencing or single nucleotide polymorphism (SNP) markers to afford sufficient resolution to provide greater insight into the demographic history, taxonomy and conservation decisions (Funk et al. 2019)

Conclusions

Our genetic assessment of the Florida bonneted bat identified that regional populations are small, appear to be demographically stable, and are genetically divergent despite being separated by small geographic distances. Among the three western populations, genetic structure appeared to be driven largely by the polygynous social system in bonneted bats, and long-term persistence will likely require the maintenance of intact habitat corridors with suitable roosting options to promote additional establishment of new colonies. Given the lack of gene flow between populations, evidence of genetic drift, and small population sizes, western Florida bonneted bat populations should be managed as separate distinct population segments.

These results provide novel insight into the dynamics and history of this endangered species. One limitation of our current study is that we collected samples only from regions of southern Florida where bonneted bats have been previously captured. Expanded sampling efforts covering areas between regions would facilitate an assessment of the geographic extent of distinct mitochondrial lineages. Samples from currently unsampled regions, including the natural areas in the proximity of Miami Dade County, would help to elucidate connectivity across the range and detail the distribution of genetically distinct lineages of bats. Similarly, broadening the extent of geographic sampling of *Eumops* throughout

the Caribbean and Mexico/Central America may uncover additional cryptic genetic structuring that will help to clarify the population history of the two lineages in Florida. Moving forward, genomic approaches will provide a clearer picture of the demographic history of Florida bonneted bats and estimate adaptive divergence among Florida and Caribbean populations. These additional insights may prove critical for evaluating potential conservation actions, including whether the translocation of bonneted bats will be an effective management tool.

Declarations

Funding: Funding for this work came from the Florida Fish and Wildlife Conservation Commission.

Conflicts of interest/Competing interests: none

Availability of data: Raw genotype data is available on Dryad. Sequences generated have been submitted to Genbank under accession numbers OK165510-OK165558.

Code availability: NA

Authors' contributions: JDA and JAG conceived of the study. JDA, JSH and CMC were involved with data generation and performed data analyses. JAG, FNR, EBT contributed to field collections. JDA drafted the initial manuscript and all authors contributed to writing and editing.

Ethics approval: Samples were collected under a cooperative agreement between the Florida Fish and Wildlife Conservation Commission and the US Fish and Wildlife Service, under Section 6 of the Endangered Species Act.

Consent to participate N/A

Consent for publication N/A

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Tables

Table 1. Summary statistics of genetic diversity for collections of Florida bonneted bat grouped by geographic location: Babcock Webb Wildlife Management Area (BW), Avon Park Air Force Base (PC), Miami-Dade County (MD), and Collier County (CC), Florida, USA. Statistics represent mean (±SE) across loci for microsatellite genotypes.

	BW	PC	MD	CC
Genotypes	60	15	19	31
A (±SE)	3.58 (0.28)	2.87 (0.29)	3.28 (0.25)	3.74 (0.31)
A_{r15} (±SE)	3.71 (0.57)	3.00 (0.58)	3.40 (0.51)	3.96 (0.64)
A_p	12	3	10	9
H_o (±SE)	0.487 (0.05)	0.491 (0.07)	0.553 (0.05)	0.499 (0.05)
H_e (±SE)	0.497 (0.04)	0.468 (0.05)	0.537 (0.04)	0.507 (0.05)
uH_e (±SE)	0.501 (0.04)	0.482 (0.05)	0.552 (0.04)	0.517 (0.05)
F_{IS} (±SE)	0.025 (0.02)	-0.038 (0.04)	-0.029 (0.03)	0.007 (0.03)
Sequences	11	8	13	17
Polymorphic sites	2	2	1	6
K	1.018	1.071	0.538	1.500
π	0.00109	0.00115	0.00058	0.00161

A, Mean number of alleles across 22 loci; A_{r15}, Allelic richness, standardized to 15 genotypes; A_p, count of private alleles across 22 loci; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; *K*, average number of nucleotide differences; π, average number of nucleotide differences per site between two sequences.

