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Phylogeny of *Opuntia* s.s. (Cactaceae): Clade delineation, geographic origins, and reticulate evolution[1]

Lucas C. Majure[2,3,6], Raul Puente[4], M. Patrick Griffith[5], Walter S. Judd[3], Pamela S. Soltis[3], and Douglas E. Soltis[2,3]

[2]Department of Biology, University of Florida, Gainesville, Florida 32611 USA; [3]Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611 USA; [4]Desert Botanical Garden, Phoenix, Arizona 85008 USA; and [5]Montgomery Botanical Center, Miami, Florida 33156 USA

- *Premise of the study:* The opuntias (nopales, prickly pears) are not only culturally, ecologically, economically, and medicinally important, but are renowned for their taxonomic difficulty due to interspecific hybridization, polyploidy, and morphological variability. Evolutionary relationships in these stem succulents have been insufficiently studied; thus, delimitation of *Opuntia* s.s. and major subclades, as well as the biogeographic history of this enigmatic group, remain unresolved.
- *Methods:* We sequenced the plastid intergenic spacers *atpB-rbcL, ndhF-rpl32*, *psbJ-petA*, and *trnL-trnF*, the plastid genes *matK* and *ycf1*, the nuclear gene *ppc*, and ITS to reconstruct the phylogeny of tribe Opuntieae, including *Opuntia* s.s. We used phylogenetic hypotheses to infer the biogeographic history, divergence times, and potential reticulate evolution of Opuntieae.
- *Key results:* Within Opuntieae, a clade of *Tacinga*, *Opuntia lilae*, *Brasiliopuntia*, and *O. schickendantzii* is sister to a well-supported *Opuntia* s.s., which includes *Nopalea*. *Opuntia* s.s. originated in southwestern South America (SA) and then expanded to the Central Andean Valleys and the desert region of western North America (NA). Two major clades evolved in NA, which subsequently diversified into eight subclades. These expanded north to Canada and south to Central America and the Caribbean, eventually returning back to SA primarily via allopolyploid taxa. Dating approaches suggest that most of the major subclades in *Opuntia* s.s. originated during the Pliocene.
- *Conclusions: Opuntia* s.s. is a well-supported clade that includes *Nopalea*. The clade originated in southwestern SA, but the NA radiation was the most extensive, resulting in broad morphological diversity and frequent species formation through reticulate evolution and polyploidy.

Key words: Cactaceae; *Consolea*; *Nopalea*; *Opuntia*; Opuntieae; polyploidy; *Tacinga*.

Cactaceae, comprising a well-supported clade (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Nyffeler, 2002, 2007; Edwards et al., 2005) apparently sister to Anacampserotaceae (Nyffeler and Eggli, 2010a), are endemic to the New World except for the occurrence of one species, *Rhipsalis baccifera* (Mill.) Stearn in the Old World tropics (Benson, 1982). Other Cactaceae have been introduced, however, to locations around the world (Britton and Rose, 1920; Anderson, 2001). Although no reliable fossils have yet been found, the clade is suggested to represent a young radiation that evolved as a result of aridification in the Americas at the end of the Eocene through the beginning of the Miocene, ca. 30 million years ago (Ma) (Hershkovitz and Zimmer, 1997). This date has been corroborated by the phylogenomic analyses of Arakaki et al. (2011) who estimated an age of ca. 35 Ma for the origin of Cactaceae. Arakaki et al. (2011) also suggested that many of the major radiations within Cactaceae were initiated at the end of the Miocene (ca. 10–5 Ma), concomitant with increased atmospheric CO_2 and aridity in the Americas.

Cactaceae comprise ca. 1500–1800 species (Anderson, 2001), which have been divided variously into 3–6 subfamilies (Crozier, 2004). Pereskioideae were generally considered to be sister to the rest of the family, but Edwards et al. (2005), Bárcenas et al. (2011), and Hernández-Hernández et al. (2011) have shown that this subfamily is paraphyletic, forming two separate clades that are the successive sisters to the rest of the family (Edwards et al., 2005). Currently, two primary subfamilies are recognized within the "core cacti" (i.e., those that generally have very reduced leaves and primarily rely on stem photosynthesis: sensu Mauseth, 2006), Cactoideae and Opuntioideae (Edwards et al., 2005).

Opuntioideae encompass *Opuntia* Mill. s.l. and four associated genera (*Cumulopuntia* F. Ritter s.l., *Maihueniopsis* Speg. s.l., *Pterocactus* K. Schum., *Puna* R. Kiesling s.l.; [Griffith and Porter, 2009]), although, *Opuntia* s.l. (e.g., Benson, 1982) was shown through molecular phylogenetic studies to be polyphyletic (Wallace and Dickie, 2002; Griffith and Porter, 2009).

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[6]Author for correspondence (e-mail: lmajure@ufl.edu)

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Thus, *Opuntia* (hereafter *Opuntia* s.s.) has been reduced drastically in size with many segregate genera [e.g., *Austrocylindropuntia* Backeb., *Brasiliopuntia* (K. Schum.) A. Berger, *Cylindropuntia* (Engelm.) F. M. Knuth] now recognized (Anderson, 2001; Wallace and Dickie, 2002; Hunt, 2006; Griffith and Porter, 2009). Currently, five tribes (Wallace and Dickie, 2002), and 15 (Anderson, 2001), 16 (Stuppy, 2002), or 18 (Hunt, 2006) genera are recognized within Opuntioideae.

Tribe Opuntieae (platyopuntioids) is a well-supported clade within Opuntioideae (Wallace and Dickie, 2002; Griffith and Porter, 2009; Hernández-Hernández et al., 2011) that consists of *Brasiliopuntia* (K. Schumann) A. Berg., *Consolea* Lemaire, *Miqueliopuntia* Frič ex F. Ritter, *Nopalea* Salm-Dyck, *Opuntia* s.s., *Salmiopuntia* Frič ex Guiggi (Guiggi 2010), *Tacinga* Britton & Rose, and *Tunilla* Hunt and Illiff. The platyopuntioids were so named by Britton and Rose (1920) for the flat, photosynthetic stem segments (i.e., cladodes) characteristic of most members, although they did not include *Miqueliopuntia*, *Tacinga*, *Tunilla*, *Nopalea*, or *Salmiopuntia* in the group. Species of *Maihueniopsis* s.l. were also recovered in Opuntieae (Griffith and Porter, 2009), but this genus is often placed in tribe Cumulopuntieae (Hunt, 2002).

DNA studies have provided conflicting results regarding the placement of *Consolea* (outside of *Opuntia* s.s. or nested within *Opuntia* s.s.), but the morphologically distinct genus *Nopalea* has consistently been nested within *Opuntia*. However, due to low resolution and/or insufficient taxon sampling, the circumscription of *Opuntia* s.s. remains unclear (Wallace and Dickie, 2002; Griffith and Porter, 2009; Bárcenas et al., 2011; Hernández-Hernández et al., 2011). *Opuntia* s.s. (nopales, prickly pears; excluding *Consolea*) is the largest genus in Opuntioideae and the most widespread genus in Cactaceae, distributed natively from Canada to Argentina (Anderson, 2001). There are 150 (Stuppy, 2002) to 180 recognized species (including *Nopalea*; Anderson, 2001; Hunt, 2006) within the genus, which is suggested to have originated as recently as 5.6 (±1.9) mya (Arakaki et al., 2011).

Members of *Opuntia* s.s. are cultivated worldwide as fruit and vegetable crops (Inglese et al., 2002) and are increasingly used as forage and fodder for livestock in arid areas of the world, such as parts of Brazil, Mexico, western Asia, and northern and southern Africa (Nefzaoui and Salem, 2002). Medicinally, *Opuntia* polysaccharides have been shown to protect brain tissue from glucose and oxygen deprivation (Huang et al., 2008). *Opuntia ficus-indica* (L.) Mill. has been used to protect the liver from harmful organophosphorous pesticides (Ncibi et al., 2008), and various *Opuntia* species have shown hypoglycemic effects in diabetic patients, returning blood glucose to normal levels (Trejo-González et al., 1996; Laurenz et al., 2003). *Opuntia streptacantha* Lem. has even been used as a bioaccumulator in lead-contaminated waters (Miretzky et al., 2008).

Species of *Opuntia* are also known as some of the most highly invasive species in arid areas of their nonnative range such as Australia (Freeman, 1992), the Mediterranean region (Vilá et al., 2003), and Africa. Millions of hectares invaded by *Opuntia stricta* (Haw.) Haw. (Dodd 1940) were eventually brought under control in Australia using a well-known biological control agent, *Cactoblastis cactorum* Berg (Zimmermann et al., 2000). This moth is now wreaking havoc in the native range of prickly pears in North America (Simonsen et al., 2008). The nutritive tissues and high production rates of *O. stricta,* introduced into Kruger National Park (South Africa), make it irresistible to the native fauna, primarily baboons and elephants; thus, this species is easily dispersed, increasing its invasion in the park (Reinhardt and Rossouw, 2000; Foxcroft et al., 2004; Foxcroft and Rejmanek, 2007). In its native range, *Opuntia* s.s. provides food for innumerable herbivores, including tortoises, iguanas, birds, rabbits, deer, bats, sloths, squirrels, coyotes, bears, pigs, and bison (Mellink and Riojas-López, 2002); this also clearly underscores the ecological importance of prickly pear. *Opuntia* also is culturally important. In Mexico, where species of *Opuntia* have been cultivated for at least the last 14 000 yr (Casas and Barbera, 2002), they represent an iconic national figure, illustrated on the country's flag. The large, tree-like *Opuntia* species, *O. megasperma*, *O. echios*, and *O. galapaegia,* are some of the most conspicuous species of the Galápagos Islands. Even Charles Darwin could not resist the intrigue of *Opuntia* when he collected the first specimen of *O. galapaegia* (later described by Henslow, 1837).

Polyploidy is a common phenomenon throughout tribe Opuntieae, which has been well studied cytologically (Pinkava, 2002; Majure et al., 2012; L. C. Majure et al., unpublished manuscript). In fact, diploids ($2n = 2x = 22$) are relatively rare in the tribe making up only 26.2% of the 164 species with reported chromosome counts (L. C. Majure et al., unpublished manuscript). Polyploid taxa within *Opuntia* range from triploid ($2n = 3x = 33$) to octoploid ($2n = 8x = 88$), and many species have multiple ploidal levels (Pinkava, 2002; Majure et al., 2012; L. C. Majure et al., unpublished manuscript). Species limits are still poorly understood, as a result of the high frequency of polyploid taxa, morphological variability, poor representation in herbaria, and frequent interspecific hybridization in *Opuntia* s.s. (Cota and Philbrick, 1994; Rebman and Pinkava, 2001; Pinkava, 2002; Majure et al., 2012).

Furthermore, there is no comprehensive phylogeny of *Opuntia* s.s., so limits of major clades are largely unknown. Numerous morphological and cytological studies have been conducted on large groups of taxa and species complexes (e.g., Doyle, 1990; Parfitt, 1991; Leuenberger, 2001; Majure et al., 2012), but *Opuntia* s.s. has not been studied comprehensively using molecular data. Griffith and Porter (2009) included 28 species of *Opuntia* s.s. in their molecular phylogeny of Opuntioideae but were unable to resolve relationships within *Opuntia* s.s. using ITS and the plastid intergenic spacer *trnL-F*. Hernández-Hernández et al. (2011) and Bárcenas et al. (2011) recovered South American *Opuntia* s.s. species and South American species of *Opuntia* plus *Tunilla erectoclada* (Backeb.) Hunt & Illiff, respectively, as sister to the rest of *Opuntia* s.s. However, Hernández-Hernández et al. (2011) only surveyed seven species of *Opuntia*, and Bárcenas et al. (2011) had no resolution among clades. In addition, although a number of *Opuntia* s.l. species have been shown to be interspecific hybrids using molecular data (Mayer et al., 2000; Griffith, 2003), the prevalence of reticulation in this group has not been extensively surveyed.

We broadly sampled species in tribe Opuntieae using nuclear and plastid sequence data and produced a phylogeny of the clade to (1) determine the circumscription of *Opuntia* s.s. and the major clades within it, (2) resolve the placement of the problematic genera *Consolea* and *Nopalea*, (3) investigate the geographic origin and subsequent spread of *Opuntia* s.s., and (4) survey for potential reticulate evolution.

MATERIALS AND METHODS

***Taxon sampling*—**We sampled 112 taxa (98 species) of *Opuntia*, nine species of *Nopalea*, six species of *Consolea*, four species of *Tacinga,* and *Brasiliopuntia brasiliensis* (Willd.) Berg. Our sampling includes members from all 29

series of subgenus *Platyopuntia* recognized by Britton and Rose (1920) and thus represents a broad sampling of the most likely members of *Opuntia* s.s. Other members of Opuntieae, *Maihueniopsis* cf. *ovata* (Pfeiffer) F. Ritter, *Miqueliopuntia miquelii* (Monville) F. Ritter, *Salmiopuntia salmiana* (J. Parmentier ex Pfeiffer) Guiggi, and *Tunilla corrugata* (Salm-Dyck) Hunt and Illiff were used as outgroups based on Griffith and Porter (2009) and Hernández-Hernández et al. (2011). GenBank accession numbers and voucher data are given in Appendix 1.

