Genetic Diversity and Structure in Florida's Threatened Bletia purpurea (Orchidaceae): Insights from Target Capture Sequencing and Implications for Conservation

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Abstract

Urban expansion and land development around the Greater Everglades Ecosystem have resulted in fragmentation and decline of *Bletia purpurea* populations, a terrestrial orchid native to South Florida. As habitat loss continues, assessment of genetic diversity and structure in remnant populations is critical for developing effective conservation strategies. Although differences in phenology, habitat preferences, and reproductive traits such as self-fertilization have been documented in Florida populations, comprehensive genetic data for this species had not been obtained prior to this study. In this work, a target capture sequencing approach utilizing the Orchidaceae963 bait set was applied to evaluate genetic diversity and structure across eight wild populations in Collier, Miami-Dade, and Palm Beach counties. Results indicate a northwest corridor of genetic connectivity among some populations, despite distances exceeding 95 km, likely reflecting a formerly continuous range prior to anthropogenic fragmentation. Three populations of particular conservation concern were identified: Site S1 formed a distinct genetic cluster suggestive of isolation; Site E3 exhibited elevated inbreeding that may compromise population viability; and Site E4 was determined to be at risk of extirpation due to small size and proximity to urban development. These findings demonstrate the utility of targeted sequencing in conservation planning and provide essential baseline data to inform habitat restoration, population management, and preservation of genetic diversity in this state-threatened orchid.

INTRODUCTION

Few plant families command as much scientific and conservation attention as orchids, renowned for their floral diversity, intricate ecological relationships, and global distribution. Orchidaceae comprises over 25,000 species—around 8% of all vascular plants (Stokstad 2015)—making it one of the largest and most diverse plant families. Yet they face mounting threats from habitat loss, overcollection, and climate change (Christenhusz and Byng 2016). All wild-collected orchids are regulated under Appendix II of the Convention on International Trade in Endangered Species (CITES), reflecting global concern over population declines (Hinsley et al. 2018). Although such protections are important, persistence ultimately depends on conservation strategies informed by robust biological and genetic data.

Because of their dependence on mycorrhizal fungi and specialized pollinators, orchids are sensitive indicators of ecosystem health (Swarts and Dixon 2009), and highly vulnerable to disturbances such as microhabitat alteration, habitat fragmentation, and pollinator decline (Phillips et al. 2020). They are often among the first species to disappear in degraded habitats, and fragmentation can reduce gene flow, allelic diversity, and long-term viability—especially in species with narrow niches (Willoughby et al. 2015). Understanding these dynamics increasingly relies on fine-scale population genetic data, which remain limited across much of the species-rich family.

Florida exemplifies this conservation challenge as the most orchid dense state in the United States, home to more than half of the country's native species. Over 70 of its 106 extant orchids are state-listed as threatened or endangered (De Stefano et al. 2022). In the Greater Everglades Ecosystem—the nation's

orchid diversity hotspot—wetlands have declined by more than half due to drainage and urban expansion over the past century (National Park Service, 2021; Congressional Research Service 2025). Additional pressures, including fire suppression, invasive species, stochastic events, and ongoing development have further subdivided habitats, isolating populations and undermining viability (Borrero et al. 2023). Yet despite these threats, the genetic structure of Florida's orchids remain poorly documented, constraining efforts to prioritize populations for conservation.

One such case is *Bletia purpurea* (Pine-Pink orchid), a terrestrial, deciduous species widespread across the Neotropics and according to Royal Botanic Gardens, Kew (2025) is at its northeastern range limit in Florida. Globally the species is secure (G5), yet in Florida it is ranked vulnerable (S3) and state-listed as threatened (Wunderlin et al. 2022; NatureServe 2025). While the genus is centered in Mexico (Palestina and Sosa 2002), recent phylogenetic work has refined its taxonomy (Sosa and Chase 2020), emphasizing the importance of investigating peripheral populations like those in South Florida, where range limits may expose species to unique ecological pressures and shape distinct evolutionary trajectories (Eckert et al. 2008; Borrero et al. 2023).

Some Florida populations exhibit a mixed-mating system, producing both cleistogamous (closed, self-pollinating) and chasmogamous (open, potentially outcrossing) flowers within the same season (North American Orchid Conservation Center n.d). Reduced or absent rostellum in some individuals facilitates autonomous selfing, increasing inbreeding risk (Catling 1990). This flexibility, rare elsewhere in the range, may buffer against pollinator scarcity at range margins (Tremblay et al. 2005). Like many orchids, *B. purpurea* employs food deception, offering no nectar reward to its bee pollinators, which reduces visitation and opportunities for outcrossing (Johnson et al. 2004; Phillips et al. 2020). In fragmented habitats, heightened selfing can intensify genetic erosion, effects compounded by hydrological change and climate-driven shifts in flowering phenology (Pellegrino et al. 2015).

Marked ecological and phenological variation across Florida populations further complicates conservation. Most populations bloom December-March in open, disturbed habitats such as meadows and trails (Small 1928; Austin et al. 1990). In contrast, some Gulf Coast populations flower later, in April-May, and occur in shaded, seasonally flooded cypress domes, sometimes even rooting semi-aquatically on tree bases or floating logs (Brown 2005). These regional differences may reflect phenotypic plasticity or local adaptation, distinctions that are critical for restoration and translocation because mixing divergent gene pools can compromise fitness and persistence (Gentili et al. 2018; de Lima et al. 2024).

Although locally abundant in some sites, most Florida populations of *B. purpurea* are now confined to protected lands and parks (The Institute for Regional Conservation n.d.). Natural germination rates in orchids are extremely low (< 5%) and depend on fungal symbionts for establishment (Rao 1977). These recruitment constraints, combined with collection pressures and habitat loss, elevate extinction risk (Kartzinel et al. 2013; Liang et al. 2024). Yet the genetic diversity and structure of Florida populations remain undocumented, limiting efforts to anticipate vulnerability and guide management.

To address this gap, we assessed genetic variation and population subdivision in *B. purpurea* using target capture sequencing with the Orchidaceae963 bait set developed by the Atlanta Botanical Garden (Eserman et al. 2021). This method targets 963 single-copy nuclear genes, producing high-resolution single nucleotide polymorphism (SNP) datasets suitable for population analyses. Target capture is especially well-suited to orchid research: it is cost-effective, works with degraded DNA, and avoids issues such as allelic dropout in RADseq (Morin and McCarthy 2007; Gautier et al. 2012). Unlike microsatellites, it does not require species-specific markers, yielding scalable and repeatable data (Jones and Good 2016) across diverse orchid lineages. The Orchidaceae963 probes are particularly effective for the Epidendroideae subfamily (Eserman et al. 2021), which includes *B. purpurea*, ensuring high locus recovery and cross-taxon compatibility.

While prior studies have examined *Bletia* morphology (Palestina and Sosa 2002) and distribution (Beltrán-Nambo et al. 2012; Hellmuth 2018), population genetic structure—particularly in Florida's fragmented range—remains unexplored. Here, we present the first such assessment, analyzing eight populations across Collier, Miami-Dade, and Palm Beach counties. We hypothesized that habitat isolation and a mixed-mating system would promote reduced connectivity and pronounced genetic subdivision despite wind-mediated seed dispersal. The results provide a genetic baseline for conserving *B. purpurea* and broader guidance for managing threatened terrestrial orchids within the Greater Everglades Ecosystem.

MATERIALS AND METHODS

Sample Collection

A total of 271 *Bletia purpurea* leaf samples were collected from eight locations across three South Florida counties: 4 in Palm Beach, 3 in Collier, and 1 in Miami-Dade (Fig. 2), spanning > 95 km to reflect part of the species' historic range before Everglades fragmentation. Sites were located on conservation lands and residential canal banks, with all necessary permits or permissions obtained.

Within each location, 1–4 distinct sampling sites were defined by clear habitat breaks or distances > 5 km. Leaf samples (< 40 mm) were clipped from plants ≥ 1.5 m apart to minimize sampling clones; 20–30 individuals were sampled at larger sites (> 50 plants), and all individuals were sampled at smaller sites (≤ 20 plants). Sampling effort varied according to plant abundance and permit restraints. When multiple habitat types (e.g., meadow, canal bank, trail, cypress dome, roadside) occurred at a site, samples were taken from each. GPS coordinates were recorded where permitted. Leaf samples were dried on silica gel and stored at -18 ℃.