Table 2. Upper table: Pairwise F_{ST} (below diagonal) and D_{Jost} (above diagonal) among regional genetic clusters of Florida bonneted bat based on 22 microsatellite loci (see Table 1 for a description of abbreviations). All values were significantly greater than zero (all P < 0.001). Lower table: Directional gene flow (*Nm*) estimates between regional samples. Rows reflect directional migration from the row into the column regional sample. Bold indicates *Nm* is significantly different from the alternative direction of migration.

	BW	PC	MD	CC
BW	-	0.123	0.218	0.080
PC	0.154	-	0.218	0.140
MD	0.224	0.226	-	0.212
CC	0.095	0.171	0.213	-
BW	-	0.398	0.345	**1.0**
PC	0.419	-	0.292	0.527
MD	0.254	0.182	-	0.306
CC	0.747	0.395	0.232	-

Table 3. The average number of nucleotide differences (above diagonal) and Pairwise G_{ST} (below diagonal), for mtDNA sequence data collected from Florida bonneted bats (see Table 1 for a description of abbreviations) and related GenBank specimens from Cuba, Jamaica, and Mexico. Values in parentheses represent a shared sequence length of 705 bp between Florida and non-Florida collections.

	BW	PC	MD	CC	Cuba	Jamaica	Mexico
BW (11)	–	1.068 (2.115)	11.538 (9.571)	1.439(2.068)	— (10.852)	— (11.667)	— (13.132)
PC (8)	0.012 (0.124)	–	11.538 (9.571)	1.838(2.029)	— (10.786)	— (11.667)	— (11.786)
MD (14)	0.311 (0.247)	0.290 (0.295)	–	11.362(9.395)	— (2.000)	— (2.238)	— (6.857)
CC (17)	0.047 (0.017)	0.164 (0.254)	0.317 (0.292)	–	— (10.710)	— (11.490)	— (13.580)
Cuba (14)	— (0.163)	— (0.201)	— (0.145)	(0.205)	–	— (1.881)	— (8.071)
Jamaica (3)	— (0.181)	— (0.279)	— (0.177)	(0.203)	— (0.028)	–	— (9.083)
Mexico (14)	— (0.111)	— (0.177)	— (0.110)	(0.140)	— (0.054)	— (0.089)	–

Table 4. Estimates of contemporary effective population sizes (N_e) of Florida bonneted bat under assumptions of random mating and monogamy.

	Random			Monogamy		
	Lowest Allele Frequency Used			Lowest Allele Frequency Used		
	0.05	0.02	0.01	0.05	0.02	0.01
BW						
# comparisons	1935	2056	2182	1935	2056	2182
N_e	37.2	41.3	39.1	76	84.1	79.9
95% CI (parametric)	30.5–46.2	33.7–51.6	32.3–48.3	62.7–94.0	69.0–104.7	66.2–98.1
95% CI (jacknife)	26.5–55.4	29.7–60.7	28.7–56.1	54.6–112.2	61.0–123.0	50.0–113.8
PC						
# comparisons	821	860	860	821	860	860
N_e	12.9	15.7	15.7	27.5	33.0	33.0
95% CI (parametric)	7.6–24.9	9.1–33.5	9.1–33.5	17.2–51.5	20.0–68.6	20.0–68.6
95% CI (jacknife)	6.3–34.7	8.0–43.8	8.0–43.8	14.8–71.1	18.0–89.2	18.0–89.2
MD						
# comparisons	1324	1324	1324	1324	1324	1324
N_e	80.9	76.7	76.7	163.7	155.2	155.2
95% CI (parametric)	31.6–∞	30.8–∞	30.8–∞	64.7–∞	63.2–∞	63.2–∞
95% CI (jacknife)	17.7–∞	19.6–∞	19.6–∞	37.1–∞	40.7–∞	40.7–∞
CC						
# comparisons	1773	2178	2372	1773	2178	2372
N_e	94.1	109.0	126.3	189.7	219.6	254.2
95% CI (parametric)	51.7–332.8	59.4–419.5	66.2–667.8	104.9–667.4	120.4–841.7	133.9–1339.6
95% CI (jacknife)	37.8–∞	45.7–∞	57.5–∞	77.3–∞	93.0–∞	116.5–∞