DNA extraction, PCR, sequencing, sequence editing, and alignment— Total genomic DNA was extracted using a modified CTAB method (Doyle and Doyle, 1987). Although cacti have highly mucilaginous tissues, we successfully extracted high-quality DNA from live plants, silica-dried material, or herbarium specimens using this method. When possible, we used the small, ephemeral leaves, which are produced as new cladodes develop. This produced the highest quality and cleanest DNA of any samples used. Otherwise we used epidermal tissue with the cuticle removed (cf. Griffith and Porter, 2003).

We sampled four plastid intergenic spacers (*atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, and *trnL-F*, following Mavrodiev et al. [2010], M. J. Moore, Oberlin College [unpublished data], Shaw et al. [2007], and Taberlet et al. [1991], respectively), the plastid gene *matK* (http://www.kew.org/barcoding/update.html), ca. 900 bp from the 5′ end of the plastid gene *ycf1* (K. Neubig, Florida Museum of Natural History, unpublished data), the nuclear gene *ppc* (Hernández-Hernández et al., 2011), and the nuclear ribosomal internal transcribed spacers (ITS; following White et al., 1990). We designed new primers for *atpB-rbcL, ndhF-rpl32*, the 3′ end of the *psbJ-petA* spacer, *ycf1*, and *ppc* after the initial sequencing of those PCR products (Table 1). A sequence of *matK* for *Tacinga funalis* Britton & Rose was downloaded from GenBank (Appendix 1).

Mixtures for 25-μL amplification reactions were as follows: 0.5–1 μL of template DNA, 9.4 μL H_2O, 5 μL of 5× buffer, 2.5 μL of 25 mmol/L $MgCl_2$, 1 μL of 2.5 mmol/L DNTPs, 2 μL betaine, 2 μL each 5 μmol/L primer, and 0.1 μL *Taq* polymerase (produced in the Soltis lab from *E. coli* producing the *Taq* gene). PCR cycling conditions for the plastid intergenic spacers and *matK* followed Shaw et al. (2007), although the initial annealing temperature was modified to 55°C and the number of cycles was increased to 35. PCR cycling conditions for ITS were an initial denaturation at 95°C for 2 min; followed by 5 cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 2 min; followed by 40 cycles of 95°C for 1 min, 48°C for 1 min, and 72°C for 2 min; with a final extension step at 72°C for 12 min. PCR cycling conditions for *ppc* were 95°C for 5 min; followed by 44 cycles of 94°C for 1 min, 55°C for 1 min increasing 0.3°C/cycle, and 72°C for 2.5 min; with a final extension of 72°C for 10 min. PCR cycling conditions for *ycf1* followed Neubig et al. (2008) with modification of the initial annealing temperature from 60°C to 63°C. Plastid *ycf1* and nuclear *ppc* were only sequenced for diploid *Opuntia* taxa.

All PCR products were initially sequenced directly, except for presumed hybrids and polyploid taxa surveyed from each clade (discussed later). We searched for nucleotide polymorphisms in sequence chromatograms of ITS, especially in polyploid *Opuntia*, and cloned those products using the TOPO TA (Invitrogen, Carlsbad, California, USA) or Stratagene cloning kit (Stratagene, La Jolla, California). We also cloned at least one polyploid member from each major clade recovered in our "diploids only" analysis (described later) and any taxa thought to be of hybrid origin. Eight clones per accession were directly sequenced at the Interdisciplinary Center for Biotechnology Research at the University of Florida using bacterial primers (T3–T7) from the kits. A subset of polyploid taxa was cloned and sequenced for *ppc* to ascertain the degree of nucleotide polymorphism among taxa. However, the use of *ppc* for analysis of polyploids was discontinued, as sequence divergence in this gene was less than that of ITS.

Sequences were edited either in the program Sequencher 4.2.2 (Gene Codes, Ann Arbor, Michigan, USA) or Geneious Pro 5.1 (Biomatters Ltd., Auckland, New Zealand) and automatically aligned using the program Muscle (Edgar, 2004); this alignment was then adjusted manually in the program Se-Al v2.0 (Rambaut, 2007). All gaps introduced during alignment were coded as missing data.

TABLE 1. DNA regions and associated primers used in this study.

Region	Primer name: sequence or reference
atpB-rbcL	atpB.Op: 5′-GTAAACTATGTCGAAATTCTTTGC-3′
	rbcL.Op: 5′-ACAACAAAACAACAAGGTCTACTC-3′
matK	matKx: (http://www.kew.org/barcoding/update.html)
	matK5: (http://www.kew.org/barcoding/update.html)
ndhF-rpl32	ndhF.Op: 5′-TGCTGAATAGACAGCTTCA–3′
	rpl32.Op: 5′-TGGTCAAACGAATCTTTG–3′
psbJ-petA	psbJ: (Shaw et al., 2007)
	petA.Op: 5′-CAACATCAAGTTCGTAACAAG-3′
trnL-F	trnE: (Taberlet et al., 1991)
	trnF: (Taberlet et al., 1991)
ycf1	ycf1.Op118F: 5′-CTTATCTCTTACTTCTCCAAGCTC-3′
	ycf1.Op1330R: 5′-GCGGCTAAACTAGGTGGATGTG-3′
nrITS	ITS4: (White et al., 1990)
	ITS5: (White et al., 1990)
ppc	ppc.Op.19F:5′-GAGATGAGGGCAGGGATGAGTTACTTCC-3′
	ppc.Op.569R.2: 5′-CTAGCCAACAAGCAAACATC-3′

Phylogenetic analyses—*Opuntia* has been well studied cytologically (see Pinkava, 2002), and we have made extensive chromosome counts, adding 31 new counts of previously uninvestigated taxa (L. C. Majure et al., unpublished manuscript). Using this cytological information, we established multiple data sets: (1) nuclear data for diploids, (2) ITS for all cytotypes, (3) plastid data for diploids, (4) plastid data for all cytotypes, (5) combined nuclear and plastid data for diploids, and (6) combined nuclear and plastid data for all cytotypes (total evidence). We conducted separate analyses of diploids only (1) because allopolyploids do not arise via cladogenesis, and their inclusion in phylogenetic analyses can result in misleading results (Rieseberg et al., 1996; Soltis et al., 2008), and (2) to test the parentage of potential allopolyploids using phylogenetic methods (Mavrodiev et al., 2008; Soltis et al., 2008). All data sets were analyzed separately using maximum parsimony (MP) in the program PAUP* 4.0 (Swofford, 2002), Maximum likelihood (ML) using the program RAxML (Stamatakis, 2006), and Bayesian methods (BI) in the program MrBAYES (Huelsenbeck and Ronquist, 2001).

The MP analyses were conducted on all data sets with 10 000 random addition sequence replicates, and support was evaluated by running 1000 nonparametric bootstrap (bs) pseudoreplicates, each with 10 random addition sequence replicates. The ML analyses were carried out in RAxML by partitioning each region under 25 rate categories using the GTR model of molecular evolution and carrying out 10 000 nonparametric rapid bootstrap pseudoreplicates for the separate and combined data sets. For BI analyses, models of molecular evolution for each marker were determined using the program ModelTest (Posada and Crandall, 1998) and the Akaike information criterion (AIC). Analyses were carried out by partitioning the data by marker, each with its corresponding model of molecular evolution, and using four heated chains for 20 million generations, sampling a tree every 1000 generations. We determined stationarity and thus the number of generations considered "burn-in" using the program Tracer v. 1.5 (http://tree.bio.ed.ac.uk/software/tracer/).

Incongruence length difference (ILD) tests (Farris et al., 1995) between plastid and nuclear data sets were carried out in PAUP* (Swofford, 2002). We initially ran analyses using plastid and nuclear data separately with only known *Opuntia* diploids. Visual inspection of tree topologies of separate nuclear vs. plastid data analyses (MP, ML, BI) also was used to determine whether any strong incongruence existed between nuclear and plastid data sets that justified not combining data (Johnson and Soltis, 1998; Fishbein et al., 2001). Due to the lack of resolution along the backbone of the phylogenies using either plastid or nuclear data alone and the resolution of many of the same clades using the data sets separately, hard incongruence (sensu Seelanan et al., 1997) using a bootstrap value of ≥70% was not apparent, so we combined our diploid plastid and nuclear data sets for further MP, ML, and BI analyses.

We then ran separate plastid and nuclear analyses using all of the aforementioned phylogenetic methods with all taxa sampled, including polyploids, to determine from which putative progenitors (at the clade level) many of the polyploid taxa within *Opuntia* s.s. may have originated. We also analyzed ITS haplotypes from the combined diploid/polyploid data set in the program TCS v1.21 (Clement et al., 2000) to take into account potential incomplete lineage sorting in ITS and inherent problems with the inclusion of reticulate taxa in a bifurcating phylogeny. Polyploid taxa that were recovered in disparate clades using nuclear or plastid data alone in phylogenetic analyses or that were found to have ITS haplotypes from more than one putative progenitor or the same haplotype of a taxon whose relationship differed from the polyploid's placement in plastid phylogenetic analyses were considered interclade allopolyploids. Morphological characters of the putative interclade hybrids and distributions of taxa also were compared with members of putative progenitor clades to provide further evidence for their hypothesized parentage. We then

removed interclade allopolyploids from further analyses. Polyploid taxa inferred to be intraclade polyploids (i.e., polyploids derived from within a given clade) were not removed from our total evidence phylogenetic analyses (i.e., intraclade phylogeny), because we were interested primarily in clade delimitation and not necessarily species delimitation, which may be obscured by the inclusion of intraclade allopolyploids when employing both nuclear and plastid markers in a combined analysis.

***Biogeographic analysis and divergence time estimation*—**We used the programs Mesquite v. 2.73 (Maddison and Maddison, 2010) and RASP (Yu et al., 2011) to infer the geographic origin of *Opuntia* s.s. and major clades by coding all diploid taxa for geographic distribution based on literature (Britton and Rose, 1920; Anderson, 2001) and personal experience. We coded seven geographic areas for diploid *Opuntia* taxa and outgroups based on generalized distributions of the diploid taxa. Those geographic areas were (1) southwestern South America (western central Chile, Chaco + Monte regions), (2) eastern South America (Caatinga), (3) western South America (Central Andean valleys), (4) northern South America (Caribbean region), (5) Central America (including tropical dry forest of southern Mexico and the Caribbean), (6) North American desert region, (7) and the southeastern United States. Geographic areas for South America are based on Sarmiento (1975).

In Mesquite v. 2.73, we implemented the maximum likelihood Mk1 model (using our diploid ML topology), which is a Markov *k*-state 1-parameter model that allows for an equally probable change from one character state to the next (Lewis, 2001; Maddison and Maddison, 2010), but without allowing polymorphic states for taxa. In RASP, we used the Bayesian binary Markov chain Monte Carlo (MCMC) analysis method (Olsson et al., 2006; Sanmartín et al., 2008; Yu et al., 2011) by implementing the JC model with equal rates (Sanmartín et al., 2008) and 50 000 MCMC cycles with 10 chains using the trees from our Bayesian analysis of diploid taxa as input. We built a condensed (consensus) tree from those BI input trees to use as a final tree for ancestral area reconstruction.

We also used RASP to perform a DIVA (Ronquist, 1996) analysis and infer dispersal scenarios based on our Bayesian trees.

Divergence time estimates were obtained using the program r8s v.1.71 (Sanderson, 2003) and implementing the penalized likelihood method (Sanderson, 2002) using the TN algorithm. We calculated smoothing using the cross-validation technique (Sanderson, 2003). No fossils are known in Cactaceae (e.g., Hershkovitz and Zimmer, 1997), so we used a fixed age of 5.6 (± 1.9) Myr for the crown node of *Opuntia* s.s. based on dates proposed by Arakaki et al. (2011), which coincides with an inferred late Miocene increase in lineage diversification rates in the clade (Arakaki et al., 2011). We fixed the age of our outgroup node at 15 (± 2.9) Myr, which is the inferred age of the crown node of Opuntioideae, to test the effect of that calibration on subsequent age estimates within *Opuntia* s.s. We also constrained the divergence time of the North American clade with a minimum age of 3 Myr based on the proposed timing for the closure of the Isthmus of Panama (Marshall et al., 1979), which would support migration rather than long-distance dispersal of the most recent common ancestor of the North American clade into North America.

RESULTS

We observed very low sequence divergence among the plastid and nuclear sequences in diploid data sets (Table 2), and very little nucleotide polymorphism was observed in directly sequenced ITS products from polyploid taxa. Neither nuclear nor plastid data for diploid taxa alone fully resolved relationships among major clades, but many of the major clades were recovered using either data set separately, although our ILD tests showed a significant difference between all nuclear compared to all plastid sequences ($P = 0.01$). It is well known, however, that the ILD test is extremely sensitive and used alone should not be an indicator of data set combinability (e.g., Yoder et al., 2001). Rate heterogeneity among sites and small numbers of parsimony-informative characters may result in rejecting congruence among data sets (Darlu and Lecointre, 2002). There was no hard incongruence based on comparison of the nuclear vs. plastid trees using a bootstrap cut-off of 70% using either MP or ML.

TABLE 2. Statistics of regions sequenced in this study based on the diploid data sets. The length (bp) of aligned sequences includes gaps introduced during alignment.