DNA Extractions and Quantification

Approximately 0.05 g of dried leaf tissue was homogenized with zirconia/silica beads and garnet particulates (FastPrep 24 Cycler, MP Biomedicals) and extracted using a modified 2%

cetyltrimethylammonium bromide (CTAB) protocol adapted from Doyle and Doyle (1987) and Storchova et al. (2000). DNA concentration was measured using the Qubit 4.0 fluorometer 1x dsDNA Broad-Range assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and fragment size distribution was visualized on agarose gels. Samples with DNA concentration > 10 ng/uL were retained for library preparation. Any leaf sample not used in DNA isolation remains preserved on silica gel in the Atlanta Botanical Garden Conservation DNA Biorepository.

Illumina Library Preparation and Sequencing

Genomic DNA was enzymatically sheared to ~ 550 bp fragments (New England Biolabs, Ipswich, MA) and dual-indexed libraries were prepared with the KAPA HyperPrep Kit (Roche, Basel, Switzerland) using primers from BadDNA (Athens, GA). Libraries were amplified for 12 PCR cycles and quantified using the Qubit 4.0 fluorometer 1x dsDNA High-Sensitivity assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The amount of sequenceable DNA within a subset of samples was assessed using qPCR (BioRad, Hercules, California, USA).

Libraries (130–170 ng in 7 uL each) were pooled in groups of 8–10 at equimolar concentrations into 9 pools from 136 high-quality samples (> 10 ng/uL). Pools were hybridized with the custom orchid-specific bait set, Orchidaceae963, (Arbor Biosciences myBaits) at 62°C for 24 hours, a temperature selected based on estimated divergence between *Bletia purpurea* and *Phalaenopsis equestris*, the reference genome used in bait set design (Cai et al., 2015; Johnson et al., 2019). Post-capture pools were amplified for 10 cycles, purified with Sera-Mag SpeedBeads (GE Healthcare, Pittsburgh, PA), and quantified using using the Qubit 4.0 fluorometer 1x dsDNA High-Sensitivity assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and qPCR (BioRad, Hercules, California, USA). Sequencing was performed on an Illumina NextSeq (PE150, 300 cycles) at Emory University's Integrated Genomics Core (Decatur, GA).

Gene Assembly and SNP Calling

A total of 136 *Bletia purpurea* samples were sequenced using the Orchidaceae963 bait set. Target capture and gene assembly were successful for 134 samples; two individuals failed during sequencing and could not be assembled. Raw reads were processed with the HybPiper pipeline (v1.3.1), which trims adaptors and low-quality bases using Trimmomatic (v0.39), retaining only paired reads. Fourteen samples sequenced in duplicate across different runs were merged prior to trimming to reduce bias and improve reproducibility. Cleaned reads were mapped to the Orchidaceae963 target genes with BWA (Li and Durbin 2009) and assemblies generated using SPAdes (v3.15.4). HybPiper extracted in-frame coding sequences (CDS) and adjacent introns, identified potential paralogs, and excluded genes present in fewer than 50% of samples.

Supercontigs were assembled for each gene, and the longest sequence from a high-coverage sample (96%, 929/963 genes) was used as the reference for SNP calling. Reads were mapped to supercontigs with BWA-MEM, and variants were called using GATK HaplotypeCaller (v4.6.0.0). Duplicate reads were removed, and sample GVCFs were merged. SNPs and indels were filtered with GATK GenotypesToPCA and VCFtools (v0.1.16), retaining one high-quality SNP per locus and excluding variants with outlier depth to minimize errors from mis-mapping or paralogs.

Three samples were excluded from downstream analysis because they recovered substantially fewer genes compared to the rest of the dataset. In total, 131 samples were retained for population genetic analysis.

Computational Analysis

All computational analyses, except for ADMIXTURE, were performed using Rstudio. For each population (n = 8 sites), we calculated standard genetic diversity statistics, including the number of genes recovered, total allelic counts, expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficients (F_{is}) using the *adegenet* package (v2.1.10). These values, summarized in Table 1, provide an overview of within and among population genetic diversity.

To visualize variation in locus recovery across individuals, a binary presence/absence matrix was constructed, with rows corresponding to individuals and columns to loci (coded as present = 1, absent = 0). The matrix was plotted with the *pheatmap* package (v1.0.12), with clustering disabled so that sample remained grouped by county. In the heatmap, unrecovered loci were shown in white, while recovered loci were color-coded by county (Palm Beach, Miami-Dade, and Collier), allowing regional differences in recovery to be readily identified (Fig. 3).

Individual inbreeding coefficients (F) were also estimated with *adegenet*. To establish thresholds for categorizing inbreeding levels, the empirical distribution of F values was examined. A histogram of all individuals revealed a distinct discontinuity between ~ 0.70 and 0.75, which was interpreted as a natural break separating typical from extreme inbreeding values. Based on this distributional criterion, individuals with $F > 0.725$ were identified as highly inbred. The final histogram was generated in *ggplot* (v3.5.1), with dashed vertical lines marking the thresholds for low ($F < 0.2$) and high ($F > 0.725$) inbreeding (Fig. 4).

Pairwise population differentiation was quantified using Nei's estimator of Wright's fixation index (F_{st}) in *adegenet*. These results, reported in Table 2, provide a measure of genetic divergence among populations.

Population structure was first evaluated using ADMIXTURE (v1.3.0), run in a Linux environment for K values ranging from 2–20 on both a single-SNP subset and the full dataset. The most likely number of ancestral populations (K) was inferred from cross-validation (CV) error scores. Principal Component

Analysis (PCA) was then conducted in R as a complementary approach to assess structure and visualize the clustering patterns suggested by ADMIXTURE. PCA was performed with the *ade4* package (v1.7–22), retaining the first three axes. Eigenvalues were inspected to assess the proportion of variation explained, and individuals were plotted along the first two principal components (PC1 and PC2), with point colors indicating population of origin (Fig. 5).

Finally, Discriminant Analysis of Principal Components (DAPC) was used to further characterize genetic structure and assign individuals to clusters. The 'find.clusters()' function in *adegenet* was used to determine the optimal number of clusters (*K*), with the Bayesian Information Criterion (BIC) supporting *K* = 6. DAPC was then performed on these clusters, and group membership was visualized with scatterplots of the discriminant axes (Fig. 6) and summarized in Table 3. While *K* = 6 was statistically supported, *K* = 3 was also examined which provided a more biologically interpretable grouping of individuals. The rationale for emphasizing *K* = 3 is presented in the Results and Discussion.

RESULTS

Genetic Diversity and Gene Recovery

Across 131 samples, an average of 920 genes (~ 96% of the bait set) was recovered per individual, exceeding the mean recovery reported for other Epidendroideae species (805 genes across 24 species; Eserman et al. 2021). Recovery was consistent across most sites, with all but one (E3) averaging > 900 genes. Site E3 showed the lowest mean recovery (888 genes per sample), though still within an acceptable range, suggesting that geographic distance among populations had little effect on capture efficiency.

From the retained samples, 38,354 SNPs were identified, of which 31,913 (~ 83%) passed quality filtering. After retaining a single SNP per locus, 903 high-quality loci remained for subsequent analyses, representing ~ 94% of the targeted gene set.

Across the eight sampling locations, observed heterozygosity (H_o) ranged from 0.029 to 0.067, while expected heterozygosity (H_e) ranged from 0.031 to 0.130, with overall means of 0.043 and 0.058, respectively. The inbreeding coefficient (F_{is}) ranged from − 0.210 to 0.490, with a mean of 0.240. Sites E4 and W1 showed nearly identical H_o and H_e values accompanied by low to moderate F_{is} (-0.21 and 0.18). Sites E1, E2, and W3 had slightly lower H_e then H_o (+ 0.010 to + 0.009), corresponding with moderate F_{is} values (E1 = 0.14, E2 = 0.066, W3 = 0.16). In contrast, sites E3, W2, and S1 had lower H_o relative to H_e, consistent with elevated F_{is} values (E3 = 0.79, W2 = 0.49, S1 = 0.23). Summary statistics for gene recovery, allele counts, and genetic diversity are shown in Table 1.

Table 1
Summary statistics of genetic diversity for *Bletia purpurea* (n = 131) across eight sampling locations. For each population, the average number of genes assembled (out of 963), total number of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{is}) are reported.

