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Spatial Patterns of Haplotype Variation in the Epiphytic Bromeliad *Catopsis nutans*

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ABSTRACT

Identifying factors governing the origin, distribution, and maintenance of Neotropical plant diversity is an enduring challenge. To explore the complex and dynamic historical processes that shaped contemporary genetic patterns for a Central American plant species, we investigated the spatial distribution of chloroplast haplotypes of a geographically and environmentally widespread epiphytic bromeliad with wind-dispersed seeds, *Catopsis nutans*, in Costa Rica. We hypothesized that genetic discontinuities occur between northwestern and southwestern Pacific slope populations, resembling patterns reported for other plant taxa in the region. Using non-coding chloroplast DNA from 469 individuals and 23 populations, we assessed the influences of geographic and environmental distance as well as historical climatic variation on the genetic structure of populations spanning >1200 m in elevation. *Catopsis nutans* revealed seven haplotypes with low within-population diversity (mean haplotype richness = 1.2) and moderate genetic structure (F_{ST} = 0.699). Pairwise F_{ST} was significantly correlated with both geographic and environmental distance. The frequency of dominant haplotypes was significantly correlated with elevation. A cluster of nine Pacific lowland populations exhibited a distinct haplotype profile and contained five of the seven haplotypes, suggesting historical isolation and limited seed-mediated gene flow with other populations. Paleodistribution models indicated lowland and upland habitats in this region were contiguous through past climatic oscillations. Based on our paleodistribution analysis and comparable prior phylogeographic studies, the genetic signature of recent climatic oscillations are likely superimposed upon the distribution of anciently divergent lineages. Our study highlights the unique phylogeographic history of a Neotropical plant species spanning an elevation gradient.

Abstract in Spanish is available with online material.

Key words: Bromeliaceae; chloroplast DNA (cpDNA); Costa Rica; Fakahatchee Strand; haplotype diversity; paleoclimate; species distribution model.

Neotropical forests are among the most biologically diverse ecosystems and intense research has focused on factors governing the origin, distribution, and maintenance of this diversity (Janzen 1967, Gentry 1982, Graham 1989, Hoorn *et al.* 2010, Rull 2011). Phylogeographic research has proven to be a powerful tool in efforts to understand the historical processes that shaped this biodiversity (Cavers & Dick 2013).

Contemporary environmental conditions and species distributions often do not reflect the historical events that gave rise to contemporary patterns of biodiversity and genetic variation. Understanding these patterns requires detailed understanding of species distributions through time (Bennett & Provan 2008, Bagley & Johnson 2014). Phylogeographic studies can provide evidence for historical bottlenecks induced by vicariance (*i.e.*, refugium-bottleneck hypothesis) or more recent changes that favored the establishment of new populations (*i.e.*, colonization-bottleneck hypothesis). In heterogeneous landscapes such investigations can also provide evidence for how genetic patterns may have been influenced by physical dispersal barriers, isolation-by-distance (IBD; inverse relationship between migration and distance between populations; Wright 1943, Avise 2001), and more complex ecological processes (Manel *et al.* 2003, Cushman *et al.* 2006, Petren 2013) such as isolation-by-environment (IBE; inverse relationship between migration and ecological dissimilarity; Orsini *et al.* 2013, Sexton *et al.* 2014). Mountain ranges, for example, present both physical and environmental impediments to dispersal and gene flow.

Phylogeographic research relating the distribution of contemporary genetic variation to historical processes has greatly contributed to our understanding of Neotropical biogeography (Cavers & Dick 2013). Such studies have revealed patterns of colonization of the Central American land bridge (Dick & Heuertz 2008), the influence of geologically active cordilleras (Cavender-Bares *et al.* 2011), the distribution of putative climatic refugia (Poelchau & Hamrick 2013a), and the implications of these factors for speciation (Cavers *et al.* 2003, 2013, Muellner *et al.* 2010). Phylogeographic analyses of multiple Central American plant species reveal similar spatial patterns of genetic variation, especially northwest–southeast genetic discontinuities that may reflect similar colonization histories, gene flow barriers, or range changes in response to climate oscillations (*e.g.*, Cavers *et al.* 2003, 2013, Trapnell & Hamrick 2004, Dick & Heuertz 2008, Cavender-Bares *et al.* 2011, Kartzinel *et al.* 2013, Poelchau & Hamrick 2013a). However, these discontinuities are only loosely concordant and often contradict expectations based on obvious contemporary

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dispersal patterns (Poelchau & Hamrick 2012), suggesting a complex suite of historical factors has shaped the Central American flora since the emergence of the Isthmus of Panama. For example, the genetic signature of early colonization patterns, often involving several conspecific lineages, may have been obscured by more recent geophysical factors and climatic fluctuations (Poelchau & Hamrick 2012, 2013a, Cavers & Dick 2013).

Development of a more unified biogeographic framework for the region remains a challenge due to the broad range of species-specific traits and responses to environmental factors displayed by the regional flora (Poelchau & Hamrick 2012, 2013b). Most investigations in the region have focused on ancient colonization routes and locations of putative climatic refugia for lowland taxa (Brown 1987, Poelchau & Hamrick 2012, 2013b). Fewer studies have considered montane plants (Kartzinel *et al.* 2013) and research on plant species with broad elevation ranges are virtually non-existent for the region. The Central American mountain ranges create broad environmental gradients, from cloud forests at the continental divide to seasonal dry forests in the Pacific lowlands (Coen 1983). Thus, historical climatic oscillations may have played a crucial role in shaping contemporary genetic patterns for plant species that occur along this elevation gradient.

One hypothesis for the similar genetic patterns documented in several plant taxa with wind-dispersed seeds in Costa Rica is that strong northeasterly winds create seed dispersal barriers (Kartzinel *et al.* 2013). Costa Rican mountain ranges form a spine that runs the length of the country and are oriented northwest–southeast, perpendicular to prevailing dry-season winds. These already strong winds are channeled between the mountains of northwestern Costa Rica (Marshall 2007), and could form a seed dispersal barrier between the Guanacaste and Tilarán mountain ranges (Kartzinel *et al.* 2013). For taxa that span a broad elevation gradient on the Pacific slope of these mountains, these strong winds should produce similar genetic discontinuities at the breaks between mountain ranges while genetically homogenizing populations across elevations within the path of the same prevailing winds.

