

INVITED SPECIAL ARTICLE

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Pleistocene aridification underlies the evolutionary history of the Caribbean endemic, insular, giant *Consolea* (Opuntioideae)

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PREMISE: The Caribbean islands are in the top five biodiversity hotspots on the planet; however, the biogeographic history of the seasonally dry tropical forest (SDTF) there is poorly studied. *Consolea* consists of nine species of dioecious, hummingbird-pollinated tree cacti endemic to the West Indies, which form a conspicuous element of the SDTF. Several species are threatened by anthropogenic disturbance, disease, sea-level rise, and invasive species and are of conservation concern. However, no comprehensive phylogeny yet exists for the clade.

METHODS: We reconstructed the phylogeny of *Consolea*, sampling all species using plastomic data to determine relationships, understand the evolution of key morphological characters, and test their biogeographic history. We estimated divergence times to determine the role climate change may have played in shaping the current diversity of the clade.

RESULTS: *Consolea* appears to have evolved very recently during the latter part of the Pleistocene on Cuba/Hispaniola likely from a South American ancestor and, from there, moved into the Bahamas, Jamaica, Puerto Rico, Florida, and the Lesser Antilles. The tree growth form is a synapomorphy of *Consolea* and likely aided in the establishment and diversification of the clade.

CONCLUSIONS: Pleistocene aridification associated with glaciation likely played a role in shaping the current diversity of *Consolea*, and insular gigantism may have been a key innovation leading to the success of these species to invade the often-dense SDTF. This in-situ Caribbean radiation provides a window into the generation of species diversity and the complexity of the SDTF community within the Antilles.

KEY WORDS Cactaceae; Caribbean Biogeography; dioecy; Greater Antilles; island gigantism; Opuntieae; polyploidy; seasonally dry tropical forest.

Although vastly important and well studied in island biogeography in animals (Dávalos, 2004; reviewed by Cadek et al., 2019), biogeographic patterns in plants have been little studied in the Greater Antilles, especially when incorporating comprehensive phylogenies of specific clades (Santiago-Valentín and Olmstead,

2004; Acevedo-Rodríguez and Strong, 2008; McDowell et al., 2009; Filipowicz and Renner, 2012; Cervantes et al., 2016; Nieto-Blázquez et al., 2017; Cano et al., 2018). The origin of the flora has been attributed to vicariance events, for instance, the GAARlandia hypothesis (Iturralde-Vinent and MacPhee, 1999),

wherein a landmass covering much of the current distribution of the Lesser Antilles served as a land bridge to the Greater Antilles from South America, and likewise, through long-distance dispersal. Although there is some evidence for the occurrence of GAARlandia, most recent analyses of plants and animals favor long-distance dispersal and subsequent in-situ radiation as the primary driver of diversity on the islands (Dávalos, 2007; Nieto-Blázquez et al., 2017; Candek et al., 2019; Crews and Esposito, 2020).

The Greater Antilles are considered a global biodiversity hotspot (Myers et al., 2000), and likewise, they are home to many native members of the family Cactaceae (ca. 94 taxa; L. C. Majure et al., unpublished data) that occur in the abundant and floristically diverse, seasonally dry tropical forests (SDTF) within the islands (Pennington et al., 2000, 2004; Acevedo-Rodríguez and Strong, 2012; Franklin et al., 2018). The Greater and Lesser Antilles house a phylogenetically diverse group of cacti, including members of the major clades, Cactoideae, Opuntioideae, and *Leuenbergeria* Lodé, and several taxa have undergone radiations there, including *Harrisia* Britton (Franck et al., 2013a, b), *Leuenbergeria* (as the northern clade of *Pereskia* Mill.; Edwards et al., 2005), *Melocactus* Link & Otto (L. C. Majure et al., unpublished manuscript), *Leptocereus* Britton & Rose, and *Consolea* Lem. However, only *Consolea* (Opuntioideae) and *Leptocereus* (Cactoideae; Barrios et al., 2020) represent endemic genera with each of these having formed in situ radiations in the Antilles. Considering the diversity of phylogenetically disparate clades of cacti in the Antilles, they are a superb group for which to test biogeographic hypotheses.

Consolea form a conspicuous component of the vegetation of SDTF in the Caribbean region. Nine currently recognized species (Areces, 2001; Negrón-Ortiz, 2007; Majure and Clase, 2018; Barrios et al., 2019) are found throughout the Greater Antilles (Cayman Islands, Cuba, Jamaica, Hispaniola, Puerto Rico, and U.S. Virgin Islands) and occur on a few of the northernmost islands of the Lesser Antilles south to Guadeloupe; one species also occurs in the Florida Keys [*C. corallicola* (Small) A. Berger] and two in the Bahamas [*C. nashii* (Britton) A. Berger and *C. millspaughii* (Britton & Rose) A. Berger]. The large tree habit exhibited by numerous taxa [e.g., *C. macracantha* (Griseb.) A. Berger, *C. microcarpa* (K. Schum.) E. F. Anderson, *C. moniliformis* (L.) A. Berger] give the appearance of the iconic Galapagos Island endemic prickly pear trees (*Opuntia*) and fit the pattern of island gigantism, as has been reported in numerous groups of insular plants and animals (Biddick et al., 2019).

Species of *Consolea* show a high degree of geographic cohesion and local endemism. *Consolea corallicola* is endemic to the Florida Keys (Pinkava, 2003), while the morphologically similar and geographically adjacent species *C. millspaughii* occurs in the Bahamas (Correll and Correll, 1982), the cays of the floristically similar eastern Cuba (Barrios et al., 2019), and in the Cayman Islands (Areces-Mallea, 2001). *Consolea macracantha* is restricted to mostly southern Cuba (although see the updated distribution of the species on Cuba by Barrios et al. [2019]), while *C. nashii* is restricted mostly to the Bahamas and east-central Cuba (those populations placed into a subspecies of *C. nashii*, *C. nashii* subsp. *gibarensis* Areces, by

Areces-Mallea [1996] based on morphological characters). *Consolea falcata* (Ekm. & Werderm.) F. M. Knuth is narrowly endemic to Hispaniola (found in both northwestern Haiti and the Dominican Republic; Majure et al., 2020), and *C. microcarpa* is endemic to the island of Hispaniola (see Majure and Clase, 2018), while *C. spinosissima* Lem. is found only on Jamaica (Areces-Mallea, 2001). *Consolea moniliformis* and *C. rubescens* Lem. are more widespread with *C. moniliformis* being found throughout Hispaniola, in parts of southeastern Cuba (circumscribed as *C. moniliformis* subsp. *guantanamoana* Areces by Areces-Mallea [2001]) and on Puerto Rico (Mona Island)—*C. rubescens* is found on Puerto Rico and in parts of the Lesser Antilles as far south as the island of Guadeloupe. There is some discussion about use of the name *C. rubescens* based on ambiguity regarding where the original material was from (Hunt et al., 2006); however, the name was neotypified by Guiggi (2007), based on material from Mona Island, Puerto Rico, which conforms to the material commonly referred to as *C. rubescens*.

Species of *Consolea* share a suite of unusual morphological characters (e.g., Fig. 1A–D). Species are small to large trees, with some species such as *C. microcarpa* reaching heights greater than 10 m (Majure and Clase, 2018), and their principal stems exhibit indeterminate growth (i.e., are monopodial and orthotropic; Fig. 1D), as in *Brasilopuntia* (Majure and Puente, 2014). Branches are often produced perpendicular to the main trunk and have delayed determinate growth (i.e., they grow indeterminately for some time before terminating and halted growth often recommences at the apex, continuing an indeterminate growth pattern); those stem segments or cladodes often also are slightly or strongly falcate or asymmetrical (Fig. 1A, C). Flowers are yellow, red, or reddish-orange (Fig. 1B), mostly scentless or with very mild odor, have a large nectar chamber below the basally expanded style, and produce copious pollen grains. These flowers are primarily pollinated by hummingbirds, the most frequent visitors to the flowers, followed by bees. Most species are dioecious or subdioecious (Strittmatter et al., 2002; Negrón-Ortiz and Strittmatter, 2004; Negrón-Ortiz, 2007)—the subdioecious species have males, females, and inconstant males. Inconstant males are identical to functionally staminate flowers of the male morph, but some flowers possess a few, viable ovules that will eventually develop into seeds, as in *C. corallicola* (Negrón-Ortiz et al., 1998; Negrón-Ortiz, 2007). Certain populations of *C. corallicola* are composed only of males and inconstant males. Several species exhibit a papillose-reticulate epidermis (Fig. 1), e.g., *C. moniliformis*, *C. spinosissima*, a character unique in tribe Opuntieae, and which has been used to separate species into morphogroups (Areces-Mallea, 1996, 2001).