Location Code	County	N	Avg # of Genes	Total # of Alleles	H_o	H_e	F_{is}
E1	Palm Beach	27	929	863	0.048	0.038	0.14
E2	Palm Beach	26	922	892	0.043	0.034	0.066
E3	Palm Beach	14	888	900	0.029	0.13	0.79
E4	Palm Beach	5	918	723	0.031	0.031	-0.21
S1	Miami-Dade	15	932	807	0.037	0.048	0.33
W1	Collier	17	913	885	0.034	0.035	0.18
W2	Collier	16	928	992	0.067	0.101	0.49
W3	Collier	11	932	748	0.056	0.047	0.16
all	-	131	920				

To visualize recovery patterns across individuals, a heatmap was generated, with samples grouped by county and color-coded accordingly (Fig. 3). Most loci were successfully recovered across individuals, with only a small subset showing low capture success. Recovery was consistent across regions, although the most pronounced gaps occurred in a subset of Palm Beach samples, supporting the robustness of the Orchidaceae963 bait set for *B. purpurea* and anticipating patterns described below.

Observed inbreeding coefficients (*F*) varied widely across individuals and sites (Fig. 4). More than half of individuals (55%, 72/131) exhibited low inbreeding (*F* = 0.0-0.2), 37.4% showed moderate inbreeding (*F* = 0.2–0.7), and 7.6% (10 individuals) were classified as highly inbred (*F* > 0.725). Notably, all of the highly inbred individuals were restricted to site E3, where they represented 71.4% of all individuals, distinguishing this site from all others.

Population Structure

Given the elevated inbreeding observed at site E3, population structure was examined to assess among-population differentiation. Pairwise F_{st} values (Table 2) indicated variable levels of heterogeneity among sites, with values ranging from minimal (< 0.05) to strong (> 0.25). The clearest signal was the pronounced differentiation of E3, which was consistently distinct from all other populations (F_{st} >0.24).

By contrast, E1 and E2 showed negligible divergence from each other and from western sites W2 and W3, suggesting a history of connectivity among these populations.

Within Palm Beach, E4 displayed a mixed pattern, closely allied with W2 but strongly diverging strongly from W3. In Collier County, W2 and W3 were nearly indistinguishable, while W1 was more moderately differentiated from both. The southern site S1 was minimally differentiated from W2 and W3 but showed higher divergence from Palm Beach populations and W1. Taken together, these patterns indicate relatively cohesive population structure and historical gene flow across regions despite some current geographical isolation, with the strongest discontinuity consistently observed between E3 and all other sites.

Table 2
Average pairwise F_{st} values among populations of *Bletia purpurea*. Strong differentiation (F_{st} >0.15) is shown in bold, minimal differentiation (F_{st} < 0.05) in italics, and moderate values in plain text.

Strata	**E1**	**E2**	**E3**	**E4**	**S1**	**W1**	**W2**
E2	*-0.018*						
E3	**0.398**	**0.412**					
E4	**0.180**	**0.241**	**0.261**				
S1	0.145	**0.200**	**0.327**	0.103			
W1	0.074	0.135	**0.387**	**0.170**	**0.189**		
W2	*-0.007*	*0.021*	**0.247**	*-0.026*	*-0.001*	0.075	
W3	*-0.012*	*0.028*	**0.303**	**0.244**	*-0.056*	0.063	*-0.058*

To visualize these relationships, a Principal Component Analysis (PCA) was performed using the filtered SNP dataset. Most individuals cluster tightly together, indicating overall genetic similarity across populations. However, samples from E3 and S1 separated from the main cluster along the first two principal components (PC1: 20.5%, PC2: 13.9%), together explain 34.4% of the total genetic variation (Fig. 5). This separation aligns with the high Fst for E3 and moderate values for S1, supporting their greater genetic isolation.

ADMIXTURE analysis identified $K = 3$ as the best-fitting model based on cross-validation scores ($K = 2–20$; Supplementary Fig. 1), revealing three major ancestral groups with varying admixture proportions across sites. To further explore genetic subdivision, a Discriminant Analysis of Principal Components (DAPC) was conducted with $K = 2–6$ (Fig. 6). The Bayesian Information Criterion (BIC) suggested $K = 6$ (Fig. 6A), but three of these clusters were represented by very small, site-specific sample sizes, likely reflecting local inbreeding or environmental variation rather than broader structure. For biological interpretation, $K = 3$ was retained, which was consistent across ADMIXTURE and PCA results.

These three DAPC clusters included: (1) a dominant group (94/131, 71.7%) present at all sites except S1; (2) a cluster concentrated in S1 but detected at five sites (18/131, 13.7%); and (3) a group linking Palm Beach and Miami-Dade counties, present in E1, E4, and S1 (11/131, 8.4%). Cluster assignments are summarized in Table 3, and their spatial and genetic relationships are shown in Fig. 6B-C.

Table 3
Assignment of *Bletia purpurea* individuals to the six genetic clusters identified in Fig. 6. Values represent the number of individuals per cluster within each sampling location. Totals and percentages across all populations are provided in the bottom rows. Bold values highlight small, distinct clusters that were excluded from downstream biological analyses but reflect the extent of genetic subdivision among Florida populations.

Strata	N	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
E1	27	21	1	5	0	0	0
E2	26	25	1	0	0	0	0
E3	14	7	0	0	**4**	**3**	0
E4	5	1	0	4	0	0	0
S1	15	0	13	2	0	0	0
W1	17	17	0	0	0	0	0
W2	16	13	2	0	0	0	**1**
W3	11	10	1	0	0	0	0
Total	*131*	*94*	*18*	*11*	*4*	*3*	*1*
%	*100*	*71.8*	*13.7*	*8.4*	*3.1*	*2.3*	*0.8*

Together these analyses reveal a largely cohesive genetic landscape for *B. purpurea* across Florida, despite considerable land-use changes over the last century, punctuated by notable differentiation and localized inbreeding in certain populations. These patterns highlight both the resilience and potential vulnerability of populations in fragmented habitats, providing a crucial context for conservation planning.

DISCUSSION

Genetic Structure

Despite growing interest in orchid conservation, the genetic composition of South Florida's native species remains poorly characterized, limiting effective restoration and recovery strategies. Most monitoring programs begin only after substantial declines are observed (Baldwin et al. 2012), leaving managers without baseline data to track long-term trends or identify drivers of population loss. This study provides the first landscape-scale population genetic assessment of *Bletia purpurea* in Florida,

revealing pronounced spatial structure, reduced diversity in some sites, and signs of localized genetic vulnerability.

Mapping genetic diversity is critical for identifying at-risk populations, particularly in regions such as South Florida where orchid declines are widespread (De Stefano et al. 2022). Three genetic clusters were detected: the largest (~ 72% of individuals) spanned over 95 km between Palm Beach and Collier counties with low differentiation, suggesting historically high gene flow; a second cluster—largely restricted to Everglades National Park (site S1)—was genetically distinct, consistent with long-term isolation; and a third cluster linked populations between Palm Beach and Miami-Dade counties. Together, these clusters indicate that *B. purpurea* once formed a more continuous metapopulation across the three counties, with present-day structure likely emerging only after recent fragmentation.

The distinctiveness of the southern Miami-Dade cluster may reflect ecological specialization or historical isolation, whereas the broader Palm Beach/Collier corridor suggests that connectivity persisted until recent habitat loss curtailed dispersal. The scale of subdivision and reduced diversity is consistent with findings in other disturbance-sensitive orchids (Chung et al. 2014; Liang et al. 2021; Ellwanger et al. 2022), where fragmentation, pollinator decline, and small population size restrict gene flow and intensify drift (Jacquemyn et al. 2008; Pellegrino et al. 2015). Maintaining habitat connectivity and safeguarding pollinator and mycorrhizal networks remain key conservation priorities (Pauw and Hawkins 2010; Kartzinel et al. 2013).

Genetic clustering was consistently supported by ADMIXTURE, PCA, and DAPC analyses. ADMIXTURE estimated ancestry proportions, while PCA and DAPC visualized population differentiation independent of Hardy–Weinberg or linkage assumptions (Patterson et al. 2006; Jombart et al. 2010). The agreement among these methods reinforces the robustness of the observed spatial structure.

Site-Specific Patterns and Conservation Concerns

Within this broader structure, site S1 emerged as especially distinctive. Despite its location inside the vast protected area of Everglades National Park (ENP), it largely formed a separate genetic cluster, suggesting that even large, protected areas can harbor small, isolated populations that diverge through drift and limited gene flow (Aguilar et al. 2008; Swartz and Dixon 2009; Chung et al. 2014). Although some admixture with northern populations were detected, the strong separation of S1 may reflect long-term isolation, reduced connectivity, or the limited footprint of our sampling, which was restricted to a single meadow.