We investigated the phylogeography of a geographically and climatically widespread epiphytic bromeliad, *Catopsis nutans* (Sw.) Griseb, which has wind-dispersed seeds and occurs across >2000 m in elevation, using chloroplast DNA (cpDNA) sequences. Maternally inherited cpDNA is exclusively dispersed by seeds in most angiosperms (Petit *et al.* 2005) thus cpDNA can provide rich insights into patterns of seed dispersal. We hypothesized that a genetic discontinuity would characterize the gap between northwestern and southwestern populations along the Pacific slope of Costa Rica, as documented in several other plant taxa (Cavers *et al.* 2013, Kartzinel *et al.* 2013, Poelchau & Hamrick 2013a). We used paleoclimate distribution models to further investigate how historical population distributions relate to the observed phylogeographic patterns.

METHODS

Study species.—*Catopsis nutans* (Bromeliaceae, subfamily Tillandsioideae) is an epiphyte that occurs from Venezuela and Ecuador to Mexico, the West Indies, and southern Florida (Morales 2000). It has a broad range of environmental tolerances, occurring throughout Costa Rica from 40 to 2150 m asl in both wet and dry forests (Morales 2000). It occurs in habitats that range from early successional to mature forest. In later successional forests with greater epiphytic bromeliad species diversity, *C. nutans* is less abundant, displays increasing demographic maturity, and occurs on a greater variety of host tree species and sizes (Cascante-Marin *et al.* 2006). It flowers nocturnally during the rainy season (June–August) in Costa Rica and is pollinated by moths. Most populations are hermaphroditic, although site-specific occurrences of dioecy have been reported in Mexico (Benzing 2000). In the vicinity of Monteverde, Costa Rica, populations are hermaphroditic (T. R. Kartzinel, pers. obs.). Each ramet is monocarpic, but 1–3 clonal ramets may be produced upon sexual reproduction. Inflorescences produce few to many fruits (>30), yielding up to 100 seeds/fruit (T. R. Kartzinel, pers. obs.). Fruit dehiscence and release of wind-dispersed seeds occur in the dry season (February–March) when trade winds (northeast to southwest) are strongest and most relentless (Coen 1983). It exhibits greater germination and fruit set in mature forest, higher survival and abundance in early succession habitats, and similar relative growth rates in open and mature habitat (Cascante-Marin *et al.* 2006, 2008, 2009). Hairy plumes may facilitate *C. nutans* seed dispersal when aloft, but microscopic barbs strongly affix seeds to substrates upon contact and may limit secondary dispersal (Benzing 2000).

Sampling.—We sampled 3–29 plants/population (mean = 20.8) from 22 *C. nutans* populations spanning an elevational gradient of 36–1237 m asl on the Pacific slope of Costa Rica (Fig. 1A). Populations were defined as all individuals within a 1–2 ha area. Most populations were located in disturbed roadside areas, pastures, and early-mid succession forests. Care was taken to avoid sampling adjacent individuals that were possible clones. Leaf samples were snap frozen in liquid nitrogen and transported to the University of Georgia for genetic analysis. Although our study populations represent only a small proportion of the species' range, the large number of populations from regions of highest known population density in Costa Rica (Figure S1), primarily spanning elevations across the Pacific Slope, provide excellent spatial coverage at this scale.

In addition to our primary investigation of *C. nutans* in Costa Rica, we also sampled a population occurring in two sloughs of the Fakahatchee Strand, Florida. This disjunct population was included to augment our understanding of the broader phylogeography of the species. *Catopsis nutans* is endangered in Florida due to historical rarity, and Fakahatchee populations are small and isolated (Coile 2000). Plants are also susceptible to invasive bromeliad weevils (Frank & Fish 2008). These plants exhibit dissimilar morphological and reproductive characteristics relative to many Central American populations (*i.e.*, smaller, fewer flowered, always monoecious) (Benzing 2000).

Genetic data.—Genomic DNA was extracted using DNeasy Plant Mini kits (Qiagen). Preliminary sequencing trials were