The phylogeny of the group is poorly understood, but it is clear that *Consolea* forms a well-supported clade (Griffith and Porter, 2009; Majure et al., 2012; Majure and Puente, 2014). Griffith and Porter (2009) using a combination of nrDNA (ITS) and the plastid intergenic spacer, *trnL-F*, showed that *Consolea* was nested within *Opuntia*, which led Nyeffeler and Eggli (2010) to synonymize *Consolea* with *Opuntia*. Majure et al. (2012) found the same topology using ITS data alone; however, on the basis of plastid data, *Consolea* was well supported as sister to the *Tacinga* + *Brasilopuntia*

FIGURE 1. Selected morphological characters of *Consolea*. (A) Reticulate epidermis of *C. moniliformis*, Sierra Martín García, Dominican Republic (Majure 6318), (B) smooth epidermis and spiny pericarpel of *C. corallicola* from the Florida Keys (DBG 1997 0397), (C) large trees of *C. macracantha* with spiny trunks from Santiago de Cuba, Cuba (Majure, Barrios, Díaz 7008), and (D) trees of *C. moniliformis* showing the very, spiny, monopodial trunks and ephemeral cladodes scattering the ground from Loma la Vigía, Dominican Republic (Majure 7810). Image credit: L. C. Majure.



(+ *Opuntia*) clade (Majure et al., 2012; Majure and Puente, 2014), and a combination of plastid and nuclear loci showed the same topology (Majure and Puente, 2014). The competing phylogenetic signal between ITS and plastid data led Majure et al. (2012) to suggest that the polyploid *Consolea* clade (see Negrón-Ortiz, 2007) may have been derived initially from deep reticulate evolution, which certainly may be accurate. However, further work in other clades within Opuntioideae has revealed similar topological incongruences between plastid and ITS data (Ritz et al., 2012; L. C. Majure et al., unpublished manuscript), especially at deeper phylogenetic levels, suggesting that either paralogy or homoplasy may play a role in those poorly supported incongruences based on ITS data in Cactaceae. More recent work using plastome data has shown that *Consolea* was likely sister to the rest of Opuntieae (Köhler et al., 2020).

Negrón-Ortiz (2007) showed through chromosome counts and flow cytometry that the species of this clade are polyploid, ranging from hexaploid ($2n = 66$) to octoploid ($2n = 88$). Baker et al. (2009) found one individual of *Consolea corallicola* to be dodecaploid ($2n = 120$). Majure et al. (2012) showed that *Consolea* had been involved in several intergeneric hybridization events presumably with *Opuntia stricta* (Haw.) Haw. s.l. ($2n = 66$) leading to the allopolyploid taxa *Opuntia acaulis* Ekm. & Werderm., *O. bahamana* Britton & Rose and *O. lucayana* Britton, these taxa being derived in each case from polyploid progenitors. Intrageneric hybridization in the *Consolea* clade has not been recorded, and Arces-Mallea (1996) mentioned the lack of interspecific hybridization in the group, although certain populations in Cuba do suggest the potential presence of hybridization between species (Barrios et al., 2019), and L. C. Majure (unpublished data) has found putatively hybrid-derived individuals on Hispaniola.

Species of *Consolea* throughout the Caribbean are in danger from anthropogenic disturbance, such as deforestation—SDTF is one of the most highly endangered forest systems worldwide (Janzen, 1988; Pennington et al., 2000, 2018)—and likewise are poised to suffer directly from the effects of sea level rise, as most species occur in low elevation SDTF. *Consolea corallicola* of the Florida Keys, a federally listed species, for example, often occurs a meter or less above sea level and has suffered in the last decades from extreme habitat loss driving the extirpation of several populations including the historical site where this species was first found (Bradley and Gann, 1999; Bradley and Koop, 2003). This species has also suffered in the last two decades from invasion by the non-native cactus moth, *Cactoblastis cactorum* Berg. (Stiling and Moon, 2001). *Cactoblastis cactorum* (Berg) also has been recorded on *C. macracantha* in Santiago de Cuba (Barrios et al., 2019), as well as in *C. macracantha* and *C. moniliformis* from Guantánamo Bay (Arces-Mallea, 2010). Species of *Consolea* (known as “asplagata” in Cuba and the Dominican Republic) in the Dominican Republic often are cut down and used to feed livestock (F. Jiménez, Jardín Botánico Nacional “Dr. Rafael M. Moscoso,” personal communication), especially in the case of *C. microcarpa*, which produces mostly spineless cladodes in age (Majure and Clase, 2018). Understanding the evolutionary history of the clade and determining the phylogenetic relationships among species would provide for a better understanding of species’ delimitations and distributions, which would greatly aid in developing and implementing conservation strategies for potentially threatened members of *Consolea*.

Majure et al. (2012) and Majure and Puente (2014) sampled six of the nine species of *Consolea* using six plastid and two

nuclear markers. Those data sets proved to be insufficient for resolving species relationships and, thus, for sufficiently determining the evolution of morphological characters and the biogeographic history and age of the clade, which appears to be phylogenetically isolated in Opuntieae (Majure and Puente, 2014). We wanted to test species relationships of the *Consolea* clade using whole plastomic data and to re-evaluate the phylogenetic position of the clade in Opuntieae. Our phylogenetic framework based on plastomic data was used for interpreting the evolution of morphological characters, clade age, and the biogeographic history of the group among the islands where they occur. Although it is relatively clear that *Consolea* evolved and diversified in situ in the Antilles, we wanted to determine the age and place of origin and subsequent dispersal patterns of the clade within the Antilles that have led to the current distribution and diversity within the clade and determine whether that diversity could have been tied to past climatic events.

MATERIALS AND METHODS

Taxon sampling, DNA extraction, sequencing

We sampled all nine species of *Consolea* from field-collected material and individuals grown at the Desert Botanical Garden (Phoenix, AZ, USA) and Montgomery Botanical Center (Miami, FL, USA) from wild sources. We also sampled other members of Opuntieae (*Opuntia* s.s., *Tacinga*, *Miqueliopuntia*, *Tunilla*), and outgroups in tribes Cyliandropuntieae, Tephrocactaceae, subfamily Cactoideae, genera *Maihuenia* and *Pereskia* s.s., and one member of the family Portulacaceae (see Appendix 1). Although we initially sampled *C. moniliformis* subsp. *guantanamana* and *C. nashi* subsp. *gibarensis*, both morphological and DNA sequence data suggest that those taxa could be of hybrid origin—those taxa will be analyzed in a subsequent manuscript, as that was out of the scope of this paper. DNA was extracted from epidermal tissue dried in silica gel for all taxa using a modified CTAB method (Doyle and Doyle, 1987). Briefly, tissues were homogenized using a mortar and pestle using sterilized sand and combined with 1.2 mL of CTAB buffer and 10 μ L of proteinase K. Samples were incubated at 55°C for up to 2 h and then 500 μ L of a 24:1 v/v solution of chloroform–isoamyl alcohol was added, samples vortexed, and centrifuged for 10–15 min. The supernatant was placed directly into an EconoSpin spin column and mixed with 20 μ L of 3 M NaAc and 450 μ L of Qiagen (Hilden, Germany) PB binding buffer, spun for 1 min, then cleaned once with Qiagen PE wash buffer. DNA was resuspended in 300 μ L of Tris-EDTA (TE) buffer (pH 8.0), and DNA quality was tested using a 1% agarose gel and quantity was analyzed on a Qubit 2.0 Fluorometer (see Neubig et al., 2014; Majure et al., 2019). Existing data used here from previous studies (Arakaki et al., 2011; Majure et al., 2019; see Appendix 1) were downloaded from GenBank (Bethesda, MD, USA). All whole genomic DNAs were sent to Rapid Genomics LLC (Gainesville, FL, USA) for library preparation and sequencing using a genome skimming approach (Straub et al., 2012) on the Illumina HiSeq X Ten platform using paired-end, 150-bp reads. Genome skimming refers to shallow sequencing of whole-genomic DNA, which preferentially retrieves high copy-number portions of genomes and is thus efficient for sequencing nrDNA and plastome fractions of whole genomic DNA (see Majure et al., 2019, 2021a; Köhler et al., 2020, for the use of genome skimming in Cactaceae). Raw reads used in our plastome and ETS/ITS assemblies from all newly sequenced

taxa for this study (see Appendix 1) were deposited in the NCBI Sequence Read Archive under Bioproject PRJNA650542 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA650542>).