Figures

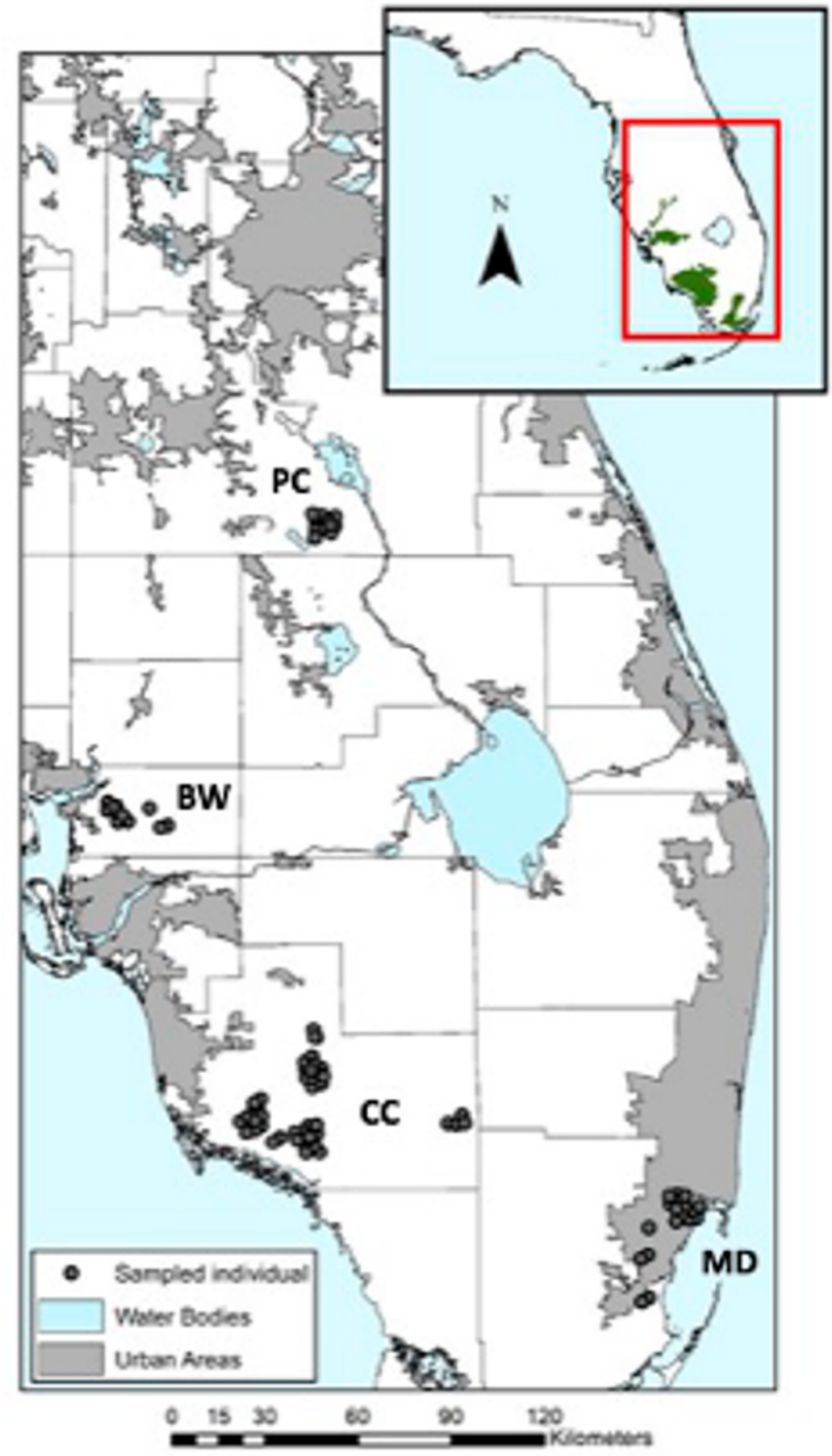

Figure 1

South Florida indicating the regional locations of known Florida bonneted bat breeding locations. In some locations only general coordinates were known and some sample coordinates were jittered to avoid overlap. Inset contains areas being considered for critical habitat designation by the USFWS.