Region	Length (bp)	No. pars. infor. characters	Model selected
atpB-rbcL	861	20	HKY
matK	905	27	F81+I+G
ndhF-rpl32	1699	43	GTR+I+G
psbJ-petA	1169	72	K81uf+I
trnL-F	441	14	K81uf
ycf1	873	51	K81uf+I+G
ITS	599	39	TVM+G
ppc	469	37	HKY+G
cpDNA combined	5948	227	—
Nuclear combined	1068	76	—
All combined	7016	303	—

Combining the diploid data sets resulted in well-supported clades in the diploids-only analysis (Fig. 1). Well-supported clades are named based on the series recognized by Britton and Rose (1920), Engelmann (1856), or a morphological feature of a given clade. Our analysis of diploids and polyploids placed many polyploid taxa in different clades in the separate ITS and plastid trees (e.g., *Opuntia tomentosa* is in the *Nopalea* clade with ITS and the *Basilares* clade with plastid data; Appendix S1A and B, see Supplemental Data with the online version of this article). Those taxa also were recovered in disparate locations in our analysis of ITS haplotypes using TCS. However, many taxa sharing ITS haplotypes were not resolved in clades together in our phylogenetic analysis of ITS due to the lack of synapomorphies for certain clades. We inferred these taxa to be interclade-derived allopolyploids (Fig. 2). Those interclade allopolyploids also reduced clade support when analyzed with the combined nuclear/plastid data set (data not shown). The intraclade phylogeny exhibits well-supported clades (bootstrap [bs] ≥ 70%) and agrees with the diploid topology, but species relationships within subclades are generally poorly supported (bs ≤ 50%; Fig. 3). BI, ML, and MP topologies are virtually identical except for reduced clade support and resolution among clades with MP.

***Relationships in Opuntieae*—**Subgenus *Platyopuntia* as recognized by Britton and Rose (1920) was paraphyletic, given that most of *Tacinga* and *Nopalea* are not included in this subgenus in their classification. *Consolea* formed a clade with both plastid and ITS data as shown in online Appendix S1A and B. However, plastid data resolved *Consolea* outside of *Opuntia* s.s. (bs = 53%), and ITS data placed *Consolea* within *Opuntia* s.s. (bs = 75%; Appendix S1A, B), placements that have been found in previous studies (Wallace and Dickie, 2002; Griffith and Porter, 2009) using plastid and ITS data, respectively. However, *Consolea* was well supported (bs = 86%) as sister to a clade containing *Brasiliopuntia, Tacinga*, and *Opuntia* s.s. in a diploids-only analysis using combined nuclear and plastid data (Appendix S2, see online Supplemental Data). *Tacinga* formed a well-supported clade (bs = 81%) that included *Opuntia lilae* Trujillo and Ponce, and *Brasiliopuntia* and *Opuntia schickendantzii* F.A.C. Weber formed a clade (bs = 87%) sister to *Tacinga*. The *Brasiliopuntia-Tacinga* clade was not recovered in MP analyses. The *Brasiliopuntia, O. schickendantzii, Tacinga* clade was resolved as sister to the well-supported *Opuntia* s.s. clade (bs = 84%). *Nopalea* was nested within

Opuntia s.s., as in other studies (e.g., Wallace and Dickie, 2002; Wallace and Gibson, 2002; Griffith and Porter, 2009; Bárcenas et al., 2011; Hernández-Hernández et al., 2011). Altogether, our phylogenetic analyses recovered 10 major clades of *Opuntia* s.s. (Figs. 1, 3), which are recognized based on high support values. These 10 major clades were recovered in BI, MP, and ML analyses.

***Opuntia s.s.*—**In the ML analyses, the *Elatae* and *Macbridei* clades of South America (Argentina-Bolivia and central Peru, respectively) were successive sisters to North American *Opuntia*, which comprised two species-rich and morphologically diverse clades (Fig. 1). However, the sister to the North American clade was unresolved with BI or MP analyses. The more morphologically extreme of the two large North American clades consists of the *Nopalea* and *Basilares* sister clades. For example, the *Nopalea* clade contains species with flowers modified for hummingbird-pollination. Subclades of the *Basilares* clade have dry-fruited species (subclade *Xerocarpa*), rhizomatous taxa (subclade *Rhizomatosa*), dioecious species, such as *O. stenopetala* (Parfitt, 1985), and the iconic and deceivingly harmless *O. microdasys* (bunny ear prickly pear) of the *Microdasys* subclade. The other of the two large North American clades consists of three subclades (*Scheerianae, Macrocentra*, and *Humifusa*), all containing taxa that, despite extensive vegetative morphological diversity, are fairly homogeneous in their floral and fruit morphology, all with fleshy fruits and open entomophilous flowers.

Of the 29 series of subgenus *Platyopuntia* of (Britton and Rose, 1920), 26 series roughly conformed to *Opuntia* s.s. (i.e., excluding *Brasiliopuntia*, *Consolea*, and *Tacinga inamoena*). Of those 26 series, no single series corresponds exactly to any clade recovered in our topology; however, there was often general agreement between clades and series composition. For example, series *Basilares* (Britton and Rose, 1920) includes *O. basilaris*, *O. rufida*, and *O. microdasys*, which formed part of the *Basilares* clade in our phylogeny (Fig. 1).

***Interclade allopolyploids and hybrids*—**We recovered 24 interclade-derived taxa. Of these, 20 are inferred to be allopolyploids (4*x*, 5*x*, 6*x*, 8*x*, and 9*x*), and one is an interclade homoploid hybrid (Table 3). We have not yet determined ploidy in *O. bella* Britton & Rose, *O. pittieri* Britton & Rose, or *O. schumannii* F.A.C. Weber ex A. Berger, but they also are inferred to be of interclade origin. Twenty of these taxa are derived from within *Opuntia* s.s., but four taxa were determined to be "intergeneric" hybrids based on current taxonomy. *Opuntia acaulis* Ekman & Werdermann, *O. bahamana* Britton & Rose, and *O. lucayana* Britton are derived from *Consolea* and *Opuntia* s.s., and *O. bella* is apparently derived from *Tacinga* and *Opuntia* s.s. It was not possible to determine the parental species of all of these allopolyploids using ITS, possibly as a result of complete concerted evolution in ITS (Álvarez and Wendel, 2003; Kovarik et al., 2005; Kim et al., 2008; Soltis et al., 2008). Concerted evolution in ITS has also been inferred in polyploid species of Galápagos *Opuntia* (Helsen et al., 2009) reducing the ability to determine relationships among those species. Furthermore, we have not sampled all extant taxa, and some parental diploids may be extinct. We discovered two or more ITS haplotypes in most cloned accessions, and certain haplotypes were not represented in any other taxa. Although, we recovered *O. leucotricha* as an interclade allopolyploid, we are uncertain about its placement, given that ITS data place the species (although poorly supported; bs = 53%) in the *Humifusa* clade, with which *O. leucotricha* neither shares morphological characters nor is sympatric (Table 3; Fig. 2).

Opuntia acaulis, *O. bahamana*, and *O. lucayana* are all derived from hybridization between members of *Consolea* and a member of the *Scheerianae* clade, most likely *O. dillenii* (Ker Gawler) Haw., which occurs sympatrically with *Consolea* species throughout their range. Morphology provides support for this interclade hybridization. *Opuntia acaulis* has the indeterminate cladode growth form of *Consolea*, but *O. bahamana* and *O. lucayana* possess the determinate cladode growth form of *Opuntia* s.s. All three taxa show strongly tuberculate areoles, which characterize certain species of *Consolea* but generally have mostly yellow spines and a shrubby growth form like *O. dillenii;* these three hybrids are mosaics, with some morphological traits from each parent, and can be distinguished from both of their putative progenitors.

Opuntia boldinghii Britton & Rose and *O.* sp. nov. (R. Puente, unpublished data) were recovered as interclade products between the *Nopalea* clade and the *Scheerianae* clade. *Nopalea* was recovered as the maternal donor and the *Scheerianae* clade as the paternal donor. Both taxa have floral characters that combine the morphologies of *Nopalea* (erect, reddish-pink tepals) and *O. dillenii* (entomophilous flowers with spreading tepals).

Opuntia cubensis Britton & Rose has long been considered a hybrid derived from *O. militaris* Britton & Rose (currently a synonym of *O. triacantha*) and *O. dillenii* (Britton and Rose, 1920). Cloned products of ITS suggest that *O. cubensis* is an interclade allopolyploid between *O. abjecta* (currently treated as a synonym of *O. triacantha*) of the *Humifusa* clade and a member of the *Scheerianae* clade, likely *O. dillenii* with which it is sympatric. *Opuntia cubensis* has a combination of yellow, smooth, flattened spines like *O. dillenii* and whitish, retrorsely barbed, cylindrical spines that turn gray in age like *O. abjecta*. The overall growth form and size of *O. cubensis* is more similar to *O. dillenii*, but *O. cubensis* demonstrates disarticulating cladodes like *O. abjecta*.

Opuntia bakeri E. Madsen, *O. bisetosa* Pittier, *O. bravoana* E. M. Baxter, *O. eichlamii* Rose, *O. ficus-indica* (L.) Mill., *O. megacantha* Salm-Dyck, *O. pillifera* F.A.C. Weber, *O. pittieri*, *O. schumannii*, and *O. tomentosa* Salm-Dyck arose from hybridizations between the *Nopalea* and the *Basilares* clades (Fig. 2). However, it is possible that additional clades from our diploids analysis, not recovered with our data for interclade allopolyploids, may have been involved in these allopolyploidization events given that many of these taxa are hexa- and octoploids (Table 3).

***Intraclade allopolyploids*—**Determining parentage of allopolyploids derived from within a given subclade of *Opuntia* s.s. was difficult because of sequence similarity among close relatives. However, certain cases were straightforward and are noted here. Hexaploid *O. aurea* McCabe ex E. M. Baxter and octoploid *O. pinkavae* Parfitt (Parfitt, 1991, 1997) are likely intraclade allopolyploids of the *Xerocarpa* clade, both involving *O. basilaris,* and members of the *O. polyacantha* complex. Parfitt (1991) suggested this relationship for *O. aurea*, but not *O. pinkavae*. Plastid data place both of these taxa with high support in the *O. polyacantha* complex (*O. pinkavae* is strongly supported as sister to *O. erinacea* Engelm. & J. M. Bigelow, and Benson included *O. pinkavae* in his concept of *O. erinacea*

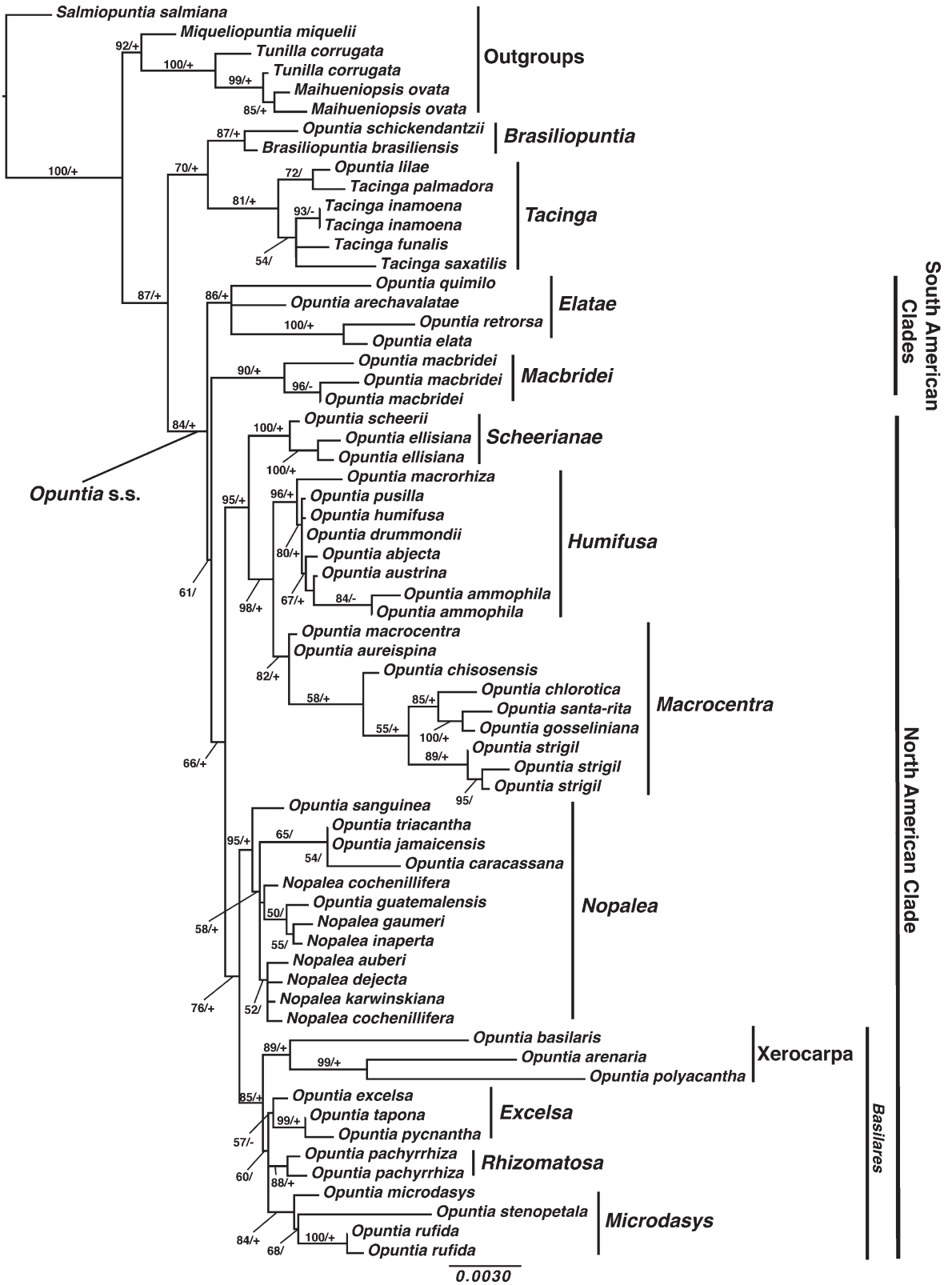

Fig. 1. Diploid phylogeny of *Opuntia* s.s. Most likely topology from our RAxML run with 10 000 bootstrap pseudoreplicates using our combined nuclear (ITS and *ppc*) and plastid data set for diploid taxa only (i.e., all presumably polyphyletic taxa excluded). *Opuntia schickendantzii* is resolved as sister to *Brasiliopuntia brasiliensis,* and *O. lilae* is resolved as sister to *Tacinga palmadora*. The *Brasiliopuntia-Tacinga* clade is sister to *Opuntia* s.s. in which *Nopalea* is deeply nested. Well-supported clades are named based on series recognized by Britton and Rose (1920), Engelmann (1856), or a morphological feature of a given clade. Bootstrap values are given to the left above branches and posterior probabilities (right) are denoted as + for values of 1.0 and—for values ≥0.95. Posterior probabilities <0.95 are not given.