Assemblies, alignment, and phylogenetic analyses

Reference-guided assemblies were carried out using raw reads based on the de novo-assembled plastome of *Cylindropuntia bigelovii* from Majure et al. (2019) and nrDNA data (ETS/ITS) based on unpublished data (L. C. Majure, unpublished data) in Geneious version 11.1.5 (Biomatters, Auckland, New Zealand) after trimming using an error probability limit of 5%. Our nrDNA data set was generated from taxa from only within subfamily Opuntioideae specifically to test for putative reticulation in *Consolea*—deep phylogenetic relationships based on ITS data appear to be affected by homoplasy in Cactaceae leading to erroneous topologies (L. C. Majure, unpublished data). Majority consensus sequences were generated from our reference guided assemblies of raw reads and were used for building our alignment with the MAFFT (Katoh and Standley, 2016) plugin in Geneious, which was then manually checked. Unalignable areas in the plastome derived from putative structural complexities or putative pseudogenes were removed from downstream phylogenetic analyses (see Majure et al., 2019). Our data sets are deposited in Dryad (Majure et al., 2021b). Our plastome (minus one copy of the inverted repeat) of 124,606 bp and nrDNA data sets (6891 bp) were analyzed using maximum likelihood (ML) undertaking 1000 bootstrap pseudoreplicates using the GTR + G model of molecular evolution in RAXML (Stamatakis, 2014) through the Geneious plugin. Likewise, due to the low level of sequence divergence among species of *Consolea*, we implemented the transfer bootstrap on our plastome data set (Lemoine et al., 2018) to test for underlying phylogenetic signal that may not have been taken into account using traditional bootstrapping analyses (Felsenstein, 1985). Likewise, to determine the number of parsimony informative characters in our plastome data set, we ran a maximum parsimony (MP) analysis in PAUP* version 4.0 (Swofford, 2003) on a “*Consolea* only” data set, “Outgroup only” data set, and the entire plastome data set for comparative purposes.

Given that initial phylogenetic results from analyses of our entire plastome and nrDNA data sets suggested that *C. moniliformis* was not monophyletic, we carried out several subsequent analyses to determine the topological effects of excluding accessions for downstream morphological, biogeographic, and divergence time estimation analyses. We reduced our plastome and nrDNA data sets to just one accession per species and re-ran our RAXML analysis on the reduced data sets. We then carried this out twice more in our plastome data set but replacing the *C. moniliformis* accession each time with a separate accession. Based on our topological results from these analyses (see Results), our reduced plastome data set with one accession per species using *C. moniliformis* LCM6318, which was resolved as sister to the rest of the *Moniliformis* clade, was used for all other downstream analyses (see below and Results).

Morphological evolution

We reconstructed the ancestral state of seven morphological characters in *Consolea* (e.g., Fig. 1) reducing our topology to just one accession per species (see above) using our RAXML topology. We coded the following characters: (1) mature cladodes with or without spines, (2) cladode epidermis reticulate or not, (3) mature trunk covered with elongated spines or not, (4) pericarpel spiny or not,

(5) growth habit (i.e., trees, shrubs, herbs), (6) growth pattern (monopodial vs. sympodial principal trunks), and (7) dioecy/subdioecy vs. monoecy/hermaphroditism (Appendix S1). Several of these characters (reticulate epidermis, spiny pericarpels) have been used to recognize morphogroups within *Consolea*, and so are considered taxonomically useful. Likewise, we wanted to test whether the tree growth form was synapomorphic for this SDTF group, a character that would likely aid these taxa in occupying and competing for resources in dense, tropical forests. A commonly cultivated form of *C. rubescens* lacks spines almost entirely (i.e., on the trunks, stems, and pericarpel; see Majure et al. 6995-DES, HAJB), but only the wild-type form was considered for our ancestral state reconstructions. Maximum likelihood (for binary character states) implementing the Mk1 model, which allows for an equally probable change from one state to another, and maximum parsimony (for polymorphic character states) were used in Mesquite v. 3.61 (Maddison and Maddison, 2017) for ancestral state reconstruction.

Divergence time estimation

Divergence time estimation analyses were carried out in BEAST 2.6.0 (Bouckaert et al., 2014) based on our one accession/species data set using the University of Florida high performance cluster (Gainesville, FL, USA). The data set was analyzed using an uncorrelated lognormal relaxed clock model (mean = 0.5, SD = 1.25) and a birth–death model of diversification (uniform prior from 0 to 3 for speciation rate and 0 to 2 for relative extinction rate; starting value were 0.1 for speciation and 0.5 for relative extinction). The GTR+I+G substitution model was selected with gamma site heterogeneity and four rate categories, based on model selection using PartitionFinder 2 (Lanfear et al., 2017). We applied a universal mutation rate to the data set to facilitate convergence in our Markov chain Monte Carlo (MCMC) runs. Because no Cactaceae fossils are known, we incorporated two secondary calibrations from Arakaki et al. (2011) in our analyses: one using the mean stem age of Cactaceae recovered in their study (35 Myr) and another with their estimate of the family’s crown age (28.6 Myr). These priors were given normal distributions centered on these ages—we assigned standard deviations of 2.6 Myr (stem age calibration) and 1.9 Myr (crown age calibration) to these distributions, matching the values recovered by Arakaki et al. (2011). For both analyses, eight independent MCMCs of 50 million generations each were run with a sampling interval of 10,000 generations. The log files and trees from eight parallel runs were combined resulting in 40,000 trees. These were subsampled at a reduced frequency using LogCombiner 2.6.0 in the BEAST package, resulting in a total of 10,000 trees. The resulting log files were examined using Tracer 1.7.1 (Rambaut et al., 2018) to assess convergence and ensure that all effective sample size (ESS) values were above 200. The first 10% of the trees were discarded as burn-in, and the resulting trees were used to generate a maximum clade credibility tree using TreeAnnotator 2.6.0, which was visualized using FigTree v.1.4.4 (Rambaut, 2016).

Biogeographic analyses

Biogeographic units were separated as follows for *Consolea* based on species’ current distributions: (1) Cuba (D), (2) Hispaniola (C), (3) Jamaica (G), (4) Puerto Rico (including Mona Island-F), (5) The Bahamas (E), (6) North America (Florida) and North America (including Central America-B), and (7) South America (A) were used

for outgroups. *Consolea corallicola*, which occurs in the Florida Keys was coded as North American. To test the biogeographic history of the clade, we used BioGeoBEARS (Maztke, 2014) implemented in RASP (Yu et al., 2015). We used the DEC model for our analysis (Ree et al., 2005; Ree and Smith, 2008) given that there is no assumption of identical range inheritance for descendants (see also Landis et al., 2013)—our analysis was implemented using our time-calibrated phylogeny based on the crown-age secondary calibration from our BEAST analysis (see above). No dispersal constraints were applied to our data set. We were principally focused on determining the origin of the *Consolea* clade and of each of the three subclades.

RESULTS

Phylogeny and morphological evolution

Raw reads per accession ranged in number from ca. 4–16 million reads; the proportion of plastid data ranged from 2–3.5% of those reads, similar to previously recorded amounts for genome

skimming in cacti (Majure et al., 2019). Overall sequence divergence in *Consolea* was minimal, as compared to other close relatives of tribe Opuntieae. Our plastome data set showed little sequence divergence within *Consolea*, with our “*Consolea* only” data set exhibiting only 71 parsimony informative characters (PICs)—our “Outgroup only” data set contained 3470 PICs, and our entire data set contained a total of 3941 PICs. Tribe Opuntieae was well supported in our plastome analyses (bootstrap support [BS] = 100), as sister to the *Cylindropuntia* + *Tephrocactaceae* clade. *Consolea* likewise, formed a well-supported clade (BS = 100) sister to a *Tacinga-Miqueliopuntia-Tunilla-Opuntia* clade (i.e., the rest of Opuntieae). Three major subclades were resolved in our phylogenetic analysis, the *Macracantha*, *Millspaughii*, and the *Moniliformis* clades. Two species included here were nonmonophyletic—*C. corallicola* was nested within *C. millspaughii*, and *C. microcarpa*, *C. rubescens*, and *C. spinosissima* were nested within *C. moniliformis*. However, *C. macracantha* from Cuba did form a well-supported clade (Fig. 2A), which was sister to the *Moniliformis* clade in our expanded data set. Based on our reduced data sets with separate accessions of