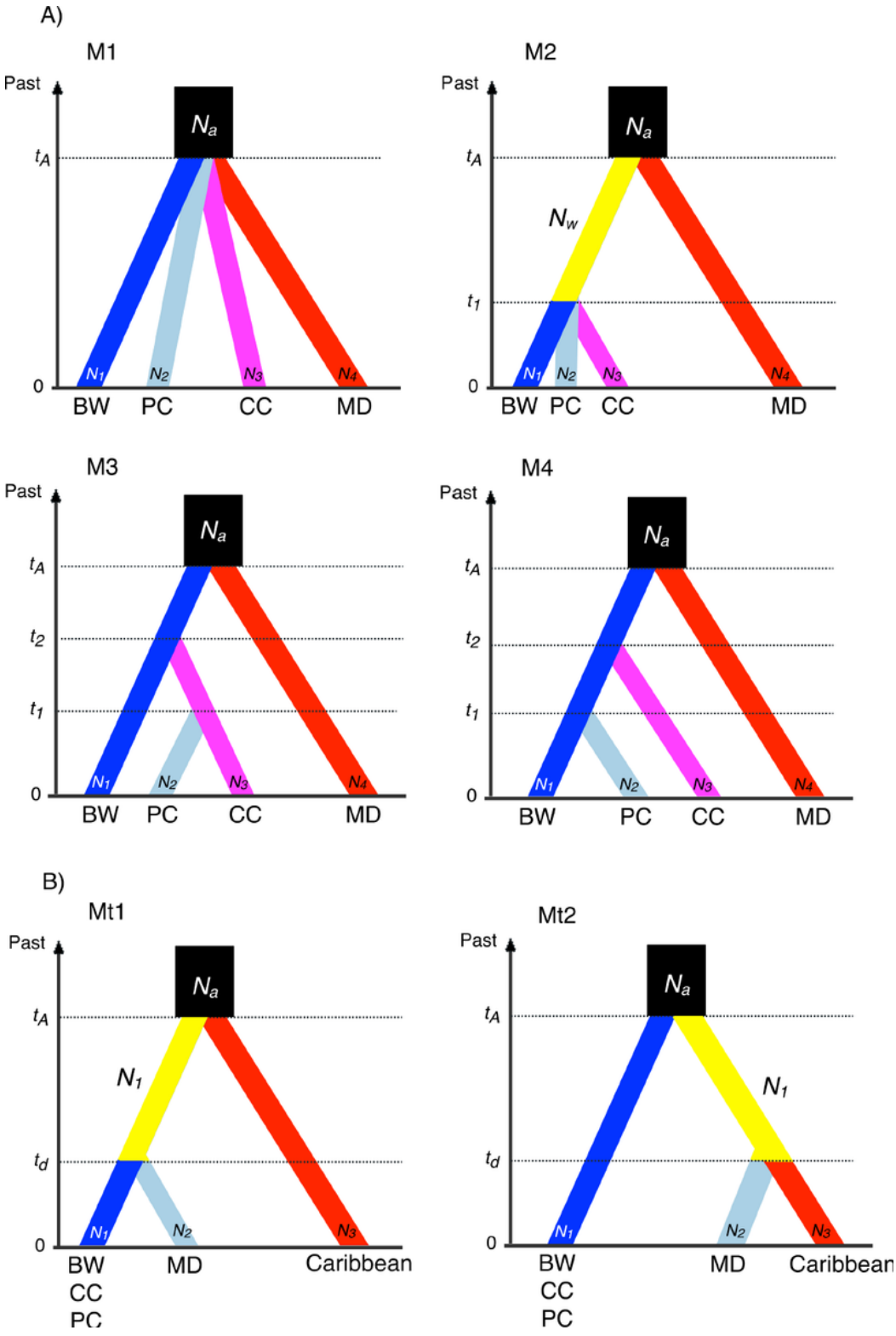

Figure 2

Demographic scenarios examined using DIYABC. Each model (M1 – M4) includes an unsampled ancestral population (Na), an initial divergence time (tA), and subsequent divergence periods moving forward in time (t2, t1), and the sizes of each regional population (CC, PC, BW, MD) since the time of the respective split. M2 included an unsampled ancestral ‘western population’ (NW) that subsequently

radiated into the three sampled populations. Bat populations are BW = Babcock Webb, PC = Polk Co., MD = Miami Dade Co., CC = Collier Co.