var. *utahensis* (Engelm.) L. D. Benson; Parfitt, 1997), but ITS sequence data do not support this relationship and rather suggest, according to haplotype analysis, a relationship with *O. basilaris* Engelm. & J. M. Bigelow. Combined plastid and ITS analyses place *O. basilaris* and *O. aurea* as subsequent sisters to the *O. polyacantha* complex (Fig. 3) and *O. pinkavae* as sister to *O. erinacea* (Fig. 3). Both *O. aurea* and *O. pinkavae* display numerous morphological characters that are mosaics between *O. basilaris* and members of the *Polyacantha* clade. *Opuntia pinkavae* exhibits pubescent cladodes and pink flowers like *O. basilaris*, and *O. aurea* exhibits pubescent cladodes like *O. basilaris* but the green stigmas, mostly yellow flowers, seeds with a broad funicular girdle, and pollen morphology similar to members of the *O. polyacantha* complex (Parfitt, 1991). *Opuntia aurea* and *O. pinkavae* are found where the geographic distributions of diploid *O. basilaris* and polyploid members of the *O. polyacantha* complex overlap (Parfitt, 1997; Pinkava, 2002).

Opuntia carstenii, *O. depressa*, and *O. robusta* were recovered within the *Basilares* clade with plastid data and a grade containing mostly members of the *Basilares* clade with ITS data (online Appendix S1A, B), but it was not possible to determine parentage of those taxa from among the four clades (i.e., *Excelsa*, *Microdasys*, *Rhizomatosa*, *Xerocarpa*).

***Biogeography and divergence time estimation of* Opuntia *s.s.*—**Our biogeographic analysis supports a southwestern South American origin for *Opuntia* s.s. with subsequent dispersals to the Central Andean Valleys of Peru and the western North American desert region (Fig. 4). The most recent common ancestor of *Brasiliopuntia* and *Tacinga* also appears to have dispersed from southwestern South America, and one lineage, *O. lilae*, dispersed to the Caribbean region of Venezuela (Fig. 4). Both ML (Mesquite) and Bayesian (RASP) results support an origin of the North American *Opuntia* radiation in the deserts of western North America. From the North American desert region, the *Nopalea* clade dispersed into the tropical dry forest of Mexico, Central America, and the Caribbean. Other North American clades continued to radiate in the North American desert region and in some cases significantly increased their ranges via the formation of polyploid taxa. For example, *O. fragilis* of the *Xerocarpa* clade moved from the southwestern United States into Canada and the upper Midwest (Parfitt, 1991; Majure and Ribbens, in press) after formation, and the *Humifusa* clade migrated from the west into the southeastern United States forming a small radiation in the Gulf Coastal Plain. Divergent diploid members of the *Humifusa* clade from the west and east eventually formed contact zones, and allopolyploid taxa expanded north after the last glacial maximum, far surpassing the distributions of diploid taxa (Majure et al., 2012).

Our divergence time estimates suggest that the North American clade originated 5.12 (± 1.6) Ma (online Appendix S3), which according to our ancestral area reconstruction would place the North American clade in the western North American desert region before the presumed closure of the Isthmus of Panama at 3 Ma (Marshall et al., 1979). Constraining the North American clade at 3 Ma had no effect on divergence time estimates. Subclades within the North American clade subsequently originated from 5–1.5 Ma (i.e., from the early Pliocene through the early Pleistocene); however, the majority of those subclades originated during the middle Pliocene (Appendix S3).

DISCUSSION

Consolea—The Caribbean genus *Consolea* consists only of hexaploid and octoploid species (L. C. Majure et al., unpublished manuscript), and the clade could have originated via an allopolyploidization event between other members of tribe Opuntieae (Negrón-Ortiz, 2007; Griffith and Porter, 2009). The conflicting placement based on ITS vs. plastid sequence data of species of *Consolea* certainly support this possibility. *Consolea* is supported as monophyletic with either ITS or plastid sequence data (Appendix S1A, B). *Consolea* is not resolved as sister to any clade of *Opuntia* in analyses of ITS data alone (ITS is insufficiently variable to illuminate relationships among clades within *Opuntia* s.s., as shown in Griffith and Porter, 2009), and plastid data place *Consolea* as sister to the *Tacinga-Brasiliopuntia-Opuntia* clade (Appendix S1A). Furthermore, combined analyses of nuclear and plastid diploid data sets place *Consolea* with strong support (bs = 86%) as sister to the *Tacinga-Brasiliopuntia-Opuntia* clade (Appendix S2), so *Consolea* should not be considered "firmly" embedded in *Opuntia* s.s., as suggested by Nyffeler and Eggli (2010b). If *Consolea* is a result of ancient reticulation, concerted evolution and subsequent ITS divergence may obscure progenitor discovery, or the putative progenitors may have since gone extinct. On the contrary, the placement of *Consolea* within *Opuntia* s.s. may represent incomplete lineage sorting or homoplasy in ITS data. Further work will be necessary to resolve the placement of *Consolea*.

Consolea shares morphological characters with numerous taxa. These include monopodial trunks, as in *Brasiliopuntia*, hairy seeds as in *Brasiliopuntia*, *Tacinga*, and some members of *Opuntia* s.s. (Stuppy, 2002), hook-shaped embryos as in *Tacinga* (Stuppy, 2002), and expanded floral nectaries for hummingbird pollination as in *Tacinga* (Taylor et al., 2002) and several *Opuntia* species (e.g., *O. quimilo, Nopalea*; Díaz and Cocucci, 2003; Puente, 2006). However, members of *Consolea* also demonstrate unique characters, which do not appear elsewhere in the Opuntieae, except in interclade allopolyploids with *Consolea* (e.g., reticulate epidermis and cryptic dioecy; Strittmatter et al., 2008). *Consolea* has diversified into at least nine species (Areces-Mallea, 2001; Negrón-Ortiz, 2007) and should not be regarded as synonymous with *Opuntia* s.s., as proposed by Nyffeler and Eggli (2010b).

Opuntia lilae *and* Opuntia schickendantzii—Previous analyses have shown that one of our outgroups, previously regarded as *Opuntia*, *Salmiopuntia salmiana*, is resolved outside of *Opuntia* s.s. (Griffith and Porter, 2009). Our analyses indicated that *O. lilae* and *O. schickendantzii* also are not members of *Opuntia* s.s. (Fig. 1). The placement of these two species outside of *Opuntia* s.s. was unexpected given that Trujillo and Ponce (1990) considered *O. lilae* to be a member of *Opuntia* series *Tunae* of Britton and Rose (1920), and *O. schickendantzii* has traditionally been considered a member of *Opuntia* series *Aurantiacae* (Britton and Rose, 1920). Our sequence data here and morphological analyses (L. C. Majure and R. Puente unpublished manuscript) indicate that *O. lilae* is a Venezuelan Caribbean member of the mostly Brazilian *Tacinga* clade. The disjunction of Cactaceae congeners from the Caatinga of eastern Brazil to the Caribbean region of northern South America has been observed previously (Sarmiento, 1975). More research is essential to determine how *O. schickendantzii* should be treated taxonomically, given that it does not share obvious morphological characters with *Brasiliopuntia* (Nyffeler and Eggli, 2010b), its sister taxon in our analyses.

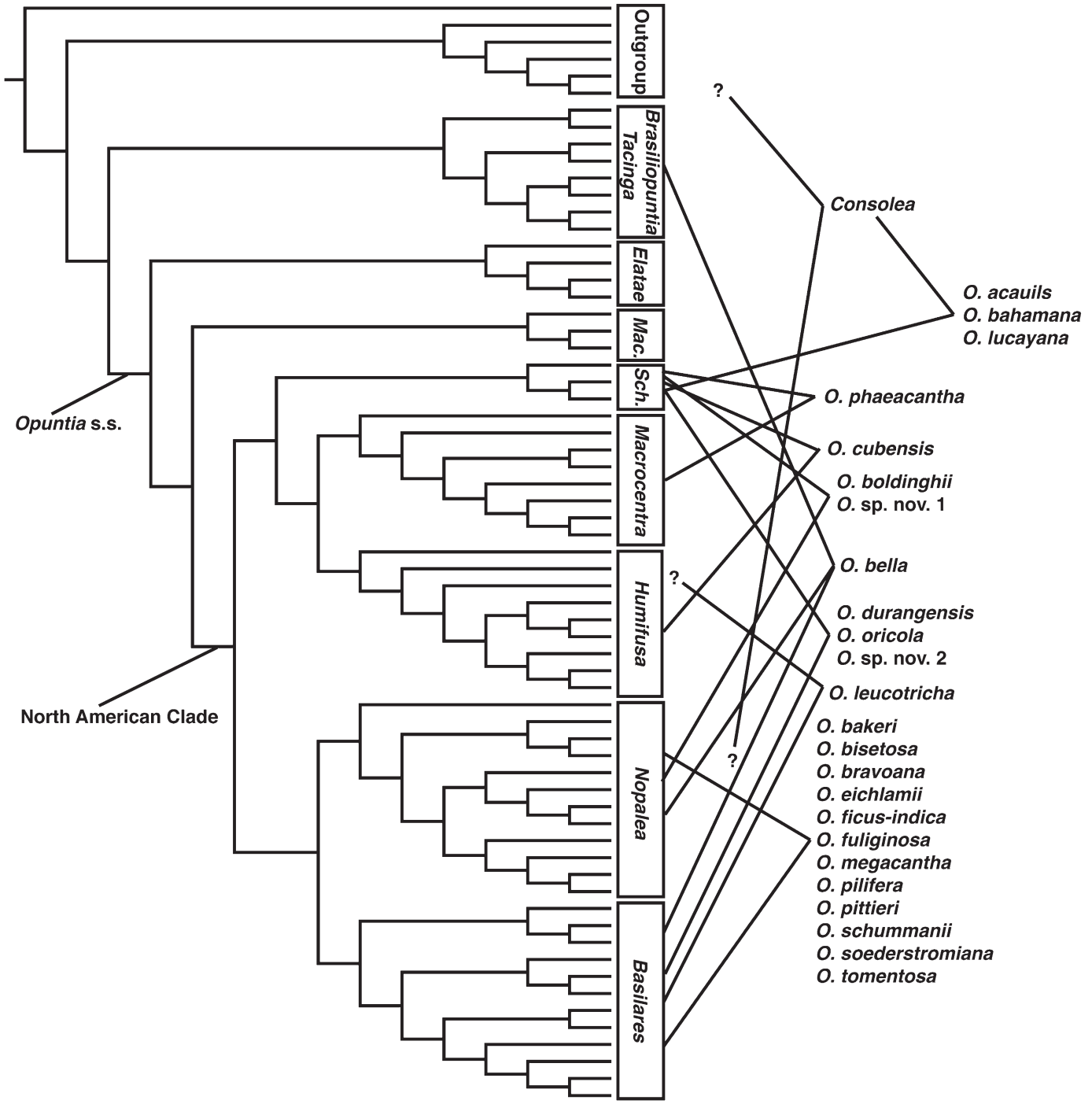

Fig. 2. Diploid phylogeny of *Opuntia* s.s. (adapted from Fig. 1) with interclade reticulate taxa mapped on their putative diploid progenitor clades. We did not discover any interclade taxa derived from the South American *Elatae* or *Macbridei* clades. Instances where putative progenitors of inferred interclade allopolyploids could not be verified are denoted as ? (e.g., *Consolea*). Interclade reticulate evolution is always associated with members of the North American *Opuntia* radiation.