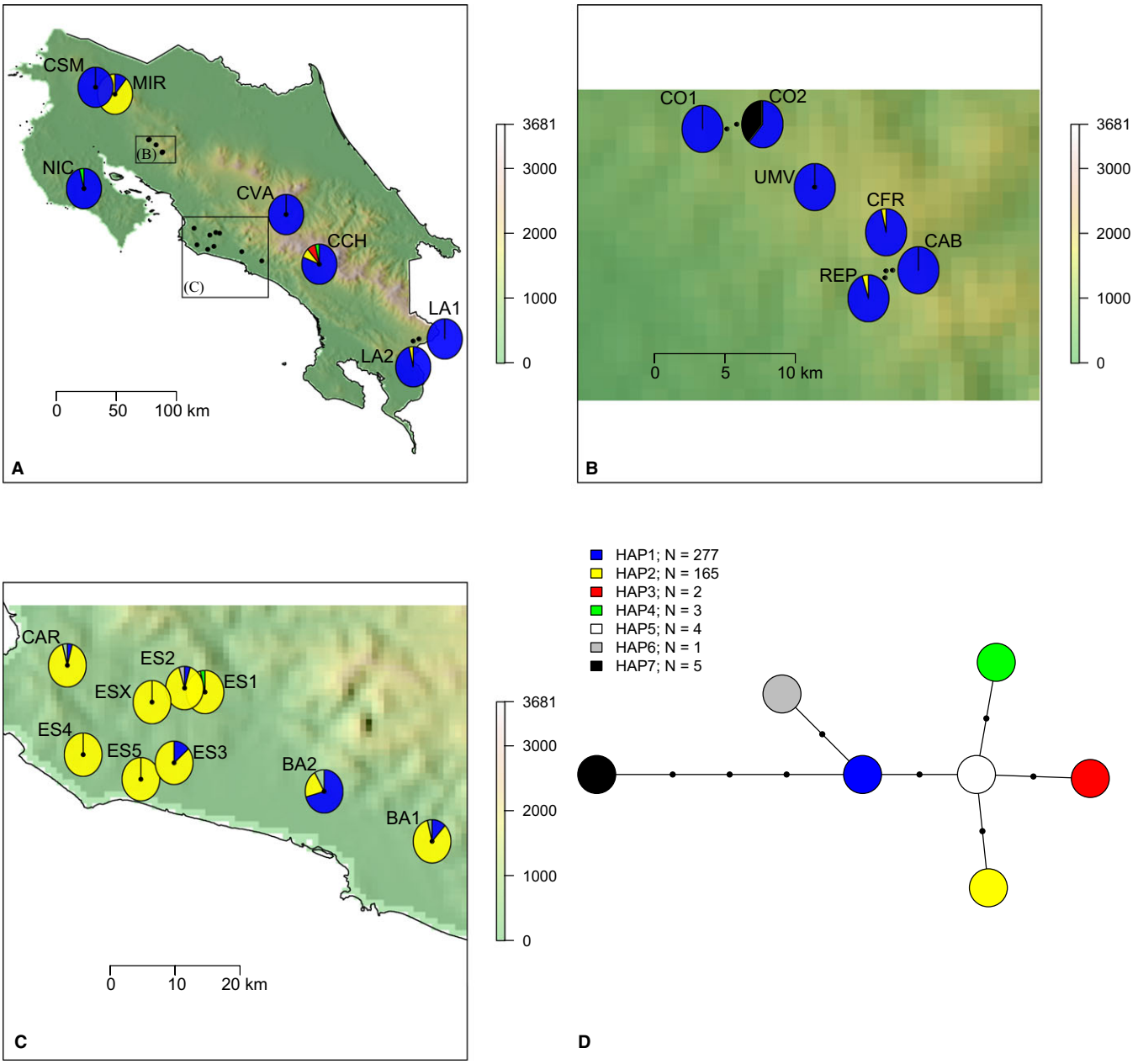

FIGURE 1. The distribution, frequency, and network of seven haplotypes sampled from 22 *Catopsis nutans* populations in Costa Rica. Pie charts show the frequency of haplotypes on (A) a topographic map of with two insets (B, C). (D) Haplotypes are color-coded, with points on lines in the network indicating the number of mutations separating haplotypes. Two haplotypes (haplotype 1 = blue; haplotype 2 = yellow) are common, with at least one of these in all populations, while the five less common haplotypes occur in only 1–3 populations. The legend shows the haplotype names and the number of each observed in Costa Rica.

conducted using nine cpDNA primer pairs (Shaw *et al.* 2005, 2007). The *psbA–trnH* intergenic region exhibited polymorphisms (SNPs and INDELs), but unfortunately the three other successfully sequenced regions revealed no variation in 15–24 individuals from 7 to 11 populations (Table S1). Sequencing these additional chloroplast markers was deemed unlikely to yield substantially more information. The non-coding *psbA–trnH* intergenic region of the chloroplast was sequenced for 469 individuals. The 12.5 μL PCR reactions comprised 1× ThermoPol Buffer (New England Biolabs; NEB, Ipswich, Massachusetts, U.S.A.), 1.0 mM $MgCl_2$ (Sigma-Aldrich, St. Louis, Missouri, U.S.A.), 0.25 mM each dNTP (NEB), 0.1 μM each primer, 0.0125 mg Bovine Serum Albumin (BSA; NEB), 10–100 ng DNA, and 0.5 units NEB *Taq* DNA polymerase. Initial denaturation was 5 min at 80°C followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 65°C for 3 min, with a

10 min final extension at 65°C. PCR products were purified with ExoSapIT (Sigma-Aldrich) and sequenced with BIGDYE v. 3.1 (Applied Biosystems, Inc., Foster City, California, U.S.A.) on an ABI3730 (Applied Biosystems) at the Georgia Genomics Facility (University of Georgia, Athens, Georgia, U.S.A.). To minimize error, amplicons were sequenced in both directions. Sequences were edited and assembled in Sequencher 4.9 (GeneCodes Corp., Ann Arbor, Michigan, U.S.A.), aligned with ClustalW (Larkin *et al.* 2007), and submitted to GenBank (accessions KJ437672–KJ438140). The final alignment spanned 412 bp.

Initially, we tested for genetic variation at nuclear microsatellite loci developed for the Bromeliaceae (Palma-Silva *et al.* 2007), but optimization for reliable use in *C. nutans* was determined to be prohibitive.

Genetic analyses.—To estimate levels of genetic diversity the number of haplotypes (H), private haplotypes (PH) and haplotype diversity (HD) were calculated in Arlequin v. 3.11 (Excoffier *et al.* 2005). Haplotype richness (HR) was estimated by rarefying the number of haplotypes to the minimum sample size ($N = 3$) using *vegan* v.2.0-7 (Oksanen *et al.* 2012) in R v.2.15.3 (R Core Development Team 2013).

Analysis of molecular variation (AMOVA) was used to partition genetic variation among populations (*i.e.*, genetic structure). Pairwise and overall differentiation (F_{ST}) was estimated using haplotype frequencies and significance was tested with 1000 permutations in Arlequin. We tested for demographic expansion using the classic Tajima's *D* and Fu's *F* statistics with 1000 permutations in Arlequin. Unfortunately, many populations contained insufficient genetic variation to perform these tests on all populations, so we pooled populations from two large, phylogeographically distinct regions (see Results). To further characterize differences among haplotypes, a haplotype network was constructed using *pegas* v. 0.4–4 (Paradis 2010) in R.

We also tested whether cpDNA differentiation is associated with geographic and/or environmental distance. Correlating pairwise $F_{ST}/(1 - F_{ST})$ with geographic distance to test for IBD could not be conducted accurately due to undefined comparisons (*i.e.*, $F_{ST} = 1$) (Rousset 1997). Instead, we performed Mantel tests and multiple matrix regressions (Wang 2013) between: (1) pairwise F_{ST} and geographic distances, and (2) pairwise F_{ST} and environmental dissimilarity. Environmental dissimilarity was calculated as Euclidean distance between populations based on a principal components analysis (PCA) that included elevation and the 19 WorldClim bioclimatic variables. These variables represent annual trends in temperature and precipitation, seasonality, and extreme or limiting environmental factors (Hijmans *et al.* 2005). Multiple matrix regression evaluates the relative strength of associations between geographic and environmental distances with processes that influence F_{ST} (*e.g.*, mutation, drift, gene flow, colonization, natural selection, demographic history). We performed univariate Mantel tests using *vegan* and multiple matrix regressions using *MMRR* (Wang 2013) in R.