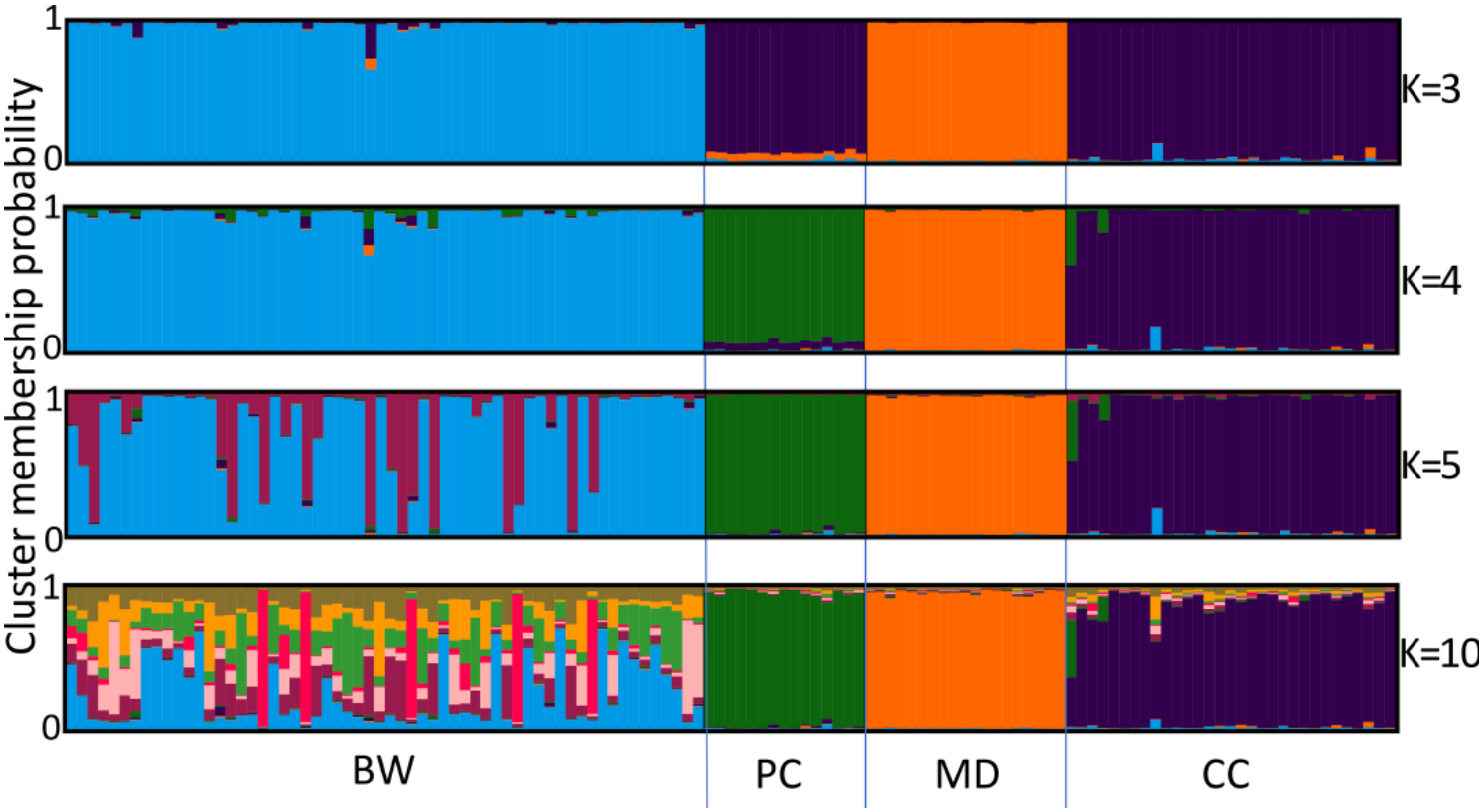

Figure 3

Individual proportional assignment (vertical bars) to one of K clusters as estimated by STRUCTURE. Bat genotypes are ordered by location (BW = Babcock Webb, PC = Polk Co., MD = Miami Dade Co., CC = Collier Co.). Results from multiple K models are shown to illustrate the three regional samples (PC, CC, MD) remained relatively homogeneous in their cluster membership, while additional clusters are apportioned among BW bats.