Nopalea—Our results indicate that the hummingbird-pollinated *Nopalea* is nested within *Opuntia* s.s., in agreement with other analyses (Wallace and Dickie, 2002; Griffith and Porter, 2009; Bárcenas et al., 2011; Hernández-Hernández et al., 2011). Hence, *Nopalea* should not be recognized at the generic level but does form a clade and could still be recognized within *Opuntia* s.s. In our combined diploid analysis, *Nopalea* forms a well-supported clade (bs = 96%) that also includes insect-pollinated *O. caracassana*, *O. guatemalensis*, *O. jamaicensis*, *O. sanguinea*, and *O. triacantha*. Shifts from insect pollination to hummingbird pollination have occurred several times in Opuntieae (e.g., *Tacinga*, *O. quimilo*, *O. stenopetala*, *Nopalea*; data not shown). In *Nopalea*, this shift resulted in pronounced floral morphological changes (e.g., short, erect tepals, and

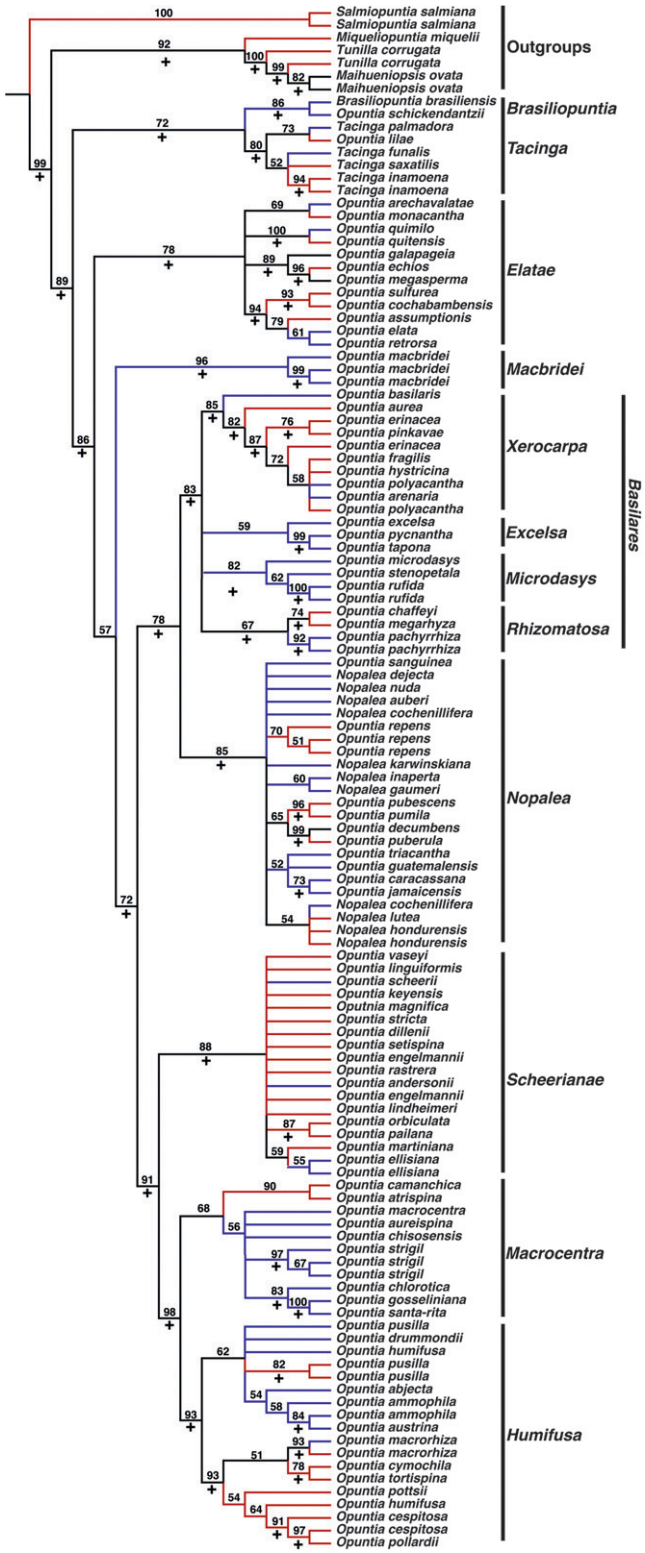

Fig. 3. Intraclade phylogeny of *Opuntia* s.s. (total evidence phylogeny excluding interclade derived taxa). The 50% majority rule consensus tree from a RAxML analysis of 10000 rapid bootstrap pseudoreplicates using our combined nuclear and plastid data set for all diploid taxa (blue) and intraclade polyploids (red). Taxa lacking ploidy information are left black. Bootstrap values are shown above branches; posterior probabilities ≥95 are represented below branches by a plus sign (+).

TABLE 3. Interclade derived taxa recovered in our analyses. Ploidy is given for each species where known based on Majure et al. (2012) and Majure et al. (unpublished manuscript).

Species	Putative progenitors	Source
O. acaulis (8*x*), *O. bahamana* (6*x*), *O. lucayana* (4*x*)	*Consolea* *Scheerianae* clade	Plastid data ITS data
O. bella (unknown)	*Basilares* clade *Nopalea* clade *Tacinga*	Plastid data ITS data ITS data
O. bakeri (9*x*), *O. bisetosa* (6*x*), *O. bravoana* (6*x*), *O. eichlamii* (6*x*), *O. ficus-indica* (8*x*), *O. fuliginosa* (8*x*), *O. megacantha* (8*x*), *O. pilifera* (8*x*), *O. pittieri* (unknown), *O. schumannii* (unknown), *O. soederstromiana* (8*x*), *O. tomentosa* (8*x*)	*Basilares* clade *Nopalea* clade	Plastid and ITS data Plastid and ITS data
O. boldinghii (6*x*), *O.* sp. nov. 1 (2*x*)	*Nopalea* clade *Scheerianae* clade	Plastid data ITS data
O. durangensis (4*x*), *O. oricola* (6*x*), *O.* sp. nov. 2 (6*x*)	*Basilares* clade *Scheerianae* clade	Plastid data ITS data
O. cubensis (5*x*)	*Humifusa* clade *Scheerianae* clade	Plastid data ITS data
O. phaeacantha (6*x*)	*Scheerianae* clade *Macrocentra* clade	Plastid data ITS data
O. leucotricha (4*x*)	*Basilares* clade *Humifusa* clade ?	Plastid data ITS data
Consolea (6*x*-8*x*)	Sister to *Tacinga*, *Brasiliopuntia*, *Opuntia* s.s. clade *Opuntia* s.s.?	Plastid data ITS data

exerted stamens and styles). Such pollinator shifts are common in angiosperms and often result in major morphological changes (e.g., Grant, 1994; Fenster et al., 2004; Penneys and Judd, 2005; Crepet and Niklas, 2009).

***South–North American disjunction in* Opuntia**—The North American *Opuntia* clade is nested within the South American *Opuntia* clades (Fig. 1); the ancestral area reconstruction for the *Macbridei* (Andean Valleys of Peru and Ecuador) + North American clade suggests that their most recent common ancestor was from southwestern South America (66% proportional likelihood; Fig. 4). Thus, our data suggest that the most recent common ancestor of North American *Opuntia* migrated north or was dispersed long distance from South America to western North America. Our DIVA analysis agrees with the long-distance dispersal scenario, although with a low probability (0.50). The disjunction of North and South American *Opuntia* has not been proposed previously, presumably because species of *Opuntia* exist throughout the Americas from Argentina to Canada (Anderson, 2001). Similar patterns of disjunctions between South America and North America can be seen in Cactoideae (Hernández-Hernández et al., 2011), elsewhere in Opuntioideae (Griffith and Porter, 2009), as well as in the close relatives of cacti, *Grahamia* (Nyffeler, 2007) and *Portulaca* (Hershkovitz and Zimmer, 2000).

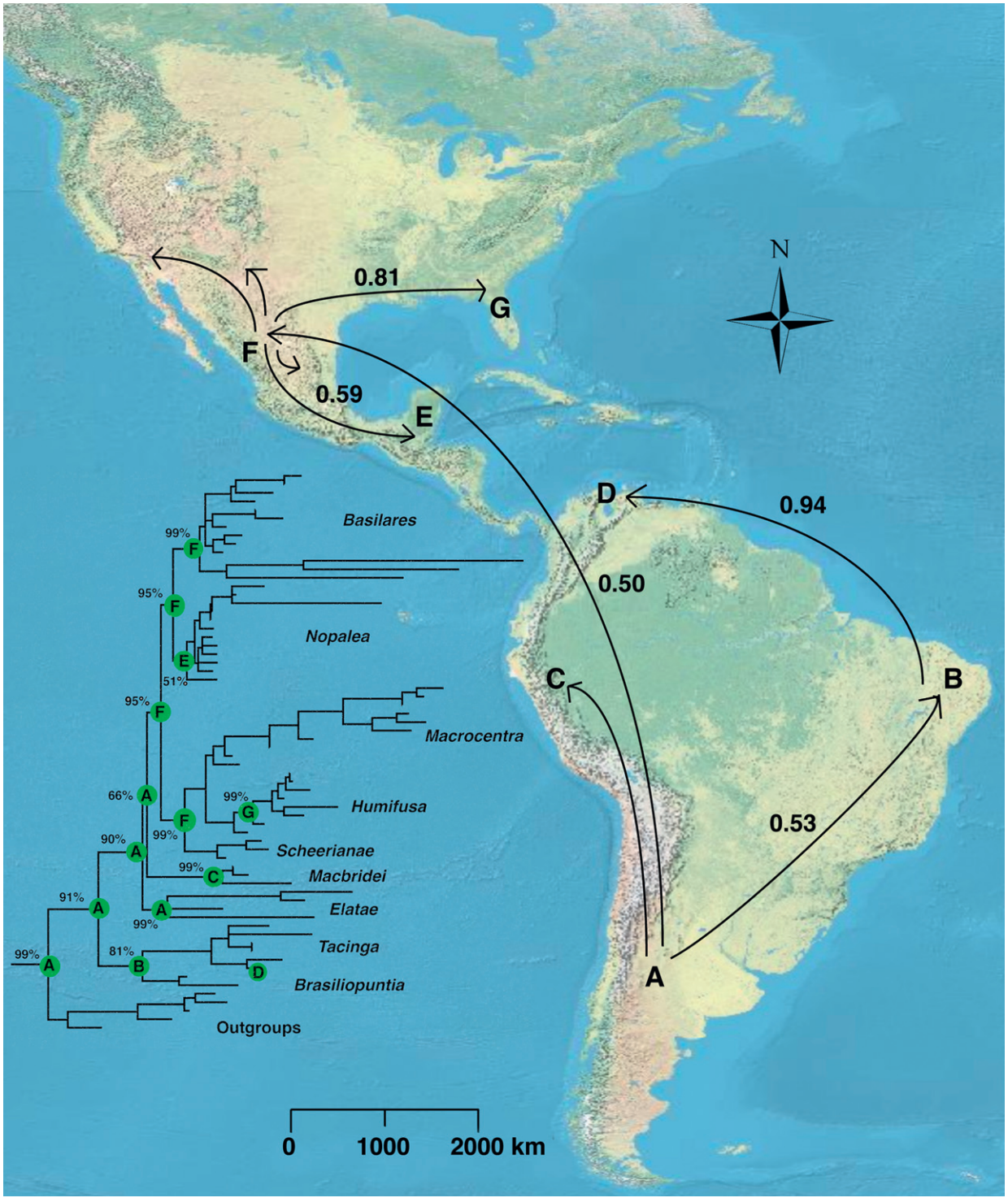

Fig. 4. Ancestral area reconstruction and putative dispersal pathways of *Opuntia* clades. Ancestral reconstructions are represented as (A) southwestern South America, (B) Caatinga, (C) Central Andean valleys, (D) northern South America (Caribbean Region), (E) tropical dry forests (Mexico, Central America, Caribbean), (F) western North American desert region, and (G) southeastern United States. Proportional likelihoods are given for each node in the phylogeny. Dispersal probabilites are given along a given pathway on the map. *Opuntia* s.s. originated in southern South America (A), and then expanded to the Central Andean Valleys (C) and the western North American desert region (F) from where it expanded in distribution and diversified into eight subclades. From the southwestern North American desert region, the *Nopalea* clade dispersed into the tropical dry forests of Mexico, Central America, and the Caribbean (E), and the *Humifusa* clade dispersed into the southeastern United States (G). The ancestor to the *Tacinga-Brasiliopuntia* clade (B), an eastern Brazilian clade of the Caatinga, also originated in southwestern South America. One lineage, *O. lilae,* dispersed to the northern South American Caribbean from the Caatinga region (D).

There are other well-known examples of similar floristic disjunctions between southern South America and the southwestern United States/northern Mexico (Johnston, 1940; Axelrod, 1948; Raven, 1963; Solbrig, 1972; Lia et al., 2001; Simpson et al., 2005; Bessega et al., 2006; Moore et al., 2006; Hawkins et al., 2007), although there is still speculation as to why such disjunctions occur (Solbrig, 1972). Many of these disjuncts also appear to have their origins in South America (Johnston, 1940). Most analyses suggest that these North–South American disjunctions must have formed via long-distance dispersal events (Raven, 1963; Simpson et al., 2005; Bessega et al., 2006; Moore et al., 2006), since very few species of the overall floras are shared between the two areas (e.g., 2%; Raven, 1972), many of these disjunct taxa are not host to the same insect faunas, and the same vertebrates often are not found in the two geographic locations (Raven, 1963, 1972).