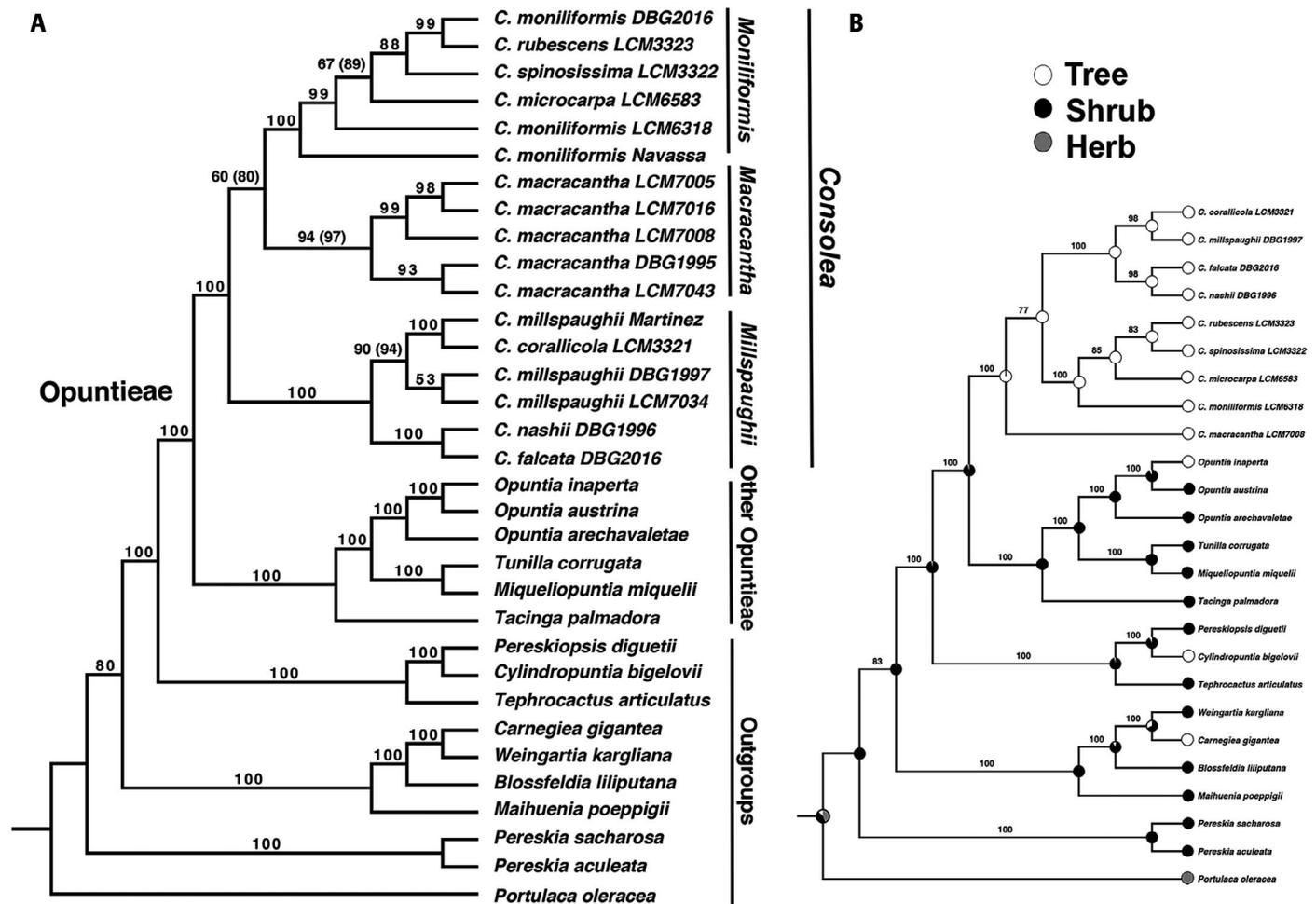


FIGURE 2. Plastome phylogeny of *Consolea* and character mapping of habit. (A) Phylogeny of *Consolea* showing the position of *Consolea* sister to the rest of tribe Opuntieae and the three major subclades, *Macracantha*, *Millspaughii*, and *Moniliformis*. Bootstrap support (BS) values are given along branches and transfer BS values are given in parentheses where they differed from traditional BS values. (B) Maximum likelihood phylogeny based on our reduced data set and reconstruction of habit is mapped onto the topology for trees (white circles), shrubs (black circles), and herbs (grey circles). The tree habit is a clear synapomorphy of the *Consolea* clade.

C. moniliformis, two accessions were always resolved as sister to the rest of the clade (*C. moniliformis* LCM6318 and *C. moniliformis* Navassa). However, our accession of *C. moniliformis* DBG2016 was resolved nested within the clade as sister to *C. rubescens* (Appendix S2). Thus, we used our accession *C. moniliformis* LCM6318 for all other analyses, given the consistency in that placement for *C. moniliformis* for two separate accessions. Our reduced data set used for analysis of morphological evolution, divergence time estimation, and biogeography were nearly identical topologically to our more inclusive data set, resolving three clades; however, the placement of *C. macracantha* shifted

from being sister to the *Moniliformis* clade to sister to the rest of *Consolea* (Fig. 2B). Likewise, our ML topology differed in topology from our BEAST analysis in the placement of *C. moniliformis*, which was sister to the rest of the *Moniliformis* clade in our reduced data set, while *C. microcarpa* was sister to the rest of the clade in our BEAST topology (Figs. 3, 4).

Based on low sequence coverage, we were unable to use two accessions of *Consolea* in our nrDNA data set (*C. moniliformis* DBG2016 and *C. millspaughii* Martínez). Our full nrDNA data set topology, like our plastome topology resolved a well-supported *Millspaughii* clade, with *C. nashii* + *C. falcata* sister to a clade

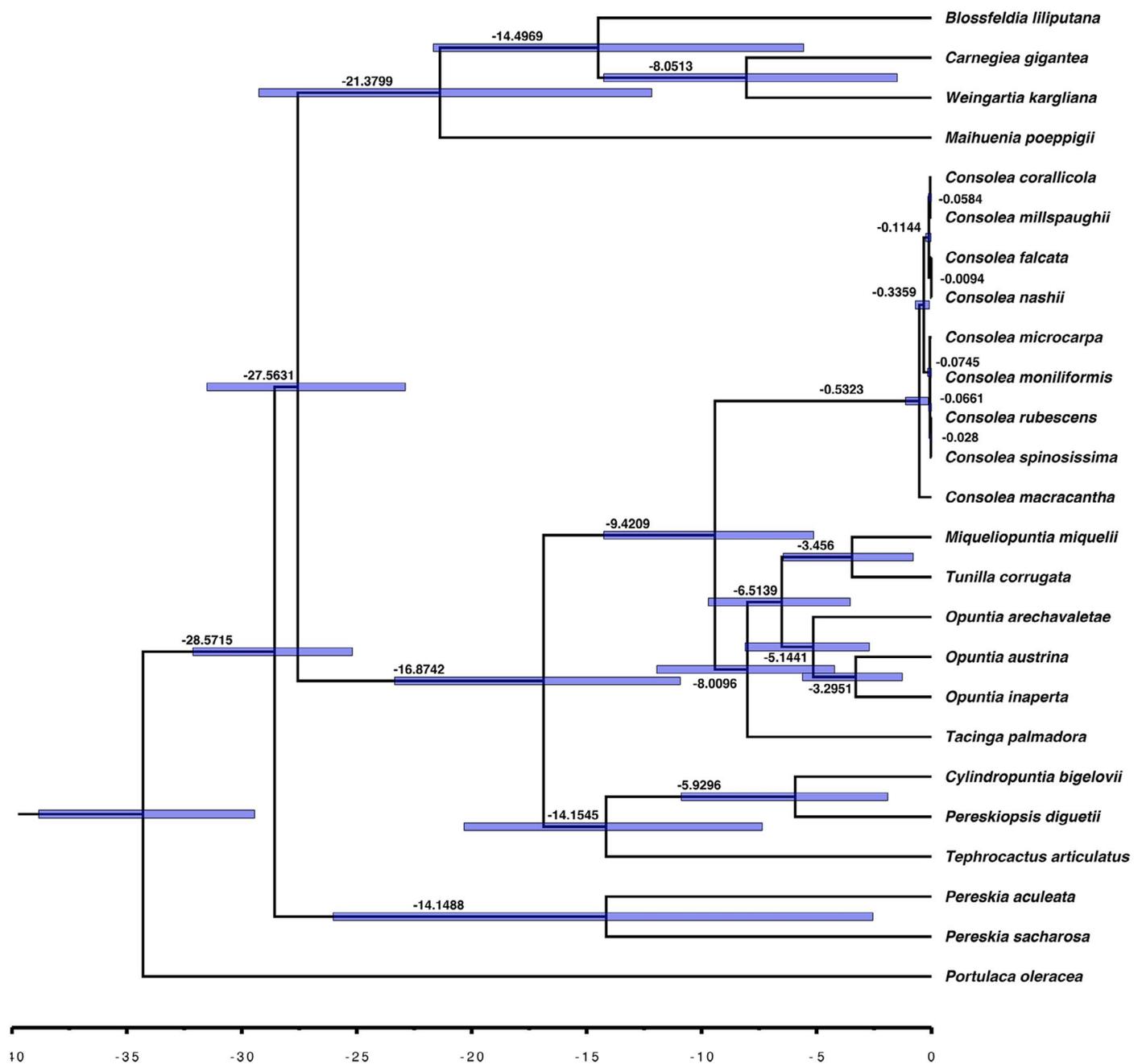


FIGURE 3. Divergence time estimation (estimates given above branches) for *Consolea*. The *Consolea* clade was estimated to have diversified in the latter part of the Pleistocene (ca. 538,000 years ago) with speciation events occurring from around 300,000 to 9000 years ago.