This meadow lies within the Hole-in-the Donut (HID) Restoration Project, which has restored > 6,000 acres of former agricultural land since 1989 (National Park Service 2022). To our knowledge, *B. purpurea* has not been artificially introduced there, suggesting persistence through past disturbance or natural recolonization, potentially facilitated by pollinator availability (Pauw and Hawkins 2011). Genetic metrics support this view: S1 showed moderately high F_{is}, moderate differentiation (F_{st}), and no individuals with extreme inbreeding ($F > 0.725$). Its distinctiveness therefore may reflect survival of a remnant lineage or

recolonization followed by drift (Tremblay et al. 2005; Phillips et al. 2012). In either case, the population's persistence within a major restoration project underscores the value of integrating genetic monitoring into habitat recovery (Mijangos et al. 2015; Ellwanger et al. 2022).

By contrast, the other restoration site (E3) clustered with Palm Beach/Collier County populations but showed extreme inbreeding ($F > 0.725$ in over 70% of individuals), with two small clusters excluded due to homozygosity artifacts. These results suggest that habitat recovery can yield very different genetic outcomes depending on historical context and surrounding land use (Tremblay and Ackerman 2001; Agiular et al. 2008; Phillips et al. 2011). At E3, exotic vegetation management began in 2008 after county acquisition in 2001, but no large-scale restoration has occurred, and the site remains boarded by agriculture. Its genetic profile likely reflects recent bottlenecks, limited pollinator movement, and reduced seed dispersal (Scobie and Wilcock 2009; Kartzinel et al. 2013). Ongoing declines in native bee communities, some of which pollinate orchids, may further exacerbate selfing and geitonogamy (Johnson et al. 2004; Ulyshen and Horn 2023).

E4, a small urban population, showed low inbreeding but was genetically distinct from nearby sites, likely due to isolation and reduced size (Jacquemyn et al. 2008; Pellegrino et al. 2015; Liang et al. 2024). Together, the contrasting outcomes at S1, E3, and E4 highlight the diverse genetic trajectories of orchid populations under restoration and urban pressures: a distinctive but relatively healthy enclave (S1), a highly inbred site despite ongoing management (E3), and a small urban isolate maintaining diversity despite fragmentation (E4). These comparisons emphasize how land-use history, habitat context, and pollinator dynamics interact to shape genetic outcomes, highlighting the need to tailor conservation strategies to site-specific conditions (Phillips et al. 2020; Ellwanger et al. 2022).

Implications for Conservation Planning

These patterns highlight both the protective value of conservation areas and the hidden risks they may harbor (Méndez et al. 2014). Even within large reserves, populations such as S1 or E3 may require targeted management, while small urban isolates like E4 retain diversity but remain vulnerable due to size and fragmentation.

Our findings support a regionally tailored conservation strategy for *B. purpurea*, integrating habitat restoration, genetically informed seed sourcing, and establishment of habitat corridors to reduce inbreeding and preserve local adaptation (Frankham et al., 2010; Phillips et al. 2020). Genetic data can also guide delineation of conservation units, ensuring restoration reflects natural genetic structure and avoids homogenization (Swartz and Dixon 2009).

For highly inbred or genetically distinct populations like E3, ex situ strategies–such as seed banking and controlled propagation–may buffer against further decline (De Stefano et al. 2022). At the same time, ongoing restoration near E3 could incorporate genetic criteria, including seed mixing and planting nectar-producing species to support pollinator recovery and outcrossing (Scoobie and Wilcock 2009; Pauw and Hawkins 2010). Integrating these approaches can enhance genetic diversity and increase the long-term

success of population reinforcement and reintroduction efforts (Méndez et al. 2014; Mijangos et al. 2014).

Methodological Insights and Future Research

This study demonstrates the value of target capture sequencing for population-level research in orchids and other non-model species with large and complex genomes (Grover et al. 2012). While commonly used in phylogenomics, this method is increasingly applied to fine-scale population analyses (Christmas et al. 2016; Noël et al. 2018). The Orchidaceae963 bait set recovered highly informative loci that resolved subtle divergence in *B. purpurea*, including at sites S1, E3, and E4. Compared to whole-genome approaches, it is a scalable and cost-effective method suited to the taxonomic complexity and conservation urgency of Orchidaceae (Jones and Good 2016; Hale et al. 2020). As demonstrated here, it provides actionable insights for both immediate conservation efforts and long-term genetic monitoring.

Herbarium specimens represent an underused but valuable extension of this framework, as target capture can yield high-quality data from degraded DNA (Morin and McCarthy 2007). Such material can establish historical genetic baselines and clarify whether divergence at sites like S1 reflects recent isolation or deeper evolutionary history. This is particularly beneficial in heavily protected areas such as Everglades National Park, where in situ sampling can be constrained by permitting. Leveraging herbarium collections can therefore complement limited field sampling and fill critical gaps in temporal and spatial genetic coverage (Swarts and Dixon 2009).

Future work should expand sampling across South Florida, especially in urban areas. Seven of the eight sites analyzed here were located within protected or managed lands, but small, unmanaged urban populations like E4 may follow different genetic trajectories. Broader urban sampling could reveal how isolation, land-use history, and pollinator decline shape orchid populations outside of formal conservation areas (Tremblay and Ackerman 2001). Comparative studies with Caribbean and Latin American *B. purpurea* populations—where the species is reportedly not autogamous—could further clarify the evolutionary history and ecological adaptations of Florida's populations.

Although *Bletia purpurea* is considered secure across much of its Neotropical range, Florida populations reveal a more precarious picture. Genetic resilience within some protected areas contrasts with high inbreeding and vulnerability in fragmented, human-modified environments. This tension between global security and local risk exemplifies the conservation challenge faced by many orchids at their range margins, where isolation and ecological pressures can drive divergence or genetic erosion (Eckert et al., 2008; Borrero et al., 2023).

Integrating genomic tools into conservation planning enables managers to identify genetically compatible seed sources, prioritize habitat corridors, and safeguard distinct lineages before they are lost (Chung et al. 2014; Liang et al. 2021; de Lima et al. 2024). These findings reinforce the importance of establishing genetic baselines not only for *B. purpurea* but also for orchids throughout Florida and the Greater Everglades Ecosystem. More broadly, they highlight the need to complement regulatory

frameworks with fine-scale genetic data, ensuring that local management sustains the evolutionary potential (Méndez et al. 2014) and long-term resilience of Orchidaceae in rapidly changing landscapes.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

Bethany A. Simpson—conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, visualization, & writing–original draft. Lauren A. Eserman—conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, software, supervision, validation, visualization, & writing–review & editing. Amanda Carmichael—data curation, investigation, project administration, resources, validation, & writing–review & editing. John D. Baldwin—conceptualization, funding acquisition, methodology, resources, supervision, validation, & writing–review & editing.

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Data Availability

The datasets generated and analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1307027: [] (https:/www.ncbi.nlm.nih.gov/sra/PRJNA1307027) [https://www.ncbi.nlm.nih.gov/sra/PRJNA1307027] (https:/www.ncbi.nlm.nih.gov/sra/PRJNA1307027)