Paleoclimate distribution modeling.—To investigate the influence of climatic variation on the distribution of *C. nutans* and its genetic variation, we developed a species distribution model (SDM). We used range-wide *C. nutans* occurrences and climatic data to produce the SDM. The SDM was then projected upon climates of the last interglacial period (LIG, ~120–140 kya), the last glacial maximum (LGM, ~21 kya), and the mid-Holocene warm period (MHW, ~6 kya).

Our SDM utilized two datasets. First, *C. nutans* occurrence records were downloaded from the Global Biodiversity Information Facility (GBIF, accessed 29 January 2014; data sources in Appendix S1). Combining our study sites with quality-checked GBIF data produced 269 location records (Fig. S1). Next, WorldClim data were extracted for each of these sites. From these, a subset of 10 bioclimatic variables that were not highly correlated ($r < 0.9$) was identified by sequentially removing variables with the largest number of correlations with other variables. Ultimately, we selected: mean annual temperature (BIO1), mean diurnal range (BIO2), isothermality (BIO3), temperature seasonality (BIO4), minimum mean temperature of the coldest month (BIO6), annual temperature range (BIO7), annual precipitation (BIO12), precipitation seasonality (BIO15), mean precipitation of the warmest quarter (BIO18), and mean precipitation of the coldest quarter (BIO19). Occurrence data and climate layers were used to produce a SDM in Maxent v.3.3 (Phillips *et al.* 2006) within the R package *dismo* (Hijmans *et al.* 2014). We used a convergence threshold of 10^{-5}, 1000 iterations, and a randomly selected 25 percent of occurrence records for model testing. Model quality was determined based on two criteria: (1) a threshold-independent receiver operating characteristic curve analysis (Anderson *et al.* 2003) and (2) a threshold-dependent assessment of the proportion of test points that fell outside the predicted range using a 10 percent intrinsic omission threshold (Phillips *et al.* 2006).

The SDM was used to evaluate historical *C. nutans* distributions in Costa Rica. We analyzed the same ten WorldClim variables obtained from calibrated paleoclimatic data (available at http://www.worldclim.org/paleo-climate). Because paleoclimatic models differ, robust interpretations require a comparison of multiple models (Poelchau & Hamrick 2013b). We compared two projections for the LGM and MHW: the Community Climate Science Model (CCSM4) (Gent *et al.* 2011) and the Model for Interdisciplinary Research on Climate (MIROC) (Watanabe *et al.* 2011). Comparable bioclimatic data for the LIG were only available from a single source, Otto-Bliesner *et al.* (2006), which we resampled to 2.5 min resolution for comparison with the other modeled time periods.

We analyzed two types of historical change: (1) Costa Rican range extent and overlap within the bounds of current sea level and (2) occurrence probabilities at our study sites. We calculated the overall predicted range size with each SDM (>10% threshold) as well as the part of that range occurring in lowlands (<500 m) and highlands (>500 m). This elevation boundary was selected based upon our finding of significant turnover of haplotypes at 500 m (see *Results*). We calculated niche overlap between current and each historical SDM using Schoener's *D* (Schoener 1968), which ranges from 0 (no overlap) to 1 (complete overlap)

(Warren *et al.* 2009). We extracted probabilities at each study site from each model and tested for differences between highland and lowland groups through time using a two-way ANOVA with interactions.

RESULTS

Levels of haplotype diversity were variable and tended to be low. Sequencing 469 samples yielded seven haplotypes that included five SNPs and two poly-A microsatellites (1. 2; Table 2). The 22 Costa Rican populations had 1–4 (mean = 1.86) haplotypes (H), with haplotype richness (HR) = 1.0–1.8 (mean = 1.2), nucleotide diversity (π) = 0.000–1.539 (mean = 0.089), and haplotype diversity (HD) = 0.000–0.513 (mean = 0.130; Table 1). Three populations (CCH, ES2, CO_2) each contained a private haplotype (Table 1). All populations had either haplotypes 1 or 2, and 10 populations had both (Fig. 1A–C). In Costa Rica, most populations had haplotype 1 (82%) and haplotype 2 (64%). Similarly most individuals sampled had either haplotype 1 (61%) or haplotype 2 (36%) in Costa Rica. Three private haplotypes (3, 6, and 7) and two rare haplotypes (4 and 5) occurred in eight populations (3% of individuals; Fig. 1; Table 2). One of these rare haplotypes occurred in both lowland and highland populations, while two occurred only in highland populations and two occurred only in lowland populations. Few mutational steps separated haplotypes; a maximum of five steps spanned the network (haplotype 7 vs. haplotypes 2, 3, and 4; Fig. 1D). An unobserved intermediate haplotype separates haplotypes 1 and 7 (Figs. 1D). Population CCH contained the most haplotypes (4), including private haplotype 3. Haplotype 4 occurred once in each of three disparate populations (Chirripó [CCH], Nicoya Peninsula [NIC], the Pacific lowlands [ES1]). Haplotype 5, the intermediate of common haplotypes 1 and 2, occurred in three lowland populations (CAR, BA1, BA2). Private haplotype 6 occurred only in the lowlands (ES2), while private haplotype 7, the most distant in the network, occurred only in the northwest (CO2). Haplotype diversity and

TABLE 1. *Locations and genetic diversity of* Catopsis nutans *populations. Population name, latitude, longitude, and elevation (m) are arranged from north to south within Costa Rica.* N = *number of sequenced individuals, H = number of haplotypes, PH = private haplotypes, HR = haplotype richness,* π = *nucleotide diversity, and HD = haplotype diversity.*