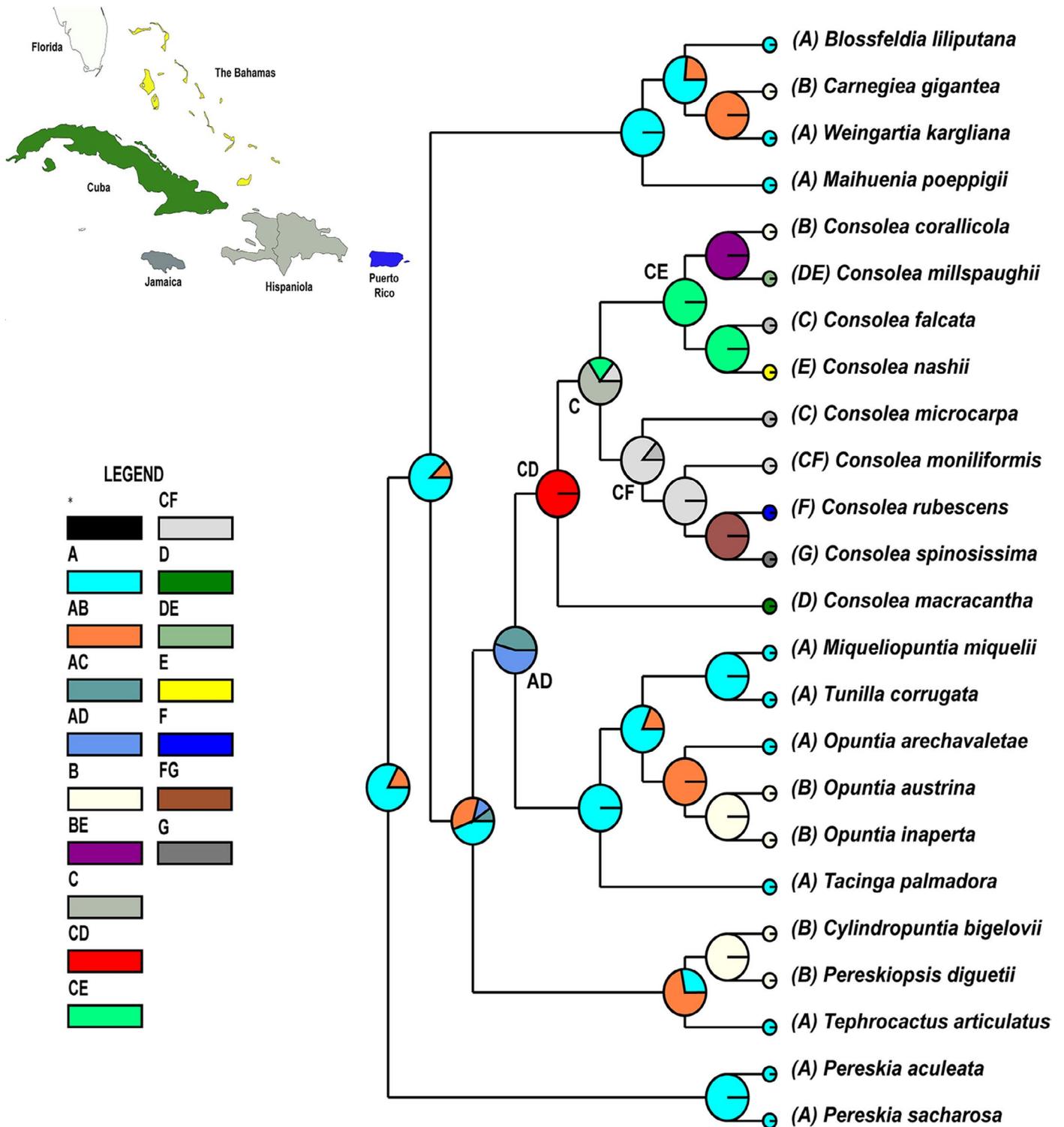


FIGURE 4. Biogeographic history of the *Consolea* clade. According to our analyses, the *Opuntieae* clade most likely originated from a South American/Cuban (AD) ancestor. The most likely ancestral area for *Consolea* was a combination of Cuba and Hispaniola (CD). The *Millspaughii* clade dispersed from Hispaniola (C) into the Bahamas (E), Cuba (D), and North America (B), and the *Moniliformis* clade subsequently dispersed into Jamaica (G) and Puerto Rico (F). South America (A) is not presented on the map.

consisting of *C. millspaughii* and *C. corallicola*; however, the *Macracantha* clade was poorly supported (BS = 56) with *C. rubescens* nested within, and the *Moniliformis* clade was not resolved.

Our reduced nrDNA topology likewise resolved a well-supported *Millspaughii* clade, but there was no resolution among a clade consisting of members of the *Macracantha* and *Moniliformis* clades.

Given the very poor resolution in our nrDNA topology, there was no strong evidence for reticulate evolution in *Consolea* based on our current taxon sampling (Appendix S3).

The tree growth form was a synapomorphy of the *Consolea* clade in both ML (Fig. 2B) and MP (not shown), as was monopodial growth of the primary trunk in tribe Opuntieae (Appendix S4A), although, this is not unique in Cactaceae. The production of trunks with dense spines using MP was equivocal in our reconstruction (Appendix S4B), although, the majority of *Consolea* species exhibit this character (e.g., Fig. 1D), which was lost in the *C. nashii*-*C. falcata* clade and is polymorphic in *C. microcarpa* and *C. rubescens* (Appendix S4B). Reticulate epidermis of the cladode surface appears to have evolved twice in the *Moniliformis* clade based on ML (Appendix S4C). Spiny pericarpels (Appendix S4D) were synapomorphic using MP for the *Millspaughii* clade and *C. rubescens* but were polymorphic for *C. moniliformis*. Non-spiny pericarpels were plesiomorphic. Spiny, mature stem segments (Appendix S4E) were plesiomorphic in Cactaceae; however, spineless, mature cladodes were equivocal for the *Consolea* clade—this character may have been lost independently in the *Macracantha* and *Moniliformis* clades. Dioecy was a clear synapomorphy for the *Consolea* clade (Appendix S4F).

Divergence time estimation—Our BEAST analyses resulted in ESS values for all parameters >200 and generated a well-supported phylogeny that was topologically identical, regarding subclades, to that found in our ML analyses based on reduced taxon sampling (Fig. 3). Our inferred crown and stem ages for Cactaceae are nearly identical to those reported by Arakaki et al. (2011). Our inferred diversification times were not surprising, given that these age estimates were inferred in part with secondary calibrations derived from that study, though it is important to recognize that secondary calibrations often result in age estimates considerably different from those reported in the original study (Schenk, 2016). Crown age estimates of major clades included here were Cactoideae (21.37 Myr), Opuntioideae (16.87 Myr), Opuntieae (9.42 Myr). The crown age of the *Tacinga* + *Opuntia* clade was estimated at 8.01 Myr. We recovered a very young crown age for *Consolea* (532,000 yr; 95% HPD = 0.13–1.129 Myr). Species divergence estimation within the clade were still younger, ranging from ca. 300,000–9000 yr in age (Fig. 3), although we treat these dates with caution given the nonmonophyly of taxa such as *C. moniliformis* and the potential for reticulate evolution in that clade.

Biogeography—Based on our DEC analysis, tribe Opuntieae was reconstructed as most likely having a South American/Cuban ancestor (55%), and the *Tacinga* + *Opuntia* clade most likely had a South American ancestral range (81%). The *Consolea* clade was suggested to have originated on Cuba/Hispaniola (66%); from Hispaniola, there was dispersal into the Bahamas, likely once (*Millspaughii* clade) and then into North America (Florida). The *Moniliformis* clade moved into Jamaica (*C. spinosissima*) and Puerto Rico (*C. rubescens* and *C. moniliformis*) from Hispaniola (Fig. 4).

DISCUSSION

Diversification driven by Pleistocene aridification

The young ages recovered here for *Consolea* have likewise been recovered in two, much larger data sets focused on the Opuntieae and Opuntioideae (M. Köhler et al., Universidade do Rio Grande

do Sul, unpublished manuscript; L. C. Majure et al., unpublished manuscript); thus, our results here provide further support for a Pleistocene age for *Consolea*. Glacial and interglacial events during the Pleistocene are well known for their global effects on speciation and diversification in many organisms, especially plants (Stebbins, 1950, 1971; Simpson, 1975; Gentry, 1982; Prance, 1982; Pennington et al., 2004; Soltis et al., 2006; Rull, 2008; Ellison et al., 2012). Pleistocene fluctuations have also been suggested to have affected diversification in numerous groups of cacti from both North and South America, including some Antillean taxa (Majure et al., 2012; Ornelas and Rodriguez-Gómez, 2015; Menezes et al., 2016; Perez et al., 2016; Franco et al., 2017; Lavor et al., 2019). In particular, the genus *Harrisia* shows a clear Caribbean radiation possibly originating in western Cuba that likely corresponds to the mid-Pleistocene transition (Franck et al., 2013a) very similar to the pattern seen here in *Consolea*.