References

1. Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: Susceptible signals in plant traits and methodological approaches. Mol Ecol 17(24):5177–5188. https://doi.org/10.1111/j.1365-294x.2008.03971.x
2. Austin DF, Jones JL, Bennett BC (1990) Endangered plants of Fakahatchee Strand State Preserve. Rhodora 92:27–35
3. Baldwin JD, Bosley JW, Oberhofer L, Bass OL, Mealey BK (2012) Long-term changes, 1958–2010, in the reproduction of Bald Eagles of Florida Bay, Southern Coastal Everglades. J Raptor Res 46:336–348. https://doi.org/10.3356/JRR-11-65.1
4. Beltrán-Nambo MA, Ortega Larrocea P, Salgado Garciglia R, Tupac Otero Ospina J, Martínez Trujillo M, Carreón-Abud Y (2012) Distribution and abundance of terrestrial orchids of the genus Bletia in sites with different degrees of disturbance, in the Cupatitzio Natural Reserve, México. Int J Biodivers Conserv 4:280–289. https://doi.org/10.5897/IJBC11.225
5. Borrero H, Oviedo-Prieto R, Alvarez JC, Ticktin T, Cisneros M, Liu H (2023) Populations of a tropical epiphytic orchid are destabilized in its peripheral range by hurricane and an exotic herbivore. Ecosphere 14(1) Article e4355. https://doi.org/10.1002/ecs2.4355
6. Brown PM (2005) Wild orchids of Florida: With references to the Atlantic and Gulf Coastal Plains. University Press of Florida, Gainesville, FL, pp 120–121
7. Cai J, Liu X, Vanneste K, Proost S, Tsai W-C, Liu K-W, Chen L-J et al (2015) The genome sequence of the orchid Phalaenopsis equestris. Nat Genet 47:65–72
8. Catling PM (1990) Auto-pollination in the Orchidaceae. In: Arditti J (ed) Orchid Biology: Reviews and Perspectives, V. Timber, pp 121–158
9. Christenhusz MJM, Byng JW (2016) The number of known plant species in the world and its annual increase. Phytotaxa 261:201. https://doi.org/10.11646/phytotaxa.261.3.1
10. Christmas MJ, Biffin E, Breed MF, Lowe AJ (2016) Finding needles in a genomic haystack: targeted capture identifies clear signatures of selection in a non-model plant species. Mol Ecol 25:4216–4233. https://doi.org/10.1111/mec.13750
11. Chung MY, Nason JD, López-Pujol J, Yamashiro T, Yang B-Y, Luo Y-B, Chung MG (2014) Genetic consequences of fragmentation on populations of the terrestrial Orchid Cymbidium Goeringii. Biol Conserv 170:222–231. https://doi.org/10.1016/j.biocon.2013.12.005
12. Congressional Research Service (2025) Everglades degradation: By the end of the 20th century, approximately half of the original Everglades wetlands had been lost to drainage and development.

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13. de Lima TM, da Silva SF, Sánchez-Vilas J, Júnior WLS, Mayer JLS, Ribeiro RV, Pinheiro F (2024) Phenotypic plasticity rather than ecotypic differentiation explains the broad realized niche of a Neotropical orchid species. Plant Biol 26(6):989–997. https://doi.org/10.1111/plb.13684
14. De Stefano D, Costa BN, Downing J, Fallahi E, Khoddamzadeh AA (2022) In vitro micropropagation and acclimatization of an endangered native orchid using organic supplements. Am J Plant Sci 13:380–393. https://doi.org/10.4236/ajps.2022.133023
15. Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bull 19:11–15
16. Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: The central–marginal hypothesis and beyond. Mol Ecol 17(5):1170–1188. https://doi.org/10.1111/j.1365-294X.2007.03659.x
17. Ellwanger C, Steger L, Pollack C, Wells R, Fant JB (2022) Anthropogenic fragmentation increases risk of genetic decline in the threatened orchid Platanthera leucophaea. Ecol Evol 12:e8578. https://doi.org/10.1002/ece3.8578
18. Eserman LA, Thomas SK, Coffey EED, Leebens-Mack JH (2021) Target sequence capture in orchids: Developing a kit to sequence hundreds of single-copy loci. Appl Plant Sci 9:e11416. https://doi.org/10.1002/aps3.11416
19. Frankham R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetics. Cambridge University Press, Cambridge, UK
20. Gautier M, Gharbi K, Cezard T, Foucaud J, Kerdelhué C, Pudlo P, Cornuet J, Estoup A (2012) The effect of RAD allele dropout on the estimation of genetic variation within and between populations. Mol Ecol 22(11):3165–3178. https://doi.org/10.1111/mec.12089
21. Gentili R, Solari A, Diekmann M, Duprè C, Monti GS, Armiraglio S, Assini S, Citterio S (2018) Genetic differentiation, local adaptation and phenotypic plasticity in fragmented populations of a rare forest herb. PeerJ 6:e4929. https://doi.org/10.7717/peerj.4929
22. Grover CE, Salmon A, Wendel JF (2012) Targeted sequence capture as a powerful tool for evolutionary analysis. Am J Bot 99(2):312–319. https://doi.org/10.3732/ajb.1100323
23. Hale H, Gardner EM, Viruel J, Pokorny L, Johnson MG (2020) Strategies for reducing per-sample costs in target capture sequencing for phylogenomics and population genomics in plants. Appl Plant Sci 8(4). https://doi.org/10.1002/aps3.11337
24. Hellmuth N (2018) Plants of Yaxha, Petén, Guatemala: Aquatic orchids (Bletia purpurea). FLARR Report
25. Hinsley A, de Boer HJ, Fay MF, Gale SW, Gardiner LM, Gunasekara RS, Kumar P, Masters S, Metusala D, Roberts DL, Veldman S, Wong S, Phelps J (2018) A review of the trade in orchids and its implications for conservation. Bot J Linn Soc 186:435–455. https://doi.org/10.1093/botlinnean/box083

26. Jacquemyn H, Brys R, Adriaens D, Honnay O, Roldán-Ruiz I (2008) Effects of population size and forest management on genetic diversity and structure of the Tuberous Orchid orchis mascula. Conserv Genet 10(1):161–168. https://doi.org/10.1007/s10592-008-9543-z
27. Johnson MG, Pokorny L, Dodsworth S, Botigué LR, Cowan RS, Devault A, Eiserhardt WL et al (2019) A universal probe set for targeted sequencing of 353 nuclear genes from any flowering plant designed using k-medoids clustering. Syst Biol 68:594–606
28. Johnson SD, Peter CI, Ågren J (2004) The Effects of Nectar Addition on Pollen Removal and Geitonogamy in the Non-Rewarding Orchid Anacamptis morio. Proceedings: Biological Sciences, 271(1541), 803–809. http://www.jstor.org/stable/4142511
29. Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94. https://doi.org/10.1186/1471-2156-11-94
30. Jones MR, Good JM (2016) Targeted capture in evolutionary and ecological genomics. Mol Ecol 25:185–202. https://doi.org/10.1111/mec.13304
31. Kartzinel TR, Shefferson RP, Trapnell DW (2013) Relative importance of pollen and seed dispersal across a Neotropical mountain landscape for an epiphytic orchid. Mol Ecol 22(24):6048–6059. https://doi.org/10.1111/mec.12551
32. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25(14):1754–1760. https://doi.org/10.1093/bioinformatics/btp324
33. Liang C, Li J, Li S, Zhang H, Zheng J, Miao J, Hao S, Wu S, Liu Z, Zhai J (2024) Human Activity Changed the Genetic Pattern of the Orchid Phaius flavus Population. Diversity. 2024; 16(11):685. https://doi.org/10.3390/d16110685
34. Liang HY, Wang XG, Chen W et al (2021) Spatial genetic structure of terrestrial orchid Cymbidium faberi in the Qinling Mountains revealed by microsatellite loci. Plant Syst Evol 307:5. https://doi.org/10.1007/s00606-020-01735-y
35. Méndez M, Vögeli M, Tella JL, Godoy JA (2014) Joint effects of population size and isolation on genetic erosion in fragmented populations: finding fragmentation thresholds for management. Evol Appl 7:506–518. https://doi.org/10.1111/eva.12154
36. Mijangos JL, Pacioni C, Spencer RJ, Craig MD (2015) Contribution of genetics to ecological restoration. Mol Ecol 24(1):22–37. https://doi.org/10.1111/mec.12995
37. Morin PA, McCarthy M (2007) Highly accurate SNP genotyping from historical and low-quality samples. Mol Ecol Notes 7:937–946. https://doi.org/10.1111/j.1471-8286.2007.01804.x
38. National Park Service (2022) *Hole-in-the-Donut Restoration Project – Restoring wetlands on abandoned agricultural lands within Everglades National Park*. U.S. Department of the Interior. Retrieved August 4, 2025, from https://www.nps.gov/ever/learn/nature/hidprogram.htm
39. National Park Service (2021) *Orchids in Everglades National Park.* U.S. Department of the Interior. Available at: https://www.nps.gov/ever/learn/nature/orchids.htm (accessed 21 June 2023)