Pop	Lat.	Long.	Elev.	*N*	H	PH	HR	π	HD
CSM	10.764	−85.303	820	26	1	0	1.00	0.000	0.000
MIR	10.713	−85.157	892	9	2	0	1.33	0.000	0.222
CO1	10.373	−84.903	821	7	1	0	1.00	0.000	0.000
CO2	10.376	−84.897	963	13	2	1	1.77	1.539	0.513
UMV	10.336	−84.847	1237	24	1	0	1.00	0.000	0.000
CAB	10.283	−84.796	1096	23	1	0	1.00	0.000	0.000
CFR	10.283	−84.801	1094	26	2	0	1.12	0.000	0.077
REP	10.278	−84.801	1056	20	2	0	1.15	0.000	0.100
NIC	10.011	−85.391	690	24	2	0	1.13	0.000	0.083
CVA	9.818	−83.866	1125	24	1	0	1.00	0.000	0.000
CAR	9.715	−84.560	442	24	3	0	1.25	0.083	0.163
ES2	9.684	−84.396	362	21	3	1	1.29	0.095	0.186
ES1	9.679	−84.367	303	25	3	0	1.12	0.000	0.080
ESX	9.665	−84.442	439	8	1	0	1.00	0.000	0.000
ES4	9.592	−84.538	36	25	1	0	1.00	0.000	0.000
ES3	9.581	−84.411	80	29	2	0	1.37	0.000	0.246
ES5	9.558	−84.457	84	3	1	0	1.00	0.000	0.000
BA2	9.541	−84.200	108	24	3	0	1.74	0.159	0.467
BA1	9.472	−84.049	172	25	3	0	1.45	0.080	0.290
CCH	9.448	−83.616	1131	26	4	1	1.56	0.000	0.348
LA1	8.892	−82.864	1189	25	1	0	1.00	0.000	0.000
LA2	8.876	−82.908	1034	26	2	0	1.12	0.000	0.077
FAK[a]	25.951	−81.360	38	12	1	0	1.00	0.000	0.000
Costa Rica mean	NA	NA	NA	20.8	1.9	0.14	1.20	0.089	0.130
Highland mean	NA	NA	NA	21.0	1.7	0.15	1.17	0.118	0.109
Lowland mean	NA	NA	NA	20.4	2.2	0.11	1.25	0.046	0.159
Overall mean	NA	NA	NA	20.4	1.8	0.13	1.19	0.085	0.124
Species total	NA	NA	NA	469	7	4	NA	0.000	0.497

[a]Coordinates are for the Fakahatchee ranger station due to the protected status of these populations.

TABLE 2. *Identity and abundance of* Catopsis nutans *haplotypes identified in each population. Colors listed correspond to those used in Fig. 1.*

Population	HAP1	HAP2	HAP3	HAP4	HAP5	HAP6	HAP7
Color	Blue	Yellow	Red	Green	White	Gray	Black
CSM	26	0	0	0	0	0	0
MIR	1	8	0	0	0	0	0
CO1	7	0	0	0	0	0	0
CO2	8	0	0	0	0	0	5
UMV	24	0	0	0	0	0	0
CAB	23	0	0	0	0	0	0
CFR	25	1	0	0	0	0	0
REP	19	1	0	0	0	0	0
NIC	23	0	0	1	0	0	0
CVA	24	0	0	0	0	0	0
CAR	1	22	0	0	1	0	0
ES2	1	19	0	0	0	1	0
ES1	0	24	0	1	0	0	0
ESX	0	8	0	0	0	0	0
ES4	0	25	0	0	0	0	0
ES3	4	25	0	0	0	0	0
ES5	0	3	0	0	0	0	0
BA2	17	5	0	0	2	0	0
BA1	3	21	0	0	1	0	0
CCH	21	2	2	1	0	0	0
LA1	25	0	0	0	0	0	0
LA2	25	1	0	0	0	0	0
FAK	12	0	0	0	0	0	0
Total	289	165	2	3	4	1	5

richness was similar between higher and lower elevation populations (above/below 500 masl; Table 1). The most common haplotype in Costa Rica (haplotype 1) was fixed in Florida (Table 1).

Interestingly, nine central Pacific lowland populations occupying a relatively limited geographic area (from just north of Jacó to just south of Quepos) have a distinct haplotype profile: they are characterized by a different dominant haplotype (2) and possess five of the seven haplotypes, two of which are found nowhere else. This level of haplotype variation is equivalent to that of the remaining 14 Costa Rican populations that span the full length of the country. Evidently, these populations have been shaped differently by historical factors.

Moderate genetic structure characterized the Costa Rican populations ($F_{ST} = 0.699$; $P \leq 0.000$; Table S2). Pairwise F_{ST} ranged from 0 to 1 while mean pairwise F_{ST} per population ranged from 0.315 to 0.594 (Table S3). Nine Costa Rican populations (41%) were fixed for a single haplotype, while 13 (59%) had multiple haplotypes. Only five populations contained >2 haplotypes: four of these populations were in the lowlands. Of the 13 populations with ≥2 haplotypes, most (77%) contained both common haplotypes, although often in dissimilar frequencies. Thus, genetic structure was strongly influenced by the distribution of haplotypes 1 and 2. Partitioning genetic variation between the nine Pacific lowland populations (Fig. 1C) and remaining 14 upland populations (Fig. 1A and B) revealed that most genetic structure is between regions (hierarchical AMOVA: $F_{CT} = 0.731$; 73.1%; $P < 0.001$; Table S2).

There was no strong evidence for demographic changes in either high- (Tajima's $D = -1.19$, $P = 0.089$; Fu's $F = -3.11$, $P = 0.057$) or low-elevation population groups (Tajima's $D = -0.77$, $P = 0.216$; Fu's $F = -1.02$, $P = 0.308$).

Mantel tests revealed significant relationships between pairwise F_{ST} and geographic distance ($r = 0.272$; $P = 0.0016$). This could indicate IBD, although results should be interpreted with caution because populations that were fixed for different haplotypes rendered $F_{ST}/(1 - F_{ST})$ undefined. Artificially assigning the next highest F_{ST} value (0.961) to these comparisons before applying the $F_{ST}/(1 - F_{ST})$ transformation similarly revealed a significant Mantel test ($r = 0.213$; $P = 0.0079$). A Mantel test between pairwise F_{ST} and environmental distance was also significant ($r = 0.416$; $P \leq 0.0001$; Fig. S2). Multiple matrix regression yielded a modest, but significant, regression coefficient for environmental distance ($\beta_E = 0.192$; $P = 0.003$), but not geographic distance ($\beta_G = -0.045$; $P = 0.152$). The first three PCA axes of WorldClim data and elevation, which were used to measure environmental distance, represented most of the variation among sites (92%; Table S4). The first, second and third axes accounted for 66.4 percent, 18.1 percent and 7.5 percent, respectively.

Distinguishing between the relative influence of geographic distance and environment is difficult, however, because they are moderately correlated ($r = 0.775$; $P \leq 0.0001$; Fig. S2) and genetic data come from a single cpDNA region. Populations at higher elevations were primarily composed of haplotype 1, while populations at lower elevations were primarily composed of haplotype 2, and the frequencies of these haplotypes significantly differed across elevations (Binomial regression $P < 0.0001$; Fig. 2). Nearly complete turnover of haplotypes 1 and 2 occurred at ~500 m asl, with two notable exceptions (MIR and BA2; Fig. 2).