Certain authors have suggested that drier conditions during Pleistocene cooling events may have led to an expansion of SDTF across Amazonia and other parts of the Neotropics (Bonatti and Gartner, 1973; Prado and Gibbs, 1993; Pennington et al., 2000, 2004, 2005), which may currently account for conspicuous disjunct distributions seen in many SDTF species across the Americas, including in the Greater Antilles. The expansion of SDTF in the Greater Antilles during that time may likewise account for the diversification of *Consolea* across the islands. The evolution of a tree growth form may have given the clade the ability to compete for resources (specifically incident photosynthetically active radiation [PAR]) alongside expanding SDTF, broad-leaf vegetation with which species of *Consolea* grow today, thus negating the necessity for restricting their distributions to open habitats. The tree growth form also would have allowed species to disperse broadly throughout dense SDTF across the Greater Antilles, at least within islands, and may account, in part, for the wide distributions of some of the most widespread species, such as *C. moniliformis* (Cuba, Hispaniola, Puerto Rico), *C. macracantha* (throughout Cuba) and *C. microcarpa* (throughout Hispaniola). Notably, the *Leptocereus* and *Leuenbergeria* clades occurring in SDTF of the Greater Antilles, also include members that form trees (Barrios et al., 2020; Majure et al., 2021a). Curiously, other members of tribe Opuntieae, as well as across Cactaceae, that occur in continental SDTF commonly form trees, such as *Pachycereus* (Cactoideae), *Brasiliopuntia* (Opuntieae) and members of the *Nopalea* clade of *Opuntia* (Majure and Puente, 2014). Monopodial (i.e., indeterminate) growth of the primary stem in *Consolea* further increases the efficiency of rapidly producing individuals large enough to penetrate the canopy produced by surrounding vegetation. Interestingly, species of *Consolea* tend to only be as tall or nearly the same height as surrounding vegetation, so *C. nashii*, which grows in relatively short SDTF in the Bahamas forms small trees to ca. 3–4 m tall, whereas *C. microcarpa*, which occurs in well-developed SDTF with large trees forms impressively large trees to ca. 10 m in height. Whether these differences are due to adaptation or phenotypic plasticity has not been investigated; the latter could be due to height convergence (Nagashima and Hikosaka, 2011).

It is clear that parts of specific landmasses in the Antilles, such as the Bahaman Bank were much larger in parts of the Pleistocene during full glacial cycles, leading to much increased habitat for a number of species (Steadman et al., 2015; Steadman and Franklin, 2015, 2017). These expanded areas likely led to increased habitat for species of *Consolea* and could have also aided the dispersal of

species across, what were then, shorter distances. Habitat expansion is especially interesting considering the distribution of the *Millspaughii* clade with members in the Bahamas and in central coastal Cuba, areas that would have been very close to one another during previous glacial events (see map of Steadman and Franklin, 2015, 2017). Likewise, our divergence time estimates support a scenario where movement during those times could have been possible, and both areas share numerous plant species (Correll and Correll, 1982; L. C. Majure, personal observation). However, owed to the ocean depth surrounding most of Hispaniola and southeastern Cuba, this phenomenon likely would have played less of a role in facilitating dispersal in those areas.

Island to mainland dispersal events, although apparently not as frequent as continental to island dispersal events, occurred several times in Cactaceae. *Consolea corallicola* clearly originated from dispersal from the islands into southern Florida (Fig. 4). Likewise, other cacti, such as *Harrisia* and *Pilosocereus* show the same pattern (Franck et al., 2013b; Lavor et al., 2019), as do a wide range of other widespread tropical species that can be found naturally in south Florida and which are shared with the Caribbean (Long, 1974; Myers et al., 2000; Negrón-Ortiz and Watson, 2003; Santiago-Valentín and Olmstead, 2004). Some animals show the same pattern, such as the short-faced bats, which initially diversified in the Caribbean before dispersing to continental North and South America (Dávalos, 2007).

No studies have been carried out to detail dispersal mechanisms in *Consolea*. Several species with spiny pericarpels (e.g., *C. corallicola*, *C. millspaughii*, *C. nashii*) produce easily disarticulating, sterile fruit that commonly act as vegetative propagules, falling to the ground, rooting, and producing clonal individuals of the parent plant (Majure and Puente, 2014). Likewise, sterile fruit produced by males of taxa with non-spiny or spiny pericarpels, such as *C. macracantha* and *C. nashii*, commonly are proliferous, producing numerous new cladodes from the apices of those sterile fruit. These new cladodes easily disarticulate, fall to the ground and root, forming clones of the parent plants (Barrios et al., 2019), so dioecy also likely drives vegetative propagation in *Consolea*. It is clear that at least short-distance dispersal could be realized through clonal propagation from sterile fruit. The sterile fruits referred to in these studies are apparently the fruits of “inconstant males” as defined above, in which case they presumably include a few viable (although apomictic) seeds. In the case of *C. corallicola*, these seeds have been observed to germinate while still inside disarticulated fruit (Negrón-Ortiz, 1998).

Although many studies have reported the consumption of fruit and potential dispersal of seeds in *Opuntia* by an array of vertebrates (Mellink and Riojas-López, 2002), there are no such studies for *Consolea*, and in general, there is little data for the dispersal of fruit in Cactaceae in the Caribbean. Barrios and Macina (2017) suggested that seeds of *Leptocereus nudiflorus* (Engelm. ex C. Wright) D. Barrios & S. Arias may be dispersed effectively, at least contemporaneously, by the iguana, *Cyclura nubila* Gray. However, they also suggested the possibility of dispersal by Pleistocene megafauna, such as the Cuban ground sloth, *Megalocnus rodens* Leidy, although they found little support for that idea. Another potential disperser during Pleistocene times could have been large tortoises (*Chelonioidis*) that once were found throughout Cuba, Hispaniola (including Navassa Island), Puerto Rico (Mona Island), as well as in the Bahamas (Williams, 1952; Auffenberg, 1967; Olson et al., 1982; Franz and Woods, 1983; Franz and Franz, 2009; Turvey et al.,

2017), most likely in open, dry habitats (Turvey et al., 2017). These giant tortoises have been considered ecosystem engineers, and modern insular species are known to interact with large cacti in the Galapagos Islands (e.g., Gibbs et al., 2010), so these species may have been key in aiding dispersal of cacti. Other authors, likewise, have suggested that megafauna may have played an important role in the dispersal and maintenance of populations of cacti (Janzen, 1986) and other large-fruited neotropical angiosperms with no apparent, effective, modern-day dispersal agents (Janzen and Martin, 1982), and their role could have been important during the Pleistocene for *Consolea* as well. Birds and bats have also been posited as potentially aiding in the dispersal of Cactaceae in the Caribbean region (Franck et al., 2013a), and likewise, birds are known to disperse seeds in *Opuntia*, so the role of birds in dispersing seeds of *Consolea* cannot be ruled out.

Majure et al. (2007) suggested that major meteorological events, such as hurricanes, could be potential drivers of maritime dispersal in *Opuntia* in the southeastern United States. Other authors have also reported dispersal of *Opuntia* via waterways and have shown their resilience to extended periods of exposure to water (Frego and Staniforth, 1985). Thus, the possibility of dispersal of vegetative propagules of *Consolea*, as well as other cacti, in and among the Caribbean Islands via meteorological events in such a hurricane prone area (Johnson, 2015) seems highly likely.

Morphology and geography

Species within clades were typically from geographically adjacent areas, although did not always share clear morphological characters. For instance, *C. microcarpa* (Hispaniola) and *C. rubescens* (Puerto Rico-Lesser Antilles), which have a nonreticulate, mostly smooth epidermis (Appendix S4C), were recovered within the *Moniliformis* clade—both *C. moniliformis* and *C. spinosissima* exhibit a strongly reticulate epidermis. Although a reticulate epidermis is reconstructed as evolving twice in the *Moniliformis* clade, it appears much more likely that character is synapomorphic for the clade and that our reconstruction is biased based on the reduced data set that does not take into account the widespread distribution and genetic differentiation among populations of *C. moniliformis* in the clade. Likewise, if our entire data set is analyzed, then a reticulate epidermis is a putative synapomorphy for the *Moniliformis* clade (data not shown). Although *C. macracantha* and *C. microcarpa* have very similar morphological features and ecology—they both form large trees, have a smooth epidermis, non-spiny pericarpels, non-spiny mature cladodes, and occur in slightly more mesic conditions, as compared to *C. moniliformis* (Majure and Clase, 2018; Barrios et al., 2019)—the two species are not closely related, with all samples of *C. macracantha* forming a clade sister to the *Moniliformis* clade, where *C. microcarpa* is resolved (Fig. 2A), or alternatively *C. macracantha* was sister to the *Moniliformis* and *Millspaughii* clades, as in our reduced data set (Fig. 2B). The overlapping morphological characters of *C. macracantha* and *C. microcarpa* are apparently a combination of plesiomorphic and homoplastic characters (Appendix S4C–E). The *Millspaughii* clade consists of taxa that have a smooth epidermis and spiny pericarpels (Appendix S4C, D), and in general form smaller trees; thus, they are more cohesive morphologically.