40. NatureServe (2025) Bletia purpurea (Pine-Pink Orchid). In NatureServe Explorer (Version 7.2). NatureServe. Retrieved July 16, 2025, from https://explorer.natureserve.org
41. Noël L, Chancerelle Y, Mariette S, Guichoux E, Dumet A, Petit RJ (2018) Targeted sequence capture and relatedness estimation in a mixed oak stand. Mol Ecol Resour 18:161–177. https://doi.org/10.1111/1755-0998.12713
42. North American Orchid Conservation Center (n.d.) Bletia purpurea (Pine Pink). Go Orchids. Retrieved June 21 (2025) from https://goorchids.northamericanorchidcenter.org/species/bletia/purpurea/
43. Palestina RA, Sosa V (2002) Morphological variation in populations of Bletia purpurea (Orchidaceae) and description of the new species B. riparia. Brittonia 54:99–111. https://doi.org/10.1663/0007-196X(2002)054[0099:MVIPOB]2.0.CO;2
44. Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. PLoS Genet 2(12):e190. https://doi.org/10.1371/journal.pgen.0020190
45. Pauw A, Hawkins JA (2010) Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. Oikos 120(3):344–349. https://doi.org/10.1111/j.1600-0706.2010.19039.x
46. Pellegrino G, Bellusci F, Palermo AM (2015) Effects of population structure on pollen flow, clonality rates and reproductive success in fragmented Serapias lingua populations. BMC Plant Biol 15., Article 222. https://doi.org/10.1186/s12870-015-0600-8
47. Phillips RD, Barrett MD, Dixon KW, Hopper SD (2011) Do mycorrhizal symbioses cause rarity in orchids? J Ecol 99(3):858–869. https://doi.org/10.1111/j.1365-2745.2011.01797.x
48. Phillips RD, Brown AP, Peakall R (2012) Low population genetic differentiation in the Orchidaceae. Mol Ecol 21(17):4078–4090. https://doi.org/10.1111/mec.12036
49. Phillips RD, Reiter N, Peakall R (2020) Orchid conservation: from theory to practice. Ann Botany 126(3):345–362. https://doi.org/10.1093/aob/mcaa093
50. Rao AN (1977) Tissue culture in the orchid industry. In: Reinert J, Bajaj YPS (eds) Applied and fundamental aspects of plant cell tissue organ culture. Springer, Berlin, pp 44–69
51. Gardens RB (2025) Kew. *Bletia purpurea*. Plants of the World Online. Retrieved August 7, 2025, from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:1218040-2
52. Scobie AR, Wilcock CC (2009) Limited mate availability decreases reproductive success of fragmented populations of Linnaea borealis, a rare, clonal self-incompatible plant. Ann Botany 103(6):835–846. https://doi.org/10.1093/aob/mcp007
53. Sosa V, Chase MW (2020) New species of Bletia (Orchidaceae) from the Caribbean Basin. Phytotaxa 456:297–306. https://doi.org/10.11646/phytotaxa.456.3.6
54. Small JK (1928) Bletia purpurea. Addisonia: Colored illustrations and popular descriptions of plants, vol 13. New York Botanical Garden, New York, pp 40–42
55. Stokstad E (2015) Orchids' dazzling diversity explained. Science. https://doi.org/10.1126/science.aad1667

56. Storchová H, Hrdličková R, Chrtek J, Tetera M, Fitze D, Fehrer J (2000) An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. Taxon 49(1):79–84. https://doi.org/10.2307/1223934
57. Swarts ND, Dixon KW (2009) Terrestrial orchid conservation in the age of extinction. Ann Botany 104(3):543–556. https://doi.org/10.1093/aob/mcp025
58. Institute for Regional Conservation (n.d.). Bletia purpurea (Lam.) DC. Pinepink. The Floristic Inventory of South Florida. https://regionalconservation.org/ircs/database/plants/PlantPage.asp?TXCODE=Bletpurp
59. Tremblay RL, Ackerman JD (2001) Gene flow and effective population size in Lepanthes (Orchidaceae): A case for genetic drift. Biol J Linn Soc 72(1):47–62. https://doi.org/10.1111/j.1095-8312.2001.tb01300.x
60. Tremblay RL, Ackerman JD, Zimmerman JK, Calvo RN (2005) Variation in sexual reproduction in orchids and its evolutionary consequences: A spasmodic journey to diversification. Biol J Linn Soc 84(1):1–54. https://doi.org/10.1111/j.1095-8312.2004.00400.x
61. Ulyshen M, Horn S (2023) Declines of bees and butterflies over 15 years in a forested landscape. Curr Biol 33(7). https://doi.org/10.1016/j.cub.2023.02.030
62. Willoughby JR, Sundaram M, Wijayawardena BK, Kimble SJA, Ji Y, Fernandez NB, Antonides JD, Lamb MC, Marra NJ, DeWoody JA (2015) The reduction of genetic diversity in threatened vertebrates and new recommendations regarding IUCN conservation rankings. Biol Conserv 191:495–503. https://doi.org/10.1016/j.biocon.2015.07.025
63. Wunderlin RP, Hansen BF, Franck AR, Essig FB (2022) *Atlas of Florida plants* (S. M. Landry & K. N. Campbell, Application development). Institute for Systematic Botany, University of South Florida. Retrieved from https://florida.plantatlas.usf.edu/plant/species/951

Figures

Figure 1

The Pine-Pink orchid (*Bletia purpurea)* in Southern Florida. (A) Inflorescence; (B) Developing fruits; (C) Cluster in a grassy meadow (Palm Beach); (D) Population in open wet pinelands (Palm Beach; orchids indicated by arrows); (E) Semi-aquatic cluster on cypress knee with exposed pseudobulbs (Collier); (F) Individual on cypress base in wooded swamp (Collier). *Photos by B.A. Simpson.*

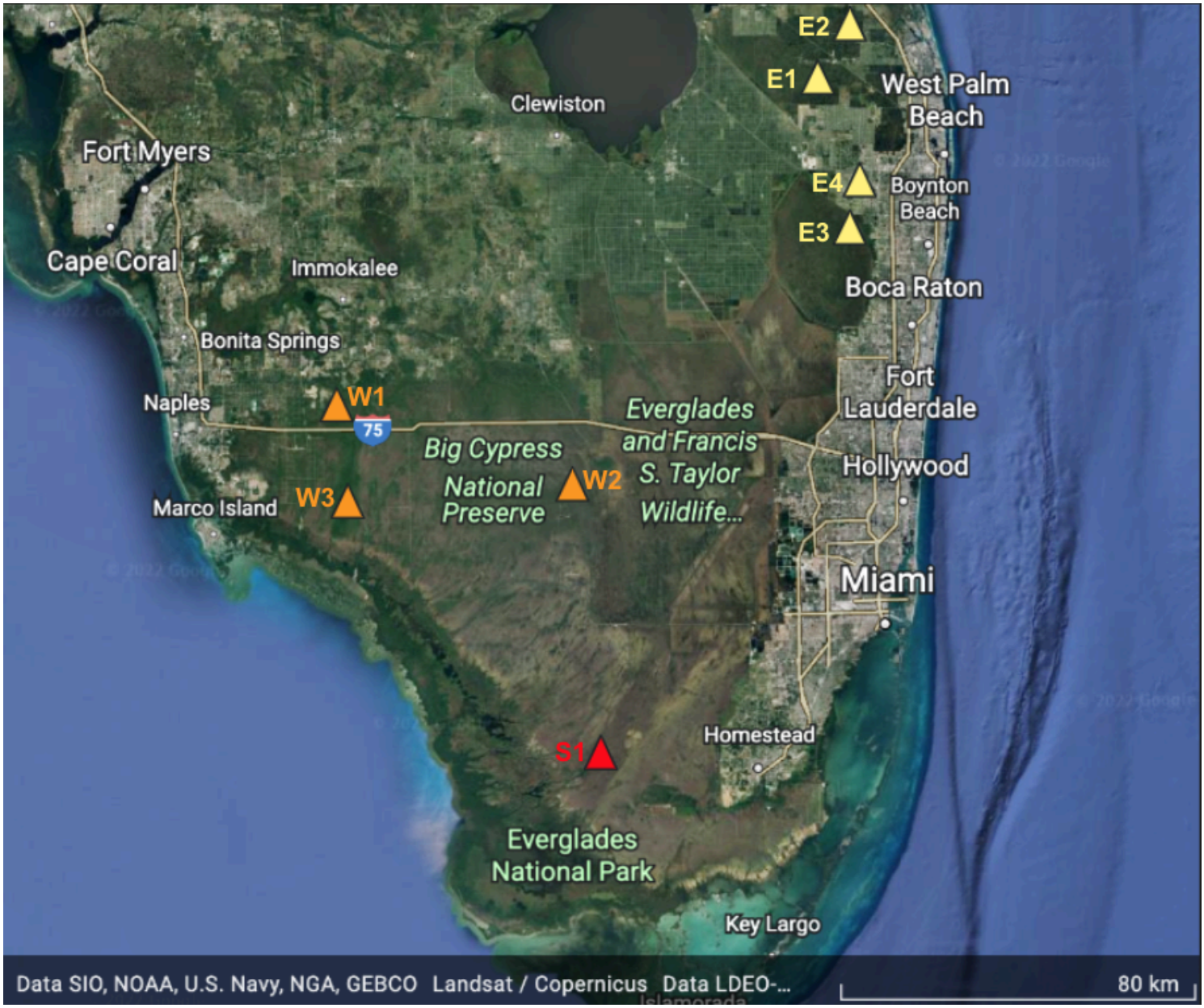

Figure 2

Locations of study sites for population-level genetic comparisons (n=8). Yellow triangles represent the sites in Palm Beach County, orange represents Collier County, and red represents Miami-Dade County. Exact locations have been obscured to protect sensitive data. *Map created by B.A. Simpson using satellite imagery from Google Earth (accessed April 2022).*