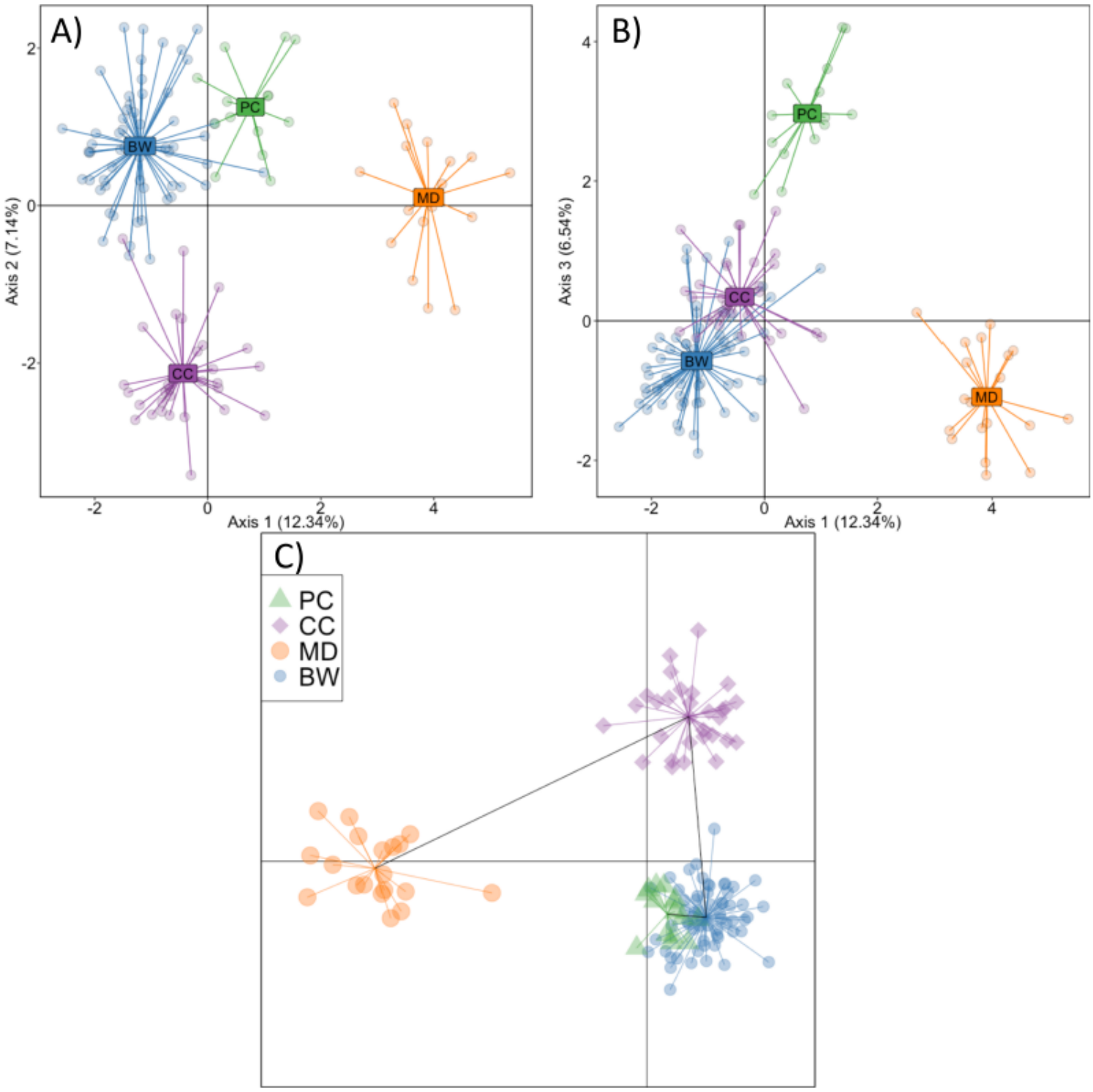

Figure 4

PCA results (A and B) and discriminant analysis of principal components (C) The line connecting the four clusters in C represents the minimum spanning tree based on squared distances among clusters.