Island gigantism is well recorded in numerous plant and animal species across island systems worldwide (Lomolino, 2005; Jaffe

et al., 2011; Cox and Burns, 2017; Biddick et al., 2019), and this pattern is present in some animals and plants of the Greater Antilles as well (Cooke et al., 2011; Turvey et al., 2017), including in the Cactaceae (e.g., *Leptocereus* s.l.; Barrios et al., 2020). Giant tortoises (*Chelonioidis*, see above) with carapace lengths of up to 60 cm long, thus comparable to the giant Galapagos tortoises, once roamed numerous Caribbean Islands (Turvey et al., 2017), and considerably large, New World monkeys, at least as compared to their continental relatives, inhabited Hispaniola (Cooke et al., 2011). Gigantism in *Consolea* is yet another example of this pattern.

The evolution of gigantism in insular biota has been discussed for some time (Darwin, 1859), and several hypotheses have been generated to try to explain the phenomenon, mostly from an animal perspective (Lomolino, 2007). Explanations for the pattern of gigantism on islands include an ecological release (from competitors, parasites, predators, etc.), resource limitation and increasing the potential for more successful dispersibility or immigration and establishment into insular areas (see Lomolino, 2007). The “island rule” in plants also seems to be maintained, although, there still are few studies that rigorously test this hypothesis (Biddick et al., 2019). The pattern of gigantism in *Consolea* seems likely to have evolved in response to environmental pressures related to the SDTF habitat, including interspecific competition with surrounding broadleaf vegetation, and likewise, potentially large herbivores, such as tortoises, could have influenced a drastic change in strategy leading to the monopodial primary growth of the stem (Appendix S4A) to reduce herbivory rates. The extremely spiny stems of numerous species of *Consolea* (Appendix S4B) could have also evolved in response to reducing herbivory from larger herbivores, such as ground sloths, and likewise, the reduction or loss of spines in mature stem segments (Appendix S4E) could be correlated with decreased herbivory rates in these large trees. Juveniles of these species have very spiny stem segments, which presumably would provide protection from abiotic (e.g., incident PAR; Nobel, 1983), as well as biotic (e.g., herbivores) pressures. It should be noted that in cacti, there are a number of gigantic species that may or may not exist on islands (e.g., saguaros in the Sonoran Desert), so it may be that gigantism in cacti is mostly under different environmental selective pressures (e.g., water storage in desert areas) based on the habitat in which they evolved.

Dioecy/subdioecy in *Consolea*

Strittmatter et al. (2006, 2008) hypothesized that cryptic dioecy/subdioecy originated once in *Consolea*. Dioecy is clearly synapomorphic for *Consolea* based on our reconstruction (Appendix S4F). Within tribe Opuntieae, dioecy occurs in three unrelated diploid taxa of *Opuntia* (Parfitt, 1985; Majure and Puente, 2014), as well as in some polyploid prickly pears (del Castillo and Trujillo-Argueta, 2009). However, hermaphroditism is clearly ancestral in the clade and likely is such for the entire Cactaceae. Intriguingly, some *Consolea* species still display this ancestral trait as occasional inconstant or weak hermaphroditic plants are present in *C. corallicola*, *C. microcarpa*, *C. millspaughii*, *C. moniliformis*, *C. nashii*, and *C. spinosissima*.

Interestingly, there does appear to be some correlation with polyploidy and dioecy in Cactaceae. Several hexaploid species of chollas (*Cylindropuntia*) are the only known dioecious species in the genus (Baker and Cloud-Hughes, 2014; Baker and Pinkava, 2018). Six dioecious species of the genus *Echinocereus* are also known to

be polyploid (Rebman, 2003; Baker, 2006; Hernández-Cruz et al., 2018). Likewise, *Mammillaria dioca*, is a hexaploid (Parfitt, 1985 reviewed by Guerrero et al., 2018). *Consolea*, however, represents the only known clade of dioecious/subdioecious species at the polyploid level. Dioecy is oftentimes seen as a mechanism for increasing outcrossing, which would be evolutionary advantageous given that polyploids may have high rates of self-compatibility. Thus, higher rates of outcrossing could lead to greater genetic diversity in recently formed polyploids that may suffer from small population sizes. This may have been a driving force for the evolution of dioecy in the putatively polyploid common ancestor of *Consolea*. Resource allocation among different sexes of the same species (Bawa, 1980) could also likely have proved important for nascent polyploid ancestors of *Consolea* in a geographically restricted island system.

It is also noteworthy that many dioecious taxa in Cactaceae are bird-pollinated. All three diploid dioecious species of *Opuntia* are hummingbird-pollinated, at least partially (Majure and Puente, 2014), as are *Consolea* (L. C. Majure, personal observations) and certain dioecious species of *Echinocereus* (Cota, 1993). However, the correlation between bird pollination and dioecy does not appear to be a general trend in angiosperms (Bawa, 1980) and may merely be a coincidence based on the location of these taxa and presence of hummingbird species. Schlessman et al. (2014) found that most dioecious species of New Caledonian plants were entomophilous rather than having more specialized pollination syndromes.

Fleshy fruit/seeds have been shown to predominate in dioecious species on islands (Schlessman et al., 2014). This predominance may correlate with increased long-distance dispersibility via long-distance traveling disperser species, such as birds, over taxa that are passively dispersed (Bawa, 1980; Givnish, 1980; Schlessman et al., 2014). This pattern certainly holds in Cactaceae and likewise in the *Consolea* clade. Although, given the restricted movement of taxa onto islands, this pattern may hold across both monoecious and dioecious taxa.

Conservation and taxonomic implications

Consolea corallicola is federally and state-listed as endangered. This species has previously been considered synonymous with *C. spinosissima* (Benson, 1982; Hunt et al., 2006; Hunt, 2014), a rather distantly related species, according to our phylogeny, and Guiggi (2012) recircumscribed *C. corallicola* as a subspecies of *C. spinosissima*. Our results clearly show *C. corallicola* nested within the morphologically very similar *C. millspaughii*. Guiggi (2007) made several new combinations within *Consolea* based on, apparently, no data—he recircumscribed *C. millspaughii* as a subspecies of *C. spinosissima* and *C. nashii* as a subspecies of *C. macracantha*—however, *C. millspaughii* is not closely related to *C. spinosissima*, and *C. macracantha* is not closely related to *C. nashii* according to our phylogenetic results (Fig. 2). Those erroneous nomenclatural changes and further taxonomic cluttering could have been avoided with an improved understanding of the underlying phylogeny, as shown here (also see Majure et al., 2019). *Consolea microcarpa* of Hispaniola has been commonly treated as synonymous with *C. macracantha* of Cuba (Hunt et al., 2006), likely owed to their overlapping morphological characters (spineless mature cladodes, spineless pericarpels; Appendix S4C–E), which apparently are plesiomorphic or homoplasious. However, these two taxa are resolved in two separate clades based on our topology (Fig. 2A, B). Hunt’s (2014) latest evaluation of *Consolea* did not recognize any member of the *Millspaughii*

clade. Careful examination of morphological characters, geography and phylogenetic relationships are necessary to circumscribe taxa within *Consolea* to help develop appropriate conservation strategies for each taxon. Presently, it is prudent to preserve as much taxonomic and morphological variability as possible, to alleviate narrow endemic species loss in this imperiled group.

Previous work by Majure et al. (2012) suggested that several species of *Consolea* were not monophyletic. The nonmonophyly of certain taxa is maintained in the results presented here, as well. *Consolea moniliformis* is the most widespread species of *Consolea*, occurring in southwestern Cuba, throughout Hispaniola, on the island of Mona, Puerto Rico, as well as on the island of Navassa off the coast of Haiti. *Consolea microcarpa*, *C. rubescens*, and *C. spinosissima* are nested within *Consolea moniliformis* based on our data, suggesting that those species may have evolved through peripheral isolate speciation, in the case of *C. microcarpa*, and potentially vicariance in the cases of *C. rubescens* and *C. spinosissima*, considering that long-distance dispersal of a *C. moniliformis*-like ancestor to Puerto Rico and Jamaica would have provided the conditions necessary for reproductive isolation and subsequent divergence between the two species. The morphological similarity of *C. moniliformis* and *C. spinosissima* (Appendix S4) add further evidence to this scenario. *Consolea microcarpa* occupies a slightly different habitat than that of *C. moniliformis*, being found in more mesic, denser forests, while the latter more often occurs directly over open, dog-tooth limestone or in low, dry forests over silty soils on Hispaniola (Majure and Clase, 2018). Thus, niche evolution possibly could be driving intra-island speciation in this group and should be further explored. *Consolea millspaughii* and *C. corallicola* also likely show a pattern of putative vicariant speciation, with an ancestral form of *C. millspaughii* dispersing to the Florida Keys and diverging into what we know consider the Florida Keys endemic, *C. corallicola*. Morphologically, those two taxa are nearly identical and likewise occur in mostly the same habitat (i.e., tropical forest over limestone at or near sea level; L. C. Majure, personal observations; Correll and Correll, 1982), and *C. corallicola* essentially represents northern populations of *C. millspaughii*. Thus, the circumscription of *C. corallicola* has been modified by Korotkova et al. (Botanischer Garten und Botanisches Museum, Berlin-Dahlem, unpublished manuscript).