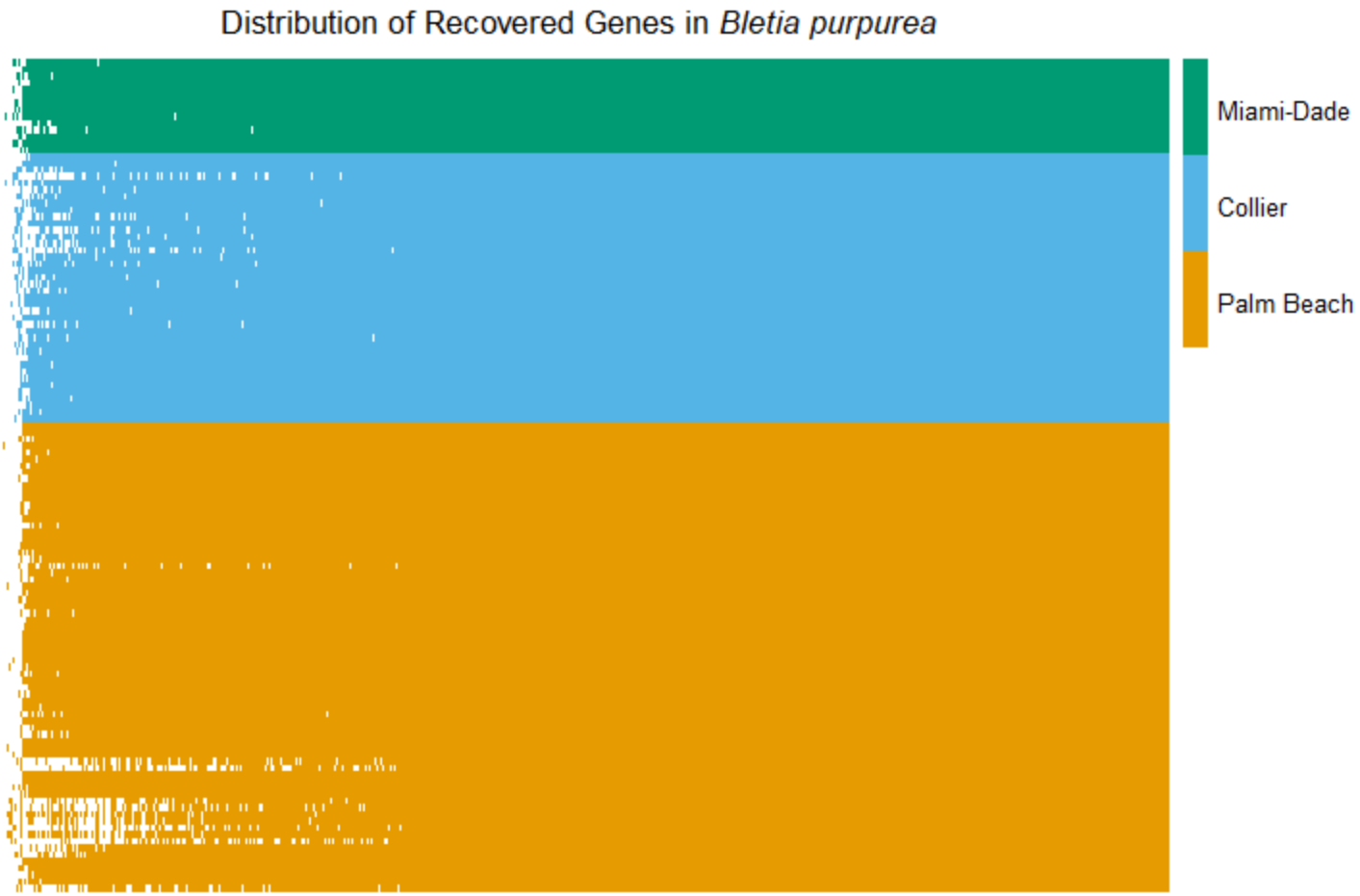

Figure 3

Presence-absence matrix of target genes in *Bletia purpurea*. Each row represents an individual, and each column represents a target gene. Colored segments indicate gene presence; white segments indicate absence. Genes are ordered left to right by increasing recovery rate. Individuals are grouped by county: Miami-Dade (green), Collier (blue), and Palm Beach (gold).

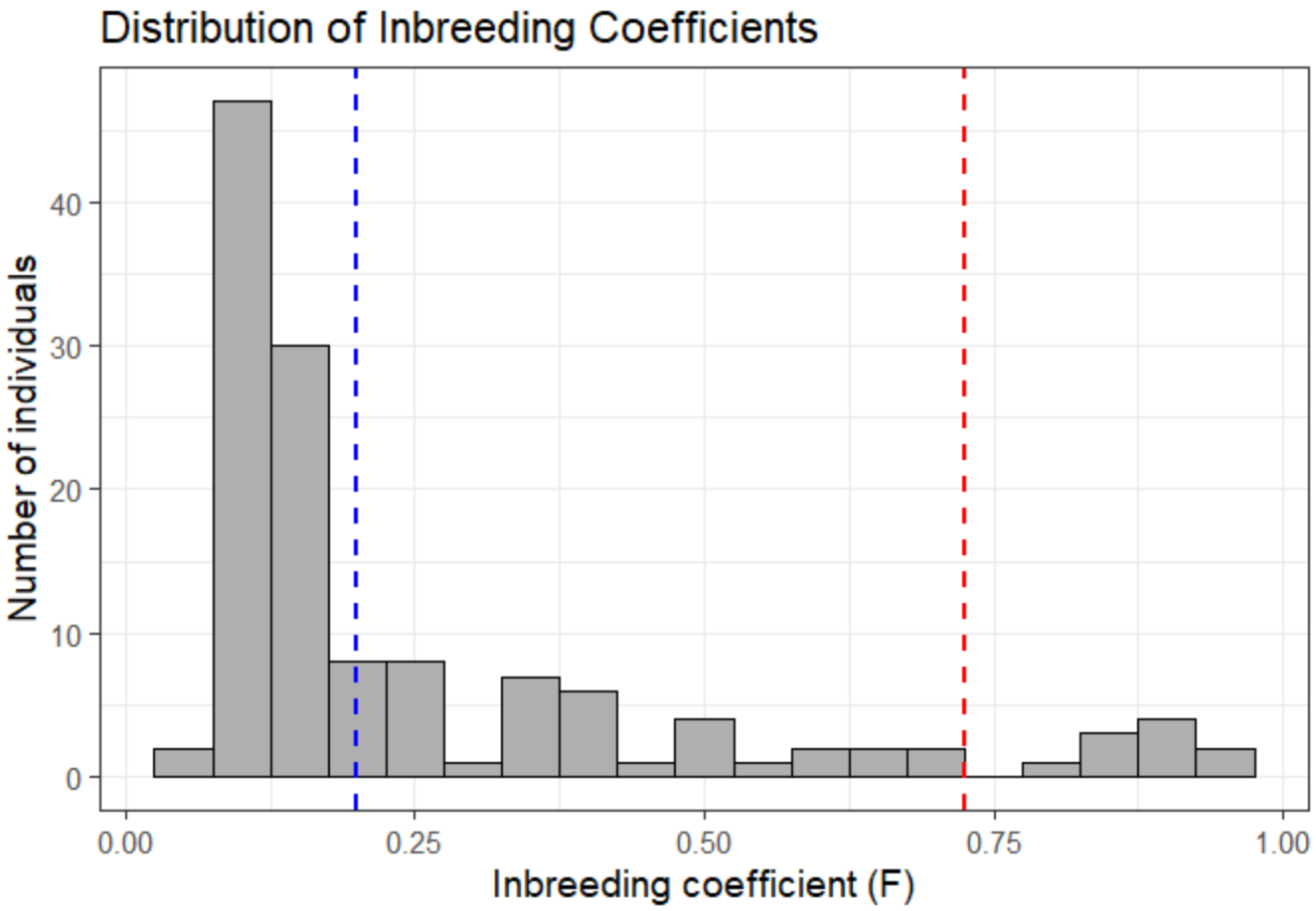

Figure 4

Histogram of individual inbreeding coefficients (*F*) for *Bletia purpurea* (n=131). The dashed blue line marks the upper threshold for low inbreeding (*F* < 0.2), and the dashed red line indicates the threshold used to classify highly inbred individuals (*F*> 0.725).