The SDM was well-fit based on the area under the curve (AUC) of 0.90 and the intrinsic omission rate of 0.16 (Fig. S1).

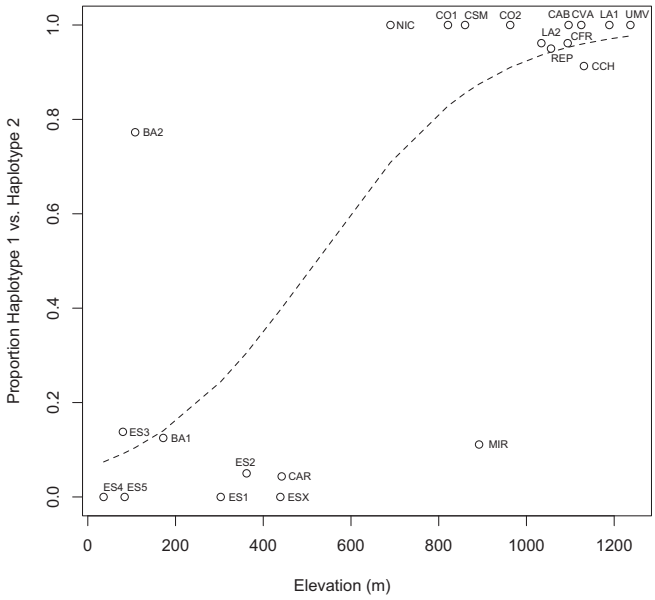

FIGURE 2. The frequency of haplotype 1 versus haplotype 2 in Costa Rican populations significantly varies with elevation. Nearly complete turnover in haplotype frequency is observed between 500 and 600 masl, with the exception of populations MIR and BA2.

On the Pacific slope, populations probably occurred in narrower bands of montane habitat during the LIG, shifting downslope during the LGM, and were relatively restricted to montane habitats in the MHW (Fig. 1). Projected range size varied across time and models, both overall (range: 41,582–53,232 km^2) and when high- (range: 16,514–18,975 km^2) and low-elevation (range: 24,490–35,060 km^2) areas were considered separately (Table 3). Niche overlap between current and all historical SDMs was moderate (0.72–0.89), and always less in the lowlands versus highlands (Table 3). The occurrence probabilities were consistently above the 10 percent threshold at our study sites, except for four lowland sites (ES2, ES4, ESX, CAR) under the MIROC-projected MHW. Probability values at our sites differed among elevation groups ($P < 0.01$), time periods ($P < 0.01$) and the elevation * time interaction ($P < 0.01$) using both CCSM4 and MIROC projections. Mean upland site probability was consistently >50 percent, with moderately higher estimates in current versus earlier climates. Models differed with regard to lowland probability: CCSM4 suggested a much higher probability than MIROC at both the LGM and MHW sites (Table 3). At the LGM, lowland sites had the highest mean probability (88%) based on CCSM4, which exceeded the mean probability for highland sites (Table 3). In contrast, the mean probability was only 33 percent based on MIROC (Table 3).

DISCUSSION

Catopsis nutans exhibited seven haplotypes, low cpDNA variation within populations, moderate genetic structure among populations, and distinct haplotype profiles along the length of Costa Rica's montane spine versus a cluster of populations in the central Pacific lowlands (Fig. 1). Thus, our data were inconsistent with the prediction that a genetic discontinuity exists between northwestern and southwestern populations based on patterns reported for other plant species in the region (*e.g.*, Trapnell & Hamrick 2004, Kartzinel *et al.* 2013, Poelchau & Hamrick 2013a).

TABLE 3. *Species distribution models for the last interglacial period (~120–140 kya; LIG), last glacial maximum (~21 kya; LGM), mid-Holocene warm period (~6 kya; MHW) and current period. Overall range size (km^2), niche overlap index (Schoener's D), and the mean probability of habitat suitability among study sites used in phylogeographic analysis are shown for the total area of Costa Rica (T) the highlands (>500 m; H) and the lowlands (<500 m; L).*

Statistic	Elevation	LIG	LGM (CCSM4)	LGM (MIROC)	MHW (CCSM4)	MHW (MIROC)	Current (Maxent)
Range size (km^2)	T	42,885	53,233	41,582	51,418	48,884	51,248
	H	18,212	18,170	17,089	18,976	16,514	18,552
	L	24,665	35,061	24,491	32,427	32,363	32,685
Overlap	T	0.83	0.81	0.83	0.84	0.81	NA
	H	0.89	0.86	0.86	0.87	0.89	NA
	L	0.80	0.78	0.79	0.84	0.73	NA
Mean probability at study sites	T	0.39 ± 0.04	0.79 ± 0.03	0.51 ± 0.04	0.61 ± 0.03	0.4 ± 0.06	0.68 ± 0.03
	H	0.53 ± 0.03	0.72 ± 0.04	0.63 ± 0.03	0.67 ± 0.04	0.62 ± 0.03	0.78 ± 0.02
	L	0.18 ± 0.02	0.88 ± 0.01	0.33 ± 0.03	0.52 ± 0.03	0.08 ± 0.03	0.54 ± 0.05

Low levels of seed flow are suggested by the haplotype diversity within populations and degree of genetic structure (F_{ST} = 0.699 vs. 0.416–0.871 interquartile range for 124 Angiosperms; Petit *et al.* 2005). This is consistent with previous reports of *C. nutans* dispersal-limitation in both mature and disturbed habitats (Cascante-Marin *et al.* 2009). Colonization by few founders, followed by *in situ* population expansion, and little seed-mediated gene flow is consistent with populations subject to disturbance and colonization (Wade & McCauley 1988, Whitlock & McCauley 1990). This is also consistent with the pattern of genetic structure in the sympatric epiphytic bromeliad (*Guzmania monostachia*) in second growth forests (Cascante-Marin *et al.* 2014). Founder effects tend to be most apparent in maternally inherited cpDNA because it has a lower effective population size than nuclear DNA and it is unaffected by post-colonization pollen flow (McCauley 1995, McCauley *et al.* 1995, Petit *et al.* 2005).