The nonmonophyly of *C. millspaughii* and *C. moniliformis* is a result, in part, of current species circumscription. Although outside the scope of this manuscript, it is quite possible that the separate accessions of *C. moniliformis* analyzed here (Fig. 2A) could represent unrecognized diversity. Given the wide distribution of the species and morphological heterogeneity of that clade, this should be explored. However, *C. spinosissima* is nearly identical to *C. moniliformis* and could thus suggest a broader circumscription of *C. moniliformis* may be necessary. Likewise, it may be necessary to amplify species boundaries in other taxa, such as in the case of *C. corallicola* and *C. millspaughii*, which are phenotypically very similar, occupy essentially the same habitat type and have the same chromosome number, i.e., $2n = 66$ (Negrón-Ortiz, 2007).

We cannot rule out that species nonmonophyly in the *Moniliformis* clade could have resulted from reticulate evolution, especially regarding the octoploid *C. rubescens*, which is poorly supported as closely related to *C. macracantha* ($BS = 56$) in our nrDNA topology (Appendix S2) and is morphologically divergent from other members of the *Moniliformis* clade (Appendix S4). Likewise, the morphologically divergent *C. microcarpa* (e.g., smooth vs. reticulate epidermis) is nested within the *Moniliformis* clade. If either

or both of these taxa may actually represent reticulate evolution events, then the presence of specific morphological characters in those taxa could represent characters obtained from putative parental taxa rather than separate evolutionary (homoplasious) events. However, morphological characters do not support this idea in *C. rubescens*, given that spiny pericarpels are not found in *C. macracantha*, but rather in the *Millspaughii* clade (Appendix S4D), which *C. rubescens* is not closely related to in either our plastome or nrDNA data set (Fig. 2A, B; Appendices S3, S4). So, although the possibility of reticulate evolution exists for these taxa that are nested within *C. moniliformis*, more data across populations and genomes will be necessary for making that determination, as our current data sets are insufficient to robustly test this hypothesis.

FUTURE WORK

The *Consolea* clade provides a window into the age and generation of biodiversity of an Antillean endemic radiation of cacti, and likewise, illuminates part of the complexity behind the composition of the flora of poorly studied SDTF in the Greater Antilles. Considered one of the most threatened forest systems in the Neotropics, with an estimate of only 10% of their original land cover still remaining (Banda-R. et al., 2016), and likewise housing a great diversity of Cactaceae, the fifth most endangered organismal group on the planet based on evaluations by the IUCN (Goetsch et al., 2015), it is clear that the SDTF of the Greater Antilles deserves far more attention and study (Majure et al., 2021a). Carrying out phylogenetic studies of numerous clades of angiosperms across SDTF throughout the Greater Antilles, which is currently underway (Barrios et al., 2020; L. C. Majure et al., unpublished manuscript), will provide us with a broader perspective on how these arid areas have shaped biodiversity throughout the region.

Increased sampling across the distribution of *Consolea* is a necessity, especially to incorporate populations of *C. millspaughii* from the Cayman Islands, and *C. moniliformis* and *C. nashii* from Cuba (L. C. Majure et al., unpublished manuscript). Population-level work in *Consolea* would also provide greater insight into potential hybridization scenarios, the level of gene flow among populations of these dioecious species, as well as finer-scale biogeographic patterns. Currently, very little population-level data has been utilized in the clade and only for one species (Cariaga et al., 2005). Likewise, our plastome and nrDNA data sets presented here are greatly limited given the low level of sequence divergence within *Consolea*. A Hybseq approach utilizing a high number of nuclear loci for comparative phylogenomics is being developed and will further our knowledge of species relationships and putative reticulation events associated with the generation of diversity in this group of taxa.

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AUTHOR CONTRIBUTIONS

L.C.M. carried out fieldwork, labwork, analyzed data, and wrote the paper. D.B. and E.D. carried out fieldwork, collected data, and helped write the paper. B.Z. carried out labwork, analyzed data, and helped write the paper. W.T. analyzed data and helped write the paper. V.N.-O. helped write the paper. Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

DATA AVAILABILITY

Raw reads from our genome skimming are deposited and available in GenBank under Bioproject PRJNA650542, and our plastome data set is deposited in Dryad <https://doi.org/10.5061/dryad.8kpr74xk7> (Majure et al., 2021b).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Character matrix of the seven characters used in our analysis of morphological evolution.

APPENDIX S2. Maximum likelihood phylogenies of reduced plastome data sets.

APPENDIX S3. Maximum likelihood phylogenies of ETS/ITS data set.

APPENDIX S4. Character state reconstruction of (A) primary stem growth pattern showing monopodial stems being synapomorphic for *Consolea* under maximum likelihood (ML), (B) the development of densely spined stems (where the trunks are obscured in densely spined species), showing the loss of spine density in *C. nashii* + *C. falcata*, and this character is polymorphic in *C. corallicola*, *C. microcarpa*, and *C. rubescens* under maximum parsimony (MP). (C) ML reconstruction of stem epidermal characters, showing reticulate epidermis to have evolved twice in the *Moniliformis* clade and smooth epidermis as plesiomorphic. (D) The plesiomorphic spineless pericarpels in *Consolea* with spiny pericarpels being synapomorphic in the *Millspaughii* clade and also acquired in the *Moniliformis* clade (under MP) at least twice. (E) MP reconstruction of mature, spineless cladodes (stems) across Cactaceae showing the loss of spines in *Consolea* being equivocal. However, it is possible that the loss of

spines in the mature cladodes in the *Macracantha* and *Moniliformis* clades is plesiomorphic. (F) ML reconstruction of dioecy/monoecy across Cactaceae showing dioecy to be synapomorphic for *Consolea*.

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APPENDIX 1. Specimens used in our phylogenetic analyses of *Consolea*. Herbarium acronyms follow Thiers (2017). Those accessions that are maintained as part of the Desert Botanical Garden (DBG) living collection are also given with their DBG accession number. Data used from other sources are cited accordingly.

Blossfeldia liliputana (Arakaki et al., 2011); *Carnegie gigantea* (Sanderson et al., 2016); *Consolea corallicola* (Majure 3321; FLAS); *Consolea falcata* (DBG 2016 0516 01; DES); *Consolea macracantha* (DBG 1995 0383 21-4); (Majure et al. 7005; DES, HAJB), (Majure et al. 7008; DES, HAJB), (Majure et al. 7016; DES, HAJB), (Majure et al. 7043; DES, HAJB); *Consolea microcarpa* (L.C. Majure 6583; DES, JBSD); *Consolea millspaughii* (DBG 1997 0355 01-1; DES), (Majure et al. 7034; DES, HAJB), (Martínez s.n.; HAJB); *Consolea moniliformis* (L.C. Majure 6318; DBG, JBSD), (DBG 2016 0510 01-2—Navassa); DES), (DBG 2016-0513-01; DES); *Consolea nashii* (DBG 1996 0257 01-1; DES); *Consolea rubescens* (Majure 3323; FLAS; Köhler et al., 2020); *Consolea spinosissima* (Majure 3322; FLAS); *Cylindropuntia bigelovii* (Baker 18286-ASU; Majure et al., 2019); *Maihueunia poeppigii* (Arakaki et al., 2011); *Miqueliopuntia miquelii* (DBG 2001 0107 01; Köhler et al., 2020); *Opuntia arechavaletae* (Majure 6959-DES, FLAS; DBG 2017 0724 01; Majure et al., 2019); *O. austrina* (Majure 4216; FLAS; Majure et al., 2019), *O. inaperta* (DBG 1997 0415 01; DES); *Pereskia aculeata* (Arakaki et al., 2011), *P. sacharosa* (Arakaki et al., 2011); *Portulaca oleracea* (Arakaki et al., 2011). *Tacinga palmadora* (DBG 1997 0392 01; Köhler et al., 2020); *Tephrocactus articulatus* (Krattemann 595, DBG 2001 0014 21; Majure et al., 2019); *Tunilla corrugata* (DBG 2001 0056 01-01; DES); *Weingartia kragliana* (Arakaki et al., 2011).