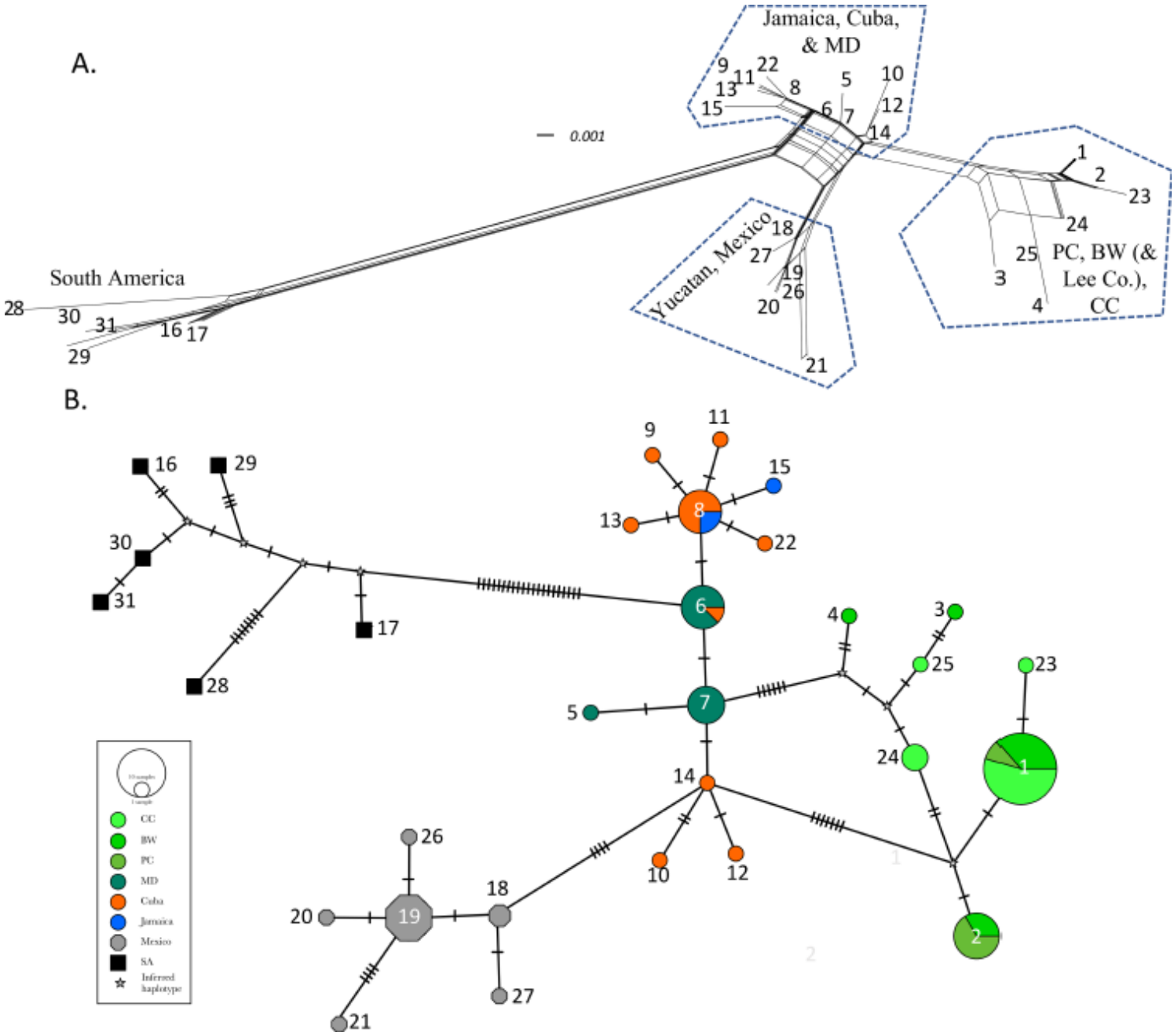

Figure 5

A) NeighborNet uncorrected p-distance network of 21 cyt b haplotypes (705 bp), including previously published Eumops from the Caribbean, Mexico and South America. The relationship of haplotypes from major geographic lineages are indicated by dotted lines. B) Parsimony network representing the most parsimonious relationships among haplotypes. Vertical hatch lines represent mutational differences separating haplotypes. Numbers for both panels are haplotype identifiers. Note that haplotypes 5 and 6 are from Lee Co. FL, near BW.

Supplementary Files

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