The spatial patterning of *C. nutans* haplotypes is discordant with phylogeographic patterns identified in most other plant species investigated in Costa Rica, including northwest–southwest splits for lowland tree species (*e.g.*, Cavers *et al.* 2003, 2013, Cavender-Bares *et al.* 2011, Poelchau & Hamrick 2013a). Patterns of cpDNA variation in a lowland epiphytic orchid with wind-dispersed seeds also revealed a north–south discontinuity (Trapnell & Hamrick 2004) that was approximately concordant with those of lowland tree species. Similarly, a montane epiphytic orchid, *Epidendrum firmum*, exhibited north–south breaks among populations on different mountain ranges, where it is sympatric with *C. nutans* (~860–1400 m asl; Kartzinel *et al.* 2013). Both of these species release wind-dispersed seeds into the same wind dispersal corridors during the dry season, when winds are strong and directionally consistent. *Catopsis nutans,* however, revealed a relatively homogeneous haplotype profile among montane populations that ranged from the northern to southern Costa Rican borders. A distinctly different, but similarly diverse, haplotype profile characterized a cluster of nine central Pacific lowland populations.

The occurrence of a distinct haplotype profile and the high level of haplotype diversity in the central Pacific lowlands suggest that this cluster represents a historically isolated lineage that has experienced limited seed-mediated gene flow. Haplotype 1 is central to the haplotype network, indicating that it is likely ancestral. This is consistent with its (1) widespread distribution (82% of Costa Rican populations and all Florida individuals), (2) high frequency in Costa Rica (61% of individuals), and (3) absence from only four clustered lowland populations. Haplotype 2, on the other hand, may have arisen more recently, perhaps in Costa Rica's Pacific lowlands from haplotype 5, which was only found in these populations. The high frequency of haplotype 2 in the cluster of central Pacific lowland populations and its relative scarcity elsewhere may be due to little spread beyond this area. The distinct regional profiles persist despite the expectation that strong northeasterly winds would facilitate dispersal of haplotype 1 into lowland populations. Furthermore, a wind rotor produced by the Talamanca mountains (east of the lowland populations) during the dry season (Coen 1983) does not appear to facilitate haplotype 2 dispersal to higher elevations (*e.g.*, CCH; Fig. 1).

What historical processes account for the Pacific lowland genetic disjunction? As with previously documented phylogeographic breaks in the region (Poelchau & Hamrick 2013b, Bagley & Johnson 2014), several historical processes likely contributed over time with each event partially obscuring the signatures of older processes: the initial colonization of Central America, the contraction and isolation of populations during climatic oscillations, and/or population isolation in local microrefugia. Because one population of the tree species *Ficus insipida* also revealed a unique haplotype profile in this narrow lowland region (Poelchau & Hamrick 2013a), we suggest that disparate plant taxa may exhibit similar patterns due to a shared phylogeographic history in the region.

At the most ancient extreme, two *C. nutans* lineages could have diverged upon, or even prior to, colonizing Central America. Long-recognized biogeographic affinities between western regions of lower Central America and western regions of Ecuador and Colombia suggest the Pacific lowland colonists could have diverged prior to colonizing Central America (Hardesty *et al.* 2010), as was the case for *Symphonia globulifera*, which arrived via long-distance oceanic dispersal (Dick & Heuertz 2008). Founder effects following long-distance wind dispersal is plausible for *C. nutans*, with limited subsequent seed flow to slowly erode the resulting genetic signature. This scenario was observed for the epiphytic bromeliad *Guzmania monostachia* at much finer spatiotemporal scales (Cascante-Marin *et al.* 2014).

A hypothesis for more recent origins of the Pacific lowland disjunction is that climate oscillations led to founder effects, coupled with limited subsequent seed-mediated gene flow, which produced distinct haplotype profiles. The bromeliad *Vriesea gigantica* from the Brazilian Atlantic Rainforest, for example, exhibited a phylogeographic split and decrease in haplotype diversity consistent with recent climate-mediated range expansion (Palma-Silva *et al.* 2009). Our results suggest that climate-induced bottlenecks may have contributed to, but cannot fully account for, the distinct haplotype profiles of *C. nutans* in Costa Rica. Paleodistribution models indicate temporal variation in lowland habitat suitability, which could produce distinct haplotype profiles through founder effects following long-distance dispersal and/or expansion from local microrefugia (Bennett & Provan 2008). Although stochastic founder effects could account for the anomalous haplotype profiles of populations BA2 (Pacific lowlands) and MIR (on Miravalles volcano) relative to neighboring populations at similar elevations (Figs. 1 and 2), recent climate-mediated bottlenecks are unlikely to account for the Pacific lowlands disjunction because: (1) regional demographic analyses did not indicate recent expansion (*i.e.*, non-significant Tajima's *D* and Fu's *F*) and (2) the comparable number of rare haplotypes in both regions is inconsistent with a lowland bottleneck. Our models suggest that climate oscillations induced elevational range shifts (Fig. 3), consistent with pollen and stable isotope records that reveal downslope shifts montane habitats during LGM cooling and subsequent upslope shifts by at least some species during MHW warming (Horn