Further supporting long-distance dispersal in *Opuntia*, the cactophagous moth, *Cactoblastis cactorum* Berg (Pyralidae), which occurs naturally in southern South America, our proposed geographic origin of *Opuntia*, does not occur naturally in North America. In fact, as aforementioned, introduced populations of *C. cactorum* are used as a biocontrol agent to destroy introduced populations of North American *Opuntia*, which have not evolved to cope with its gregarious feeding habits (Stiling, 2000; Stiling and Moon, 2001; Marisco et al., 2010). Likewise, cactophagous moths in North America (e.g., *Melitara* Walker) are in a different clade from *C. cactorum*, suggesting that the internal feeding behavior of these pyralid moths evolved several times within this lineage after the initial evolution of cactophagy in the Pyralidae (Simonsen, 2008).

It is presumed that African, Malagasy, Sri Lankan, and Indian populations of the epiphyte, *Rhipsalis* (Cactoideae), originated via long-distance dispersal by birds from their native range in South America (Thorne, 1973; Benson, 1982; Barthlott, 1983; Anderson, 2001). Long-distance dispersal of Didiereaceae from South America to Africa also has been postulated (Applequist and Wallace, 2001). Birds (e.g., species of *Geospiza*) are also known to disperse the seeds of Galápagos *Opuntia* at least for short distances (Grant and Grant, 1981). Numerous other species of birds have been recorded eating fruits and seeds of *Opuntia* in other areas as well (Dean and Milton, 2000; Mellink and Riojas-López, 2002), so there may be a link between birds and the long-distance dispersal of *Opuntia* seeds in South and North America.

Species of *Opuntia* s.s. currently exist throughout the neotropics, and it is possible that ancestral populations of the North American clade once occurred in local refugia throughout Central America, a scenario that also has been proposed for other arid-adapted disjunct taxa (Solbrig, 1972). It has been established that a desertified environment did not exist throughout the neotropics from the Miocene through the Pliocene (Axelrod, 1948; Raven, 1963), although isolated patches of "subhumid" habitats may have existed (Solbrig, 1972). These local refugia may have acted as "stepping stones" between xeric environments from South America to western North America (Raven, 1972; Solbrig, 1972), with northward-migrating populations eventually going extinct in more southerly locations. Regardless, the Isthmus of Panama did not create an impassible barrier for the continued northern migration of *Opuntia* s.s. considering that the closure of the Isthmus of Panama is proposed to have taken place 3 mya (Marshall et al., 1979), and divergence time estimates for the North American radiation (5.12 ± 1.6 Ma) place the origin of the clade before that time.

***The North American radiation*—**Our phylogeny suggests that *Opuntia* s.s. radiated rapidly with substantial morphological diversification after its movement into North America. The modern day Sonoran and Chihuahuan deserts were hotspots for the formation of new clades and rampant speciation, as evidenced by the great diversity of *Opuntia* in these regions (Gómez-Hinostrosa and Hernández, 2000; Hernández et al., 2001; Powell and Weedin, 2004). Our dating analysis indicates that the North American clade originated 5.12 (± 1.6) Ma. All subclades of the North American clade originated from 5–1.5 Ma, suggesting that diversification of the clade was facilitated by the expansion of arid habitats during the mid-Pliocene through the early Pleistocene (Axelrod, 1948) and possibly coinciding with the middle Pliocene warm period (Axelrod, 1948; Haywood et al., 2001; Haywood and Valdes, 2004). Speciation within and among North American clades was further increased by hybridization and subsequent allopolyploidy, which are common in *Opuntia* s.s. In contrast, there is little evidence for interclade allopolyploids between the South American clade and other clades, suggesting that those clades remained isolated until modern times with the human introduction of North American taxa into South America or naturally southward-migrating taxa (Kiesling, 1998; Novoa, 2006).

***Reticulate evolution in* Opuntia—**Hybridization between species and subsequent polyploidization (i.e., allopolyploidy) is a common speciation process in plants (Stebbins, 1950, 1971; de Wet, 1971; Grant, 1981; Gibson and Nobel, 1986; Ramsey and Schemske, 1998; Soltis and Soltis, 2009). In *Opuntia,* the production of allopolyploid species is very common and has led to the origin of many new species (Pinkava, 2002). These polyploids often are not completely reproductively isolated from other species (Grant and Grant, 1982). However, these new genomic combinations often result in morphologically distinct entities, which may propagate themselves indefinitely via agamospermy, vegetative apomixis, or sexual reproduction (Rebman and Pinkava, 2001).

Most crosses leading to the formation of interclade allopolyploids are natural; however, a few appear to have been human-mediated (Kiesling, 1998; Griffith, 2004; Reyes-Aguero et al., 2005). Evidence for the use of *Opuntia* in central Mexico as a foodstuff by Native Americans, where many of these polyploid taxa occur, has been found dating to at least 14 000 yr ago (Casas and Barbera, 2002). Kiesling (1998) suggested an 8000–9000-yr-old date for the domestication of the polyploid, *O. ficus-indica,* a species still cultivated and used widely as a foodstuff today (Inglese et al., 2002; Felker et al., 2005).

Many of the shrubby to arborescent allopolyploid taxa, most of which are octoploids, occurring from central Mexico through northern South America, are derivatives of the *Nopalea* clade, which contains the arborescent *Nopalea* members, and one or more of two other clades (e.g., *Basilares*, *Scheerianae*; Fig. 2). Baker (2002) noted the possible relationship between the Ecuadorian-Peruvian octoploid, *O. soderstromiana,* and the introduced central Mexican octoploid, *O. ficus-indica.* Berger considered *O. schumannii* to be intermediate between *Nopalea* and *Opuntia* (Britton and Rose, 1920). These taxa have putative progenitors in common from the *Nopalea* clade and the *Basilares* clade (Fig. 2). This was unexpected, as several South

American taxa (e.g., *O. bisetosa*, *O. boldinghii*, *O. pittieri*, *O. schumanii*) are actually derived from the North American clade, suggesting that they originated from species of *Opuntia* migrating south from North America or those being dispersed south by humans or other fauna (e.g., *O. ficus-indica*).

The common consumption of the fruit of *Opuntia* by humans and many other animals would allow for the facile dissemination of seeds and thus dispersal by migrating frugivores. Sixty-nine species of vertebrates (not including *Homo sapiens*) have been recorded eating the fruits and/or seeds of *Opuntia* species (Mellink and Riojas-López, 2002). Davis et al. (1984) found seeds of *Opuntia* in wooly mammoth (*Mammuthus*) dung, which confirms the use of *Opuntia* s.s. by Pleistocene megafauna and further emphasizes potential long-distance dispersal via migrating herbivores.

Interclade taxa involving the *Scheerianae* clade consistently have a member of the *Scheerianae* clade as the paternal donor and the other clade involved as the maternal donor (e.g., *O. acaulis*, *O. bahamana*, *O. boldinghii*, *O. cubensis*, *O. lucayana*, *Opuntia* sp. nov. 1). This is most likely the result of specialized pollination syndromes (primarily bird pollination) in *Consolea* and *Nopalea*, since hummingbirds presumably rarely visit the entomophilous flowers of *Scheerianae*. However, insects occasionally visit hummingbird-pollinated taxa, such as *O. quimilo* (Díaz and Cocucci, 2003) and *Nopalea* (Puente, 2006). In the case of the allopolyploid *O. cubensis*, the putative paternal progenitor *O. dillenii* of the *Scheerianae* clade is much larger than the putative maternal progenitor *O. abjecta* and may thus be more easily accessible to insect pollinators, leading to higher transfer rates of pollen from *O. dillenii* to receptive stigmas of *O. abjecta*. Alternatively, genetic interactions may determine whether reciprocally formed polyploids are both viable.

The precise origins of those species designated intraclade polyploids are not clear for several reasons. First, limited sequence divergence among closely related species precludes determination of the specific origins of true intraclade polyploids. Second, concerted evolution of ITS in an allopolyploid may conceal one of the putative progenitors (Álvarez and Wendel, 2003; Kovarik et al., 2005; Kim et al., 2008; Soltis et al., 2008) such that a true allopolyploid (interclade or intraclade) would not be detected as such. Finally, autopolyploidy, rather than allopolyploidy, could explain a pattern of shared sequences between diploids and polyploids. Some taxa included in our analyses are composed of more than one ploidal level (e.g., *O. macrocentra*, *O. pusilla*, *O. strigil*; Pinkava, 2002; Powell and Weedin, 2004; Majure et al., 2012); samples of different cytotypes are sometimes morphologically similar and form clades (e.g., *O. pusilla*; Fig. 3), suggesting autopolyploidy. Autopolyploids have been found elsewhere in Cactaceae, although the best documented are restricted to subfamily Cactoideae (Sahley, 1996; Hamrick et al., 2002; Nassar et al., 2003). Autopolyploids may play a much larger role in plant speciation than is currently recognized (Judd et al., 2007; Soltis et al., 2007) and may have been influential in the diversification of *Opuntia* s.s. as well. Determining the origins of all intraclade polyploids thus would be especially informative.

Conclusions—*Opuntia* s.s. is a well-supported clade, which originated in southwestern South America and quickly diversified after a northern migration or long-distance dispersal into the arid regions of western North America. Reticulate evolution and polyploidization have played a major evolutionary role in the clade by producing novel phenotypes and increasing species richness. The complexity of phylogenetic relationships among species and major clades is increased by polyploids, so determining the ploidy of all taxa is imperative to the construction of an accurate evolutionary history of the clade. Detailed phylogenetic, morphological, and field studies of taxa within each clade will be necessary to fully understand relationships and biogeographic patterns at the species level.

Given the proposed recent ages for *Opuntia* s.s. (5.6 ± 1.9 Ma; Arakaki et al., 2011) and its subclades given here, *Opuntia* s.s. shows the signature of a clade that has undergone a rapid radiation (i.e., broad distribution, high morphological and species diversity, and low molecular marker divergence; Malcomber, 2002). The nuclear and plastid data do not fully resolve species relationships within clades, and several nodes along the backbone of the phylogeny lack high bootstrap support, although the major clades of *Opuntia* s.s. are generally well supported. Rapid radiations are often constrained by the lack of support for clade relationships (e.g., Fishbein et al., 2001; Malcomber, 2002; Valente et al., 2010).

Increased taxon and marker sampling is an important next step in determining relationships among all species of *Opuntia* s.s. Species delimitation will require development of appropriate markers to allow for the discovery of intraspecific variation, using multiple accessions from each potential species described within that clade. This work will also allow for the potential discovery of morphologically cryptic species within taxa composed of multiple ploidal levels and for illuminating the origins and evolutionary role of the abundant polyploids in the clade.

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APPENDIX 1. Taxa, voucher information (collector, herbarium acronym or botanical garden), and GenBank accessions used in our study (*ndhF-rpl32*, *psbJ-petA*, *atpB-rbcL*, *trnL-F*, *matK*, *ycf1*, *ppc*, nrITS). Missing data for a given region is listed as: —. Material obtained from living collections is cited as: DBG (Desert Botanical Garden, Phoenix, AZ), HBG (Huntington Botanical Garden, San Marino, CA), KEW (Royal Botanic Gardens, UK), MBC (Montgomery Botanical Center, Coral Gables, FL), and SRSC (Sul Ross State University, Alpine, TX).