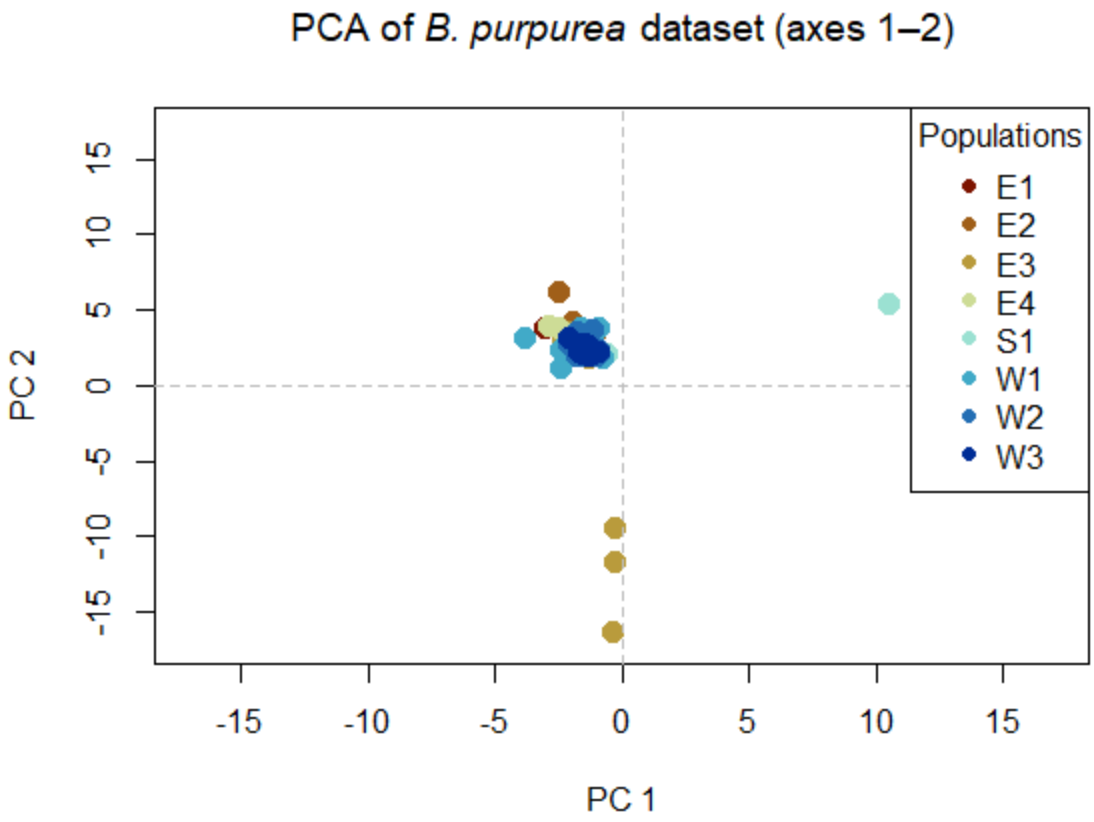

Figure 5

Principal Component Analysis (PCA) of *Bletia purpurea* individuals (n=131). Each point represents an individual, with colors indicating sampling locations. Genetically similar individuals cluster together in PCA space. The first two principal components explain 34.4% of the total genetic variation (PC1: 20.5%, PC2: 13.9%).

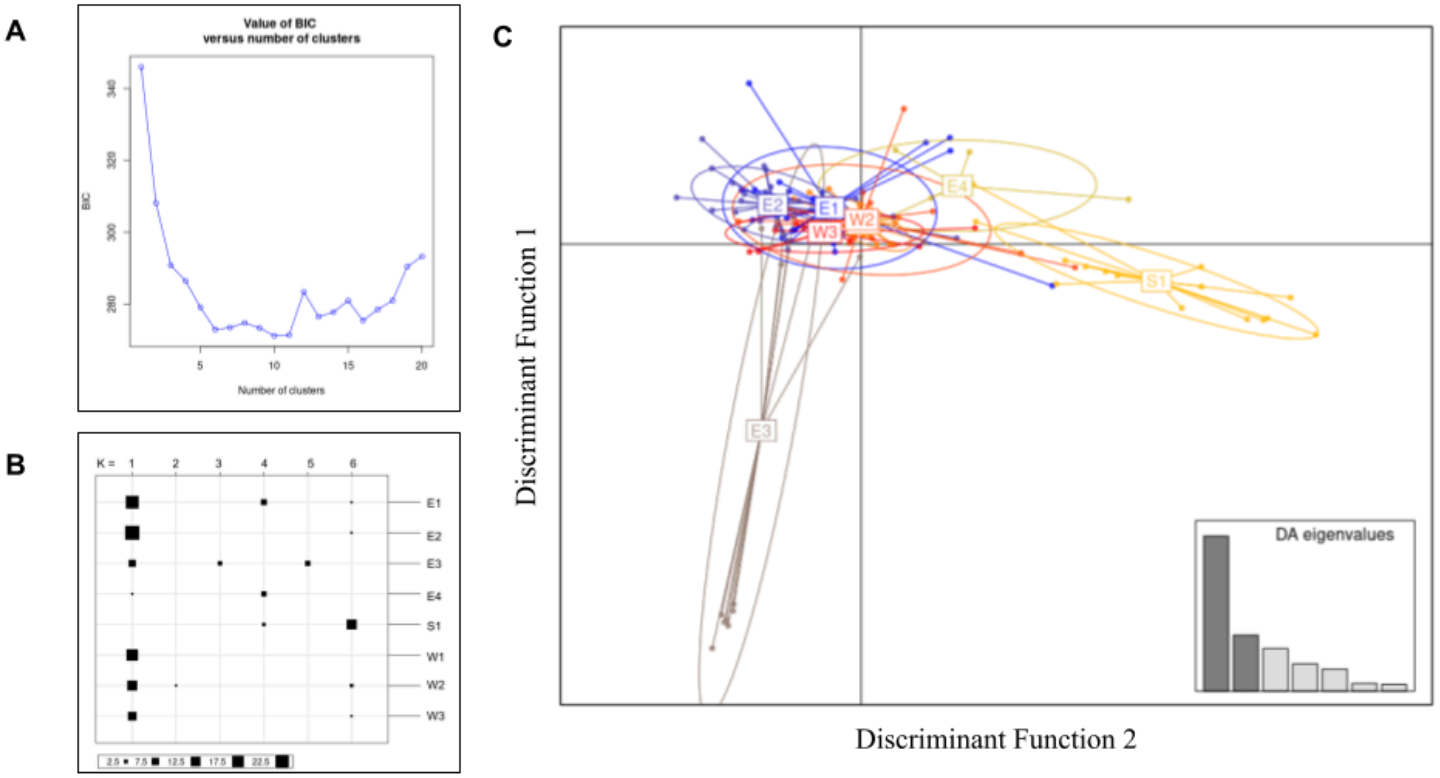

Figure 6

Discriminant Analysis of Principal Components (DAPC) revealing genetic structure in *Bletia purpurea*. **(A)**Bayesian Information Criterion (BIC) values used to determine the optimal number of genetic clusters, with *K* = 6 identified as the best fit. **(B)**Posterior membership probabilities of individuals to each cluster, with square size proportional to assignment probability. **(C)** Scatterplot of the first two discriminate functions, showing genetic clustering by sampling location. Colored inertia ellipses indicate the spatial dispersal of each cluster, and point labels denote population identity.