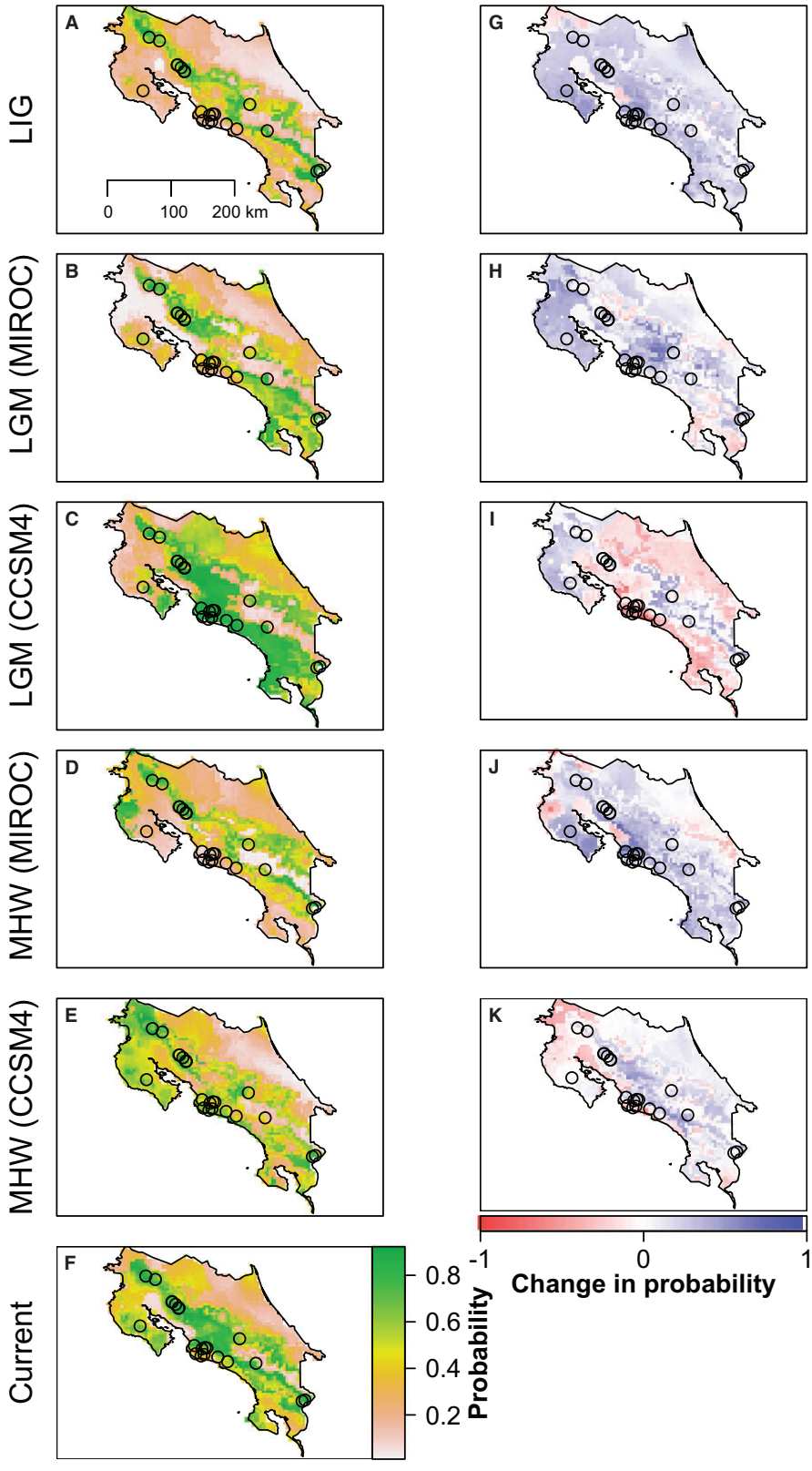

FIGURE 3. Paleoclimatic distribution models for *Catopsis nutans*. Species distribution models are shown (left) for (A) the last interglacial, (B, C) the last glacial maximum (MIROC and CCSM4, respectively), the (D, E) mid-Holocene period (MIROC and CCSM4, respectively), and (F) the current Maxent model. The difference in probability between current and each paleodistribution model are shown to the right (G–K). Each map is cropped to the current terrestrial boundaries of Costa Rica and shows study site locations.

1993, Islebe & Hooghiemstra 1997, Bagley & Johnson 2014). Yet contiguous lowland and upland habitats for *C. nutans* throughout these changes (Fig. 3) is inconsistent with the hypothesis of divergence in vicariant refugia (Bennett & Provan 2008). Thus, the distinct haplotype profiles could predate recent climate oscillations.

Despite the availability of only one cpDNA marker, we identified a clear and deep phylogeographic break among populations of a Central American plant species—a result that is notably less frequent in plant studies than animal studies in the region (Bagley & Johnson 2014). Identifying deep phylogeographic breaks in Central American plant species brings us closer to a more unified biogeographic framework for the region, and future comparative phylogeographic analyses of species across elevations could further elucidate the timing and drivers of population subdivision. Such analyses would be particularly illuminating if they encompassed both Caribbean and Pacific slopes and used both nuclear and organellar genetic markers.

The Florida population provided crucial insights regarding the haplotype network. Florida's Fakahatchee Strand is an ecotone between tropical and temperate vegetation, in which many plant species persist at their northern limit (Austin *et al.* 1990). *Catopsis nutans* has likely persisted in Florida since the Pleistocene, when Florida and the Caribbean islands were much larger and more closely connected due to lower sea level. Fixation of haplotype 1 in Florida may have resulted from a founder effect or genetic drift due to limited seed-mediated gene flow, small effective population size, and/or many generations of isolation combined with a slow Bromeliaceae cpDNA mutation rate (Givnish *et al.* 2011). Regardless of how fixation occurred in Florida, it suggests the ancestral nature and historical dominance of haplotype 1.

Costa Rican populations of *C. nutans* revealed low within-population haplotype diversity and moderate genetic structure. The historical genetic isolation of a cluster of populations on the central Pacific coast was inconsistent with the northwest–southwest discontinuity of most other plant taxa occurring in northwest Costa Rica. The observed disjunction may have arisen (1) prior to the initial colonization of Central America (*e.g.*, Dick & Heuertz 2008), (2) by re-colonization of the central Pacific lowlands after unfavorable climatic oscillations (*e.g.*, Palma-Silva *et al.* 2009), and/or (3) from the expansion of populations from local microrefugia (*e.g.*, as 'local' as individual tree canopies; Cascante-Marin *et al.* 2014). Paleodistribution models and comparable phylogeographic studies suggest the genetic signatures of recent climatic oscillations may be superimposed upon the signatures of earlier lineage divergence (Poelchau & Hamrick 2013b). This study highlights the unique population histories that may be revealed by the phylogeography of Neotropical species with broad elevation distributions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found with online material:

TABLE S1. *Results of a pilot screening for variation in* Catopsis nutans *chloroplast DNA.*

TABLE S2. *AMOVA table partitioning* Catopsis nutans *chloroplast DNA variation in Costa Rica.*

TABLE S3. *Pairwise F_{ST} for* Catopsis nutans *populations in Costa Rica.*

TABLE S4. *Loadings for the first three PCA axes measuring environmental distance among* Catopsis nutans *populations in Costa Rica.*

FIGURE S1. Species distribution maps for *Catopsis nutans.*

FIGURE S2. Correlations for *Catopsis nutans* between (A) F_{ST} and geographic distance, (B) F_{ST} and environmental distance and (C) environmental and geographic distance.

APPENDIX S1. List citing all sources accessed via the Global Biodiversity Information Facility (GBIF) on 27 January, 2015.

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