Brasiliopuntia brasiliensis (Willd.) A. Berger — DBG 1990 0559 02 *Zimmerman 2606*, Cult. (DES), JF787309, JF787469, JF787155, JF712685, JF786712, JN387143, JN387207, JF786876; ***Consolea corallicola*** Small; DBG 1997 0397 01, *L.C. Majure 3321*, United States, FL, (FLAS), JF787310-11, JF787470-71, JF787156-57, JF712686-87, JF786713-14, JQ676987-86, —, JF786877-78; ***Consolea falcata*** (Ekman & Werdermann) F.M. Knuth, DBG 1997 0394 Dominican Republic, Bayajibe (DES), JF787312, JF787472, —, JF712688, JF786715, JQ676988, —, JF786879; ***Consolea moniliformis*** (L.) A. Berger, *L.C. Majure 3909*, United States, FL, Cult., (FLAS), JF787313, JF787473, JF787158, JF712689, JF786716, JQ676989, —, JF786880; ***Consolea nashii*** (Britton) A. Berger, DBG 1996 0257 *C. Fleming s.n,* Turks & Caicos Is., South Caicos Island; DBG 1999 0025, JF787314-15, JF787474-75, JF787159-60, JF712690-91, JF786717-18, JQ676991-90, —, JF786881-82; ***Consolea rubescens*** (Salm-Dyck ex A.P. deCandolle) Lem., DBG 1997 0390 cult., MBC, *L.C. Majure 3323,* United States, FL, Cult., (FLAS), JF787316-17, JF787476/JF787606, JF787161-62, JF712692-93, JF786719-20, JQ676992-93, —, JF786883-84/JF787126-32; ***Consolea spinosissima*** (P.Mill.) Lem., DBG 1995 0389 Jamaica, Hellshire Hills; MBC, *L.C. Majure 3322*, United States, FL, Cult., (FLAS), JF787318-19, JF787477-78, JF787163, JF712694-95, JF786721-22, JQ676995-94, —, JF786885-86; ***Maihueniopsis*** cf. ***ovata*** (Pfeiffer) F. Ritter, DBG 2001 0101, DBG 2001 0102, JF787320-21, JF787479 (01), —, JF712696-97, JF786723-24, JN387144 —, JN387208- JN387209, JF786887-88; ***Miqueliopuntia miquelii*** (Monville) F. Ritter, DBG 1997 0129 *E. F. Anderson 6306,* Chile, Huasco Bajo Región. (DES), JF787322, JF787480, JF787164, JF712698, JF786725, JN387145, JN387210, JF786889; ***Nopalea auberi*** (Pfeiffer) Salm-Dyck, *M.P. Griffith 175*, (SRSC), JF787323, JF787481, JF787165, JF712699, JF786726, JN387146, JN387211, JF786890; ***Nopalea cochenillifera*** (L.) Salm-Dyck, DBG 1997 0395 Costa Rica, San Jose, *D. Lancaster s.n., L.C. Majure 2789*, United States, FL, (FLAS), JF787324-25, JF787482-83, JF787166-67, JF712700-01, JF786727-28, —JN387147, —JN387212, JF786891-92; ***Nopalea dejecta*** (Salm-Dyck) Salm-Dyck, DBG 2002 0342 0101 *R. Puente 1614* Mexico, Valles. (DES, ASU), JF787326, —, JF787168, —, JF786729, JN387148, JN387213, JF786893; ***Nopalea gaumeri*** Britton & Rose, DBG 1997 0367 0101 Mexico, Yucatan, JF787327, JF787484, JF787169, JF712702, JF786730, JN387149, JN387214, JF786894; ***Nopalea hondurensis*** (Standley) Rebman, DBG 1996 0554, DBG 1990 0544 0201 0201 *A. D. Zimmerman 2626*, Honduras, Olanchito (DES), JF787329-30, JF787486-87, JF787171-72, JF712704-05, JF786732-33, —, —, JF786896-97; ***Nopalea inaperta*** Schott ex Griffiths, DBG 1997 0367 Mexico, Yucatan, JF787331, JF787488, JF787173, JF712706, JF786734, JN387151, JN387216, JF786898; ***Nopalea karwinskiana*** (Salm-Dyck) K. Schumann, DBG, JF787332, JF787489, JF787174, JF712707, JF786735, JN387152, JN387217, JF786899; ***Nopalea lutea*** Rose, DBG 1997 0368 0102 Cult., JF787333, JF787490, JF787175, JF712708, JF786736, JN387153, —, JF786900; ***Nopalea nuda*** Backeberg, *M.P. Griffith 171*, (SRSC), JF787334, JF787491, JF787176, JF712709, JF786737, —, —, JF786901; ***Opuntia abjecta*** Small, *L.C. Majure 3908*, United States, FL, (FLAS), JF787455, JF787598, JF787300, JF712838, JF786865, JN387199, JN387264, JF787021; ***Opuntia acaulis*** Ekman & Werdermann, DBG 1997 0360, JF787335, JF787492, JF787177, JF712710, JF786738, —, —, JF786902/JF787078-83; ***Opuntia ammophila*** Small, *L.C. Majure 2753, 2826*, United States, FL, (FLAS), JF787336/JF787463, JF787493-94, JF787178-79, JF712711-12, JF786739-40, JN387154/JQ676984, JN387218-19, JF786903-04; ***Opuntia*** x ***andersonii*** H.M.Hernández, Gómez-Hin., Bárcenas, Puente 1239, San Luis Potosi, Mexico (ASU), JF787337, JF787495, JF787180, JF712713, JF786741, —, —, JF786905; ***Opuntia arechavalatae*** Spegazzini, DBG, JF787338, JF787496, JF787181, JF712714, JF786742, JN387155, JN387220, JF786906; ***Opuntia arenaria*** Engelm., *R.D.Worthington 36390*, United States, TX, (SRSC), JF787339, —, JF787182, JF712715, JF786743, JN387155, JN387220, JF786907; ***Opuntia assumptionis*** K. Schumann, DBG, *R. Puente 2010 (3)*, JF787441, JF787586, JF787286, JF712824, JF786846, —, —, JF787007; ***Opuntia atrispina*** Griffiths, *B.L. Snow 2106*, United States, TX, (FLAS), JF787340, JF787497, JF787183, JF712716, JF786744, JN387155, JN387220, JF786908; ***Opuntia aurea*** McCabe ex E.M. Baxter, *D. Woodruff 111A*, United States, UT, (FLAS), JF787341, JF787498, JF787184, JF712717, JF786745, —, —, JF786909; ***Opuntia aureispina*** (S. Brack & K.D. Heil) Pinkava & B.D. Parfitt, *M.P. Griffith 73*, (SRSC), JF787342, JF787607, JF787185, JF712718, JF786746, JN387158, JN387223, JF786910; ***Opuntia austrina*** Small, *L.C. Majure 3450,* United States, FL, (FLAS), JF787343, JF787499, JF787186, JF712719, JF786747, JQ676985, JN387224, JF786911; ***Opuntia bahamana*** Britton & Rose, DBG 1996 0298, JF787344, JF787500, JF787187, JF712720, JF786748, —, —, JF787032-37; ***Opuntia bakeri*** J.E. Madsen, DBG 1985 0571, JF787345, JF787501, JF787188, JF712721, JF786749, —, —, JF786912/ JF787092-97; ***Opuntia basilaris*** Engelm. & Bigelow var. ***basilaris***, *R. Altig s.n.,* United States, CA, (FLAS), JF787346, JF787502, JF787189, JF712722, JF786750, JN387159, JN387225, JF786913; ***Opuntia bella*** Britton & Rose, DBG 1997 0400 Colombia, Venicas Del Dagna, JF787347, JF787503, JF787190, JF712723, JF786751, —, —, JF786914; ***Opuntia bisetosa*** Pittier, DBG 1997 0396, JF787348, JF787504, JF787191, JF712724, JF786752, —, —, JF786915; ***Opuntia boldinghii*** Britton & Rose, DBG 1997 0391, JF787349, JF787505, JF787192, JF712725, JF786753, —, —, JF786916; ***Opuntia bravoana*** E.M. Baxter, DBG 1939 0094 01 H. Gates s.n. Mexico, Baja California Sur (DES) ASDM 2005 0280 01 R. Felger s.n. Mexico, Sonora (DES), JF787350-51, JF787506-07, JF787193-94, JF712726-27, JF786754-55, —, —, JF787038-45; ***Opuntia camanchica*** Engelm., *J.F. Weedin 374*, United States, TX, (SRSC), *L.C. Majure 3514*, United States, TX, (FLAS), JF787352/JF787409, JF787508/JF787556, JF787195/JF787253, JF712728/JF712788, JF786756/JF786816, —, —, JF786917/JF786973; ***Opuntia caracassana*** Salm-Dyck, DBG 1993 0667, JF787464, JF787509, JF787196, JF712729, JF786757, JN387159, JN387225, JF786918; ***Opuntia*** x ***carstenii*** R. Puente & C. Hamann, DBG *R. Puente 2901* Coahuila, Mexico. (Holotype DES), JF787353, JF787510, —, JF712730, JF786758, —, —, JF786919/JF787111-18; ***Opuntia cespitosa*** Raf., *L.C. Majure 1380*, United States, MS, *L.C. Majure 1938*, United States, TN, (MISSA), JF787354-55, JF787511-12, JF787197-98, JF712731-32, JF786759-60, —, —, JF786920-21; ***Opuntia chaffeyi*** Britton & Rose, DBG 1990 0238, JF787356, JF787513, JF787199, JF712733, JF786761, —, —, JF786922; ***Opuntia chisosensis*** (M. Anthony) D.J. Ferguson, DBG 1999 0040, JF787357, JF787514, JF787200, JF712734, JF786762, JN387159, JN387225, JF786923; ***Opuntia chlorotica*** Engelm. & Bigelow, DBG 1977 1021, JF787358, JF787608, JF787201, JF712735, JF786763, JN387162, JN387228, JF786924; ***Opuntia cochabambensis*** Cárdenas, *R. Puente 2010 (2),* DBG, JF787359, JF787609, JF787202, JF712736, JF786764, —, —, JF787046-53; ***Opuntia cubensis*** Britton and Rose, *L.C. Majure 3907, 3968*, United States, FL, (FLAS), JF787360-61, JF787515-16, JF787203, JF712737-38, JF786765-66, F786925/JF787054-60/JF786926; ***Opuntia cymochila*** Engelm. ex. Bigelow, *L.C. Majure 3530*, United States, TX, (FLAS), JF787362, JF787517, JF787204, JF712739, JF786767, —, —, JF786927; ***Opuntia decumbens*** Salm-Dyck, *M.P. Griffith 177,* (SRSC), JF787363, JF787518, JF787205, JF712740, JF786768, —, —, JF786928/JF787133-40; ***Opuntia dillenii*** (Salm-Dyck.) Ker Gawl., *L.C. Majure 3220*, United States, FL, MBC, *L.C. Majure 3319*, United States, FL, (FLAS), JF787444-45, JF787588-89, JF787289-90, JF712827-28, JF786854-55, —, —, JF787010-11; ***Opuntia drummondii*** Graham, *L.C. Majure 2094*, United States, MS, (MISSA), JF787365, JF787520, JF787207, JF712742, JF786770, JN387163, JN387229, JF786930; ***Opuntia durangensis*** Britton & Rose, DBG 1988 0166 0201, JF787366, JF787521, JF787208, JF712743, JF786771, —, —, JF786931; ***Opuntia echios*** J.T. Howell, DBG 1994 0009 *E. F. Anderson 2533*. Ecuador, Galapagos Is., JF787367, JF787522, JF787209, JF712744, JF786772, —, —, JF786932; ***Opuntia eichlamii*** Rose, DBG 2011 0005 01 *C. Hamann s.n.* Guatemala, JF787368, JF787610, JF787210, JF712745, JF786773, —, —, JF786933; ***Opuntia elata*** Link & Otto ex Salm-Dyck, *R. Puente s.n.,* United States, AZ, cult., DBG, JF787369, —, JF787211, JF712746, JF786774, JN387164, JN387230, JF786934; ***Opuntia ellisiana*** Griffiths *B.L. Snow 1083*, United States, TX, (FLAS), DBG 1999 0040 0103 cult., JF787370-71, JF787523-24, JF787212-13, JF712747-48, JF786775-76, JN387166-65, JN387232-31, JF786935-36; ***Opuntia***

engelmannii Salm-Dyck ex Engelm. var. ***engelmannii***, *L.C. Majure 3586*, United States, TX, (FLAS), *A.M. Powell 6009*, United States, TX, (SRSC), JF787372-73, JF787525-26, JF787214-15, JF712749-50, JF786777-78, —, —, JF786937-38; ***Opuntia engelmannii*** Salm-Dyck ex Engelm. var. ***lindheimeri*** (Engelm.) B.D. Parfitt & Pinkava, *L.C. Majure 3506*, United States, TX, (FLAS), JF787374, JF787527, JF787216, JF712751, JF786779, —, —, JF786939; ***Opuntia engelmannii*** Salm-Dyck ex Engelm. var. ***linguiformis*** (Griffiths) B.D. Parfitt & Pinkava, *L.C. Majure 3947*, United States, NM, (FLAS), JF787375, JF787528, JF787217, JF712752, JF786780, —, —, JF786940; ***Opuntia erinacea*** Engelm. & Bigelow, M. H. 658, RSA; *D. Woodruff s.n.,* United States, UT, (FLAS), JF787376-77, JF787529 (658), JF787218-19, JF712753-54, JF786781-82, —, —, JF786941 (658); ***Opuntia excelsa*** Sánchez-Mejorada, DBG 1986 0546 1001, JF787378, JF787530, JF787220, JF712755, JF786783, JN387167, JN387233, JF786942; ***Opuntia ficus-indica*** (L.) P.Mill., *L.C. Majure 3225*, United States, FL, (FLAS), *M.P. Griffith 326*, (SRSC), JF787379-80, JF787531-32, JF787221-22, JF712756-57, JF786784-85, —, —, JF786943-44/JF787101-03; ***Opuntia fragilis*** (Haw.) Nutt., *E. Ribbens 612*, United States, WI, (MWI), JF787381, JF787533, JF787223, JF712758, JF786786, —, —, JF786945; ***Opuntia fuliginosa*** Griffiths, DBG 1986 0027 1005, JF787382, JF787534, JF787224, JF712759, JF786787, —, —, JF786946; ***Opuntia galapageia*** Henslow, DBG 1994 0012 01 *E. F. Anderson 2540*, Galapagos Is., Ecuador, JF787383, JF787535, JF787225, JF712760, JF786788, —, —, JF786947; ***Opuntia gosseliniana*** F.A.C.Weber, *R. Puente 3273-B*, Sierra Mazatan, Sonora (DES, USON), JF787384, JF787611, JF787226, JF712761, JF786789, JN387169, JN387234, JF786948; ***Opuntia guatemalensis*** Britton & Rose, DBG 1990 0534 *Zimmerman 2609*, La Paz, Honduras (DES), JF787328, JF787485, JF787170, JF712703, JF786731, JN387150, JN387215, JF786895; ***Opuntia humifusa*** (Raf.) Raf., *L.C. Majure 3785,* United States, GA, (FLAS); *L.C. Majure 1833,* United States, MS, (MISSA), JF787385-86, JF787536-37, JF787227-28, JF712762-63, JF786790-91, JN387169—, JN387234—, JF786949-50; ***Opuntia hystricina*** Engelm. & Bigelow, *L.C. Majure 3529*, United States, NM, (FLAS), —, JF787538, JF787229, JF712764, JF786792, —, —, JF786951; ***Opuntia jamaicensis*** Britton & Harris, DBG 1997 0357, —, —, JF787230, JF712765, JF786793, JN387169, JN387234, JF786952; ***Opuntia keyensis*** Britton ex Small, *L.C. Majure 4156*, United States, FL, (FLAS), JF787387, —, JF787235, JF712766, JF786794, —, —, —; ***Opuntia leucotricha*** A.P. deCandolle, *L.C. Majure 3953*, United States, FL, (FLAS); DBG 1987 0448, JF787388-89, JF787539-40, JF787231-32, JF712767-68, JF786795-96, —, —, JF786953-54; ***Opuntia lilae*** Trujillo & Ponce, DBG 1997 0369 01 *Trujillo & Ponce 18643*, Venezuela, Sucre, JF787390, JF787612, JF787233, JF712769, JF786797, JN387171, JN387237, JF786955; ***Opuntia lucayana*** Britton, DBG 1997 0398, JF787391, JF787541, JF787234, JF712770, JF786798, —, —, JF786956; ***Opuntia macbridei*** Britton & Rose, HBG, DBG 1990 0601, *L.C. Majure 3848*, United States, FL, Cult., (FLAS), JF787392-93/JF787423, JF787542-43/JF787616, JF787236-37/JF787269,JF712771-72/JF712806,JF786799-00/JF786833, JN387172-73/84, JN387238-39/49, JF786957-58/JF786990; ***Opuntia macrocentra*** Engelm., United States, *L.C. Majure 3516*, United States, NM, (FLAS), JF787394, JF787544, JF787238, JF712773, JF786801, JN387174, JN387240, JF786959; ***Opuntia macrorhiza*** Engelm., United States, *L.C. Majure 3510*, United States, TX, (FLAS); *M.H. Baker 15682*, United States, NM, (FLAS), JF787395-96, JF787545-46, JF787239-40, JF712774-75, JF786802-03, — JQ676983, —JN387241, JF786960-61; ***Opuntia magnifica*** Small, *L.C. Majure 3451*, United States, FL, cult., (FLAS), JF787397, JF787613, JF787241, JF712776, JF786804, —, —, JF786962; ***Opuntia martiniana*** (L.D. Benson) B.D. Parfitt, DBG 1984 0579, JF787398, JF787547, JF787242, JF712777, JF786805, —, —, JF787061-66; ***Opuntia megacantha*** Salm-Dyck, *M.P. Griffith 1288*, (SRSC), JF787399, JF787548, JF787243, JF712778, JF786806, —, —, JF786963/JF787098-100; ***Opuntia megarhyza*** Rose, *Puente 1884-A* Rio Verde, SLP, Mexico (ASU), JF787400, JF787549, JF787244, JF712779, JF786807, JN387175, JN387242, JF786964; ***Opuntia megasperma*** J.T. Howell, DBG 1994 0075, JF787401, JF787550, JF787245, JF712780, JF786808, —, —, JF786965; ***Opuntia microdasys*** (Lehmann) Pfeiffer, *L.C. Majure 3519*, United States, NM, cult., (FLAS), JF787402, JF787551, JF787246, JF712781, JF786809, JN387175, JN387242, JF786966; ***Opuntia monacantha*** (Willd.) Haw., *L.C. Majure 3847*, United States, FL, cult., (FLAS), JF787403, JF787552, JF787247, JF712782, JF786810, —, —, JF786967; ***Opuntia orbiculata*** Salm-Dyck ex Pfeiffer, *C. Hamann s.n., cult,* (DES), JF787404, —, JF787248, JF712783, JF786811, —, —, JF786968; ***Opuntia oricola*** Philbrick, DBG 1994 0178, JF787405, JF787553, JF787249, JF712784, JF786812, —, —, JF786969; ***Opuntia pachyrrhyza*** H. M. Hernández, C. Gómez-Hinostrosa & R. T. Bárcenas, *Puente 601* Mexico, San Luis Potosi, (ASU, DES); *Puente 1260*, Queretaro, Mexico (DES), JF787406-07, JF787554-55, JF787250-51, JF712785-86, JF786813-14, JN387178-79, — JN387178, —, JF786970-71; ***Opuntia pailana*** Weingart, *R. Puente 3371*, Coahuila, Mexico (DES), JF787408, JF787614, JF787252, JF712787, JF786815, —, —, JF786972; ***Opuntia phaeacantha*** Engelm., *M.P. Griffith 214*, United States, (SRSC), JF787410, JF787557, JF787254, JF712789, JF786817, —, —, JF786974; ***Opuntia pilifera*** F.A.C. Weber, DBG, JF787411, JF787558, JF787255, JF712790, JF786818, —, —, JF786975; ***Opuntia pinkavae*** B.D.Parfitt, *D. Woodruff 118A*, United States, UT, (FLAS), —, JF787559, JF787256, JF712791, JF786819, —, —, JF786976, ***Opuntia pittieri*** Britton & Rose, DBG 1995 0319, JF787412, JF787560, JF787257, JF712792, JF786820, —, —, JF786977/JF787104-110; ***Opuntia pollardii*** Britton & Rose, *L.C. Majure 1921*, United States, MS, (MISSA), JF787413, JF787561, JF787258, JF712793, JF786821, —, —, JF786978; ***Opuntia polyacantha*** Engelm., *L.C. Majure 3526,* United States, NM, (FLAS); *D. E. Soltis 2902*, United States, WY, (FLAS), JF787465/—, JF787562/—, JF787259/—, JF712794-95, JF786822-23, —/JN387180, —/JN387245, JF786979/—; ***Opuntia pottsii*** Salm-Dyck, *A.M. Powell 6897*, United States, TX, (SRSC, FLAS), JF787414, JF787563, JF787260, JF712796, JF786824, —, —, JF786980; ***Opuntia puberula*** Pfeiffer, DBG 1993 0887 1003, JF787415, JF787615, JF787261, JF712797, JF786825, —, —, JF786981; ***Opuntia pubescens*** Wendland ex Pfeiffer, *M.P. Griffiths 300*, (SRSC), —, —, —, JF712798, —, —, —, JF786982; ***Opuntia pumila*** Rose, *R. Puente 2297*, Mexico, Oaxaca, (DES), JF787416, JF787564, JF787262, JF712799, JF786826, —, —, JF786983/JF787141-46; ***Opuntia pusilla*** (Haw.) Haw., *L.C. Majure 753*, United States, MS, (MISSA); *L.C. Majure 1091*, United States, AL, (MISSA); *L.C. Majure 1920*, United States, MS, (MISSA, MMNS), JF787417-19, JF787566-68, JF787263-65, JF712800-02, JF786827-29, JN387181——, JN387246——, JF786984-86; ***Opuntia pycnantha*** Engelm., DBG 1987 0916 01 Baja California Sur, Mexico, JF787420, JF787565, JF787266, JF712803, JF786830, JN387182, JN387247, JF786987; ***Opuntia quimilo*** K. Schumann, DBG 2003 0111 0101 Argentina, cult., JF787421, JF787569, JF787267, JF712804, JF786831, JN387183, JN387248, JF786988; ***Opuntia quitensis*** F.A.C. Weber, DBG 1988 0262 0201, cult., JF787422, JF787570, JF787268, JF712805, JF786832, —, —, JF786989; ***Opuntia rastrera*** F.A.C. Weber, DBG, JF787424, JF787571, JF787270, JF712807, JF786834, —, —, JF786991; ***Opuntia repens*** Bello, *L.C. Majure 3837*, United States, VI, (FLAS); *L.C. Majure 3838-39,* United States, PR, (FLAS), JF787425-27, JF787572-74, JF787271-73, JF712808-10, JF786835-37, —, —, JF786992-94/JF787147-54; ***Opuntia retrorsa*** Spegazzini, *J.R. Abbott 16248,* Bolivia, Santa Cruz, (FLAS), JF787428, JF787575, JF787274, JF712814, JF786839, JN387185, JN387250, JF786995; ***Opuntia robusta*** Wendland, *M.P. Griffith 327*, (SRSC), JF787429, JF787576, JF787275, JF712811, JF786838, —, —, JF786996/JF787119-25; ***Opuntia rufida*** Engelm., DBG 1990 0343 0202 United States TX, Big Bend; *Manning s.n.*,, TX, (FLAS), JF787430-31, JF787577/—, JF787276-77, JF712812-13, JN387186-87, JN387251-52, JF786840-41, —/JF786997; ***Opuntia sanguinea*** Proctor, DBG 1996 0297 0101, JF787434, JF787580, —, JF712817 JF786844,, JN387190, JN387255, JF787000; ***Opuntia santa-rita*** (Griffiths & Hare) Rose, DBG 1940 1421 0103W, JF787435, JF787617, JF787280, JF712818, JF786845, JN387191, JN387256, JF787001; ***Opuntia scheeri*** F.A.C. Weber, *R. Puente s.n.,* DBG, JF787436, JF787581, JF787281, JF712819, JF786847, JN387192, JN387257, JF787002; ***Opuntia schickendantzii*** F.A.C. Weber, DBG 2010 0049 01 Cult., JF787437, JF787582, JF787282, JF712820, JF786848, JN387192, JN387257, JF787003; ***Opuntia schumannii*** F.A.C.Weber ex A.Berger, DBG 1997 0362, JF787438, JF787583, JF787283, JF712821, JF786849, —, —, JF787004; ***Opuntia setispina*** Engelm. Ex Salm-Dyck, *Puente 3656,* Cosihuariachi, Chihuahua, Mexico (DES), JF787439, JF787584, JF787284, JF712822, JF786850, —, —, JF787005; ***Opuntia soederstromiana*** Britton & Rose, DBG 1985 0569 0101, JF787440, JF787585, JF787285, JF712823, JF786851, —, —, JF787006; ***Opuntia stenopetala*** Engelm., *M.P. Griffith* s.n., DBG, JF787442, JF787618, JF787287, JF712825, JF786852, JN387192, JN387257, JF787008; ***Opuntia stricta*** (Haw.) Haw., *L.C. Majure 1922,* United States, MS, (MISSA), JF787443, JF787587, JF787288, JF712826, JF786853, —, —, JF787009; ***Opuntia strigil*** Engelm., *A.M. Powell 6008*, (SRSC), *L.C. Majure 3515*, United States, TX, (FLAS), *Puente 3359*,

United States, TX (DES), JF787446-48, JF787590-91, JF787291-93, JF712829-31, JF786856-58, JN387195-97, JN387260-62, JF787012-14; ***Opuntia sulphurea*** Don, DBG 1995 0372, JF787449, JF787592, JF787294, JF712832, JF786859, —, —, JF787015; ***Opuntia tapona*** Engelm., DBG 1939 0093 0101 Comondu, Baja California, Mexico, JF787450, JF787593, JF787295, JF712833, JF786860, JN387198, JN387263, JF787016; ***Opuntia tomentosa*** Salm-Dyck, *M.P. Griffith 181*, (SRSC); DBG 1996 0371 0101; DBG 1978 0326 0101, JF787451-53, JF787594-96, JF787296-98, JF712834-36, JF786861-63, —, —, JF787017-19/JF787067-69; ***Opuntia tortispina*** Engelm., *L.C. Majure 3533*, United States, TX, (FLAS), JF787454, JF787597, JF787299, JF712837, JF786864, —, —, JF787020; ***Opuntia triacantha*** (Willd.) Sweet., *Mori et al. 26693*, Netherlands Antilles, Saba, (NY), JN676104, JN676105, JN676101, —, JN676103, JN387200, JN387265, JN676102; ***Opuntia*** sp. nov. 1, DBG 2003 0155 0102 *Puente 1614* Valles, San Luis Potosi, Mexico (DES), JF787456, JF787599, JF787301, JF712839, JF786866, —, —, JF787022/JF787070-77; ***Opuntia*** sp. nov. 2, *A.L. Reyna 97-292* Sonora, Mexico (ASU, ARIZ), JF787457, JF787600, JF787302, JF712840, JF786867, —, —, JF787023; ***Opuntia vaseyi*** (Coult.) Britton & Rose, DBG 1987 0049 0201, JF787458, JF787601, JF787303, JF712841, JF786868, —, —, JF787024; ***Opuntia*** cf. ***wilcoxii*** Britton & Rose, *S. Friedman 94-148* Mesiaca, Sonora (ASU, ARIZ), JF787466, JF787602, JF787304, JF712842, JF786869, —, —, JF787025; ***Salmiopuntia salmiana*** (Parmentier ex Pfeiffer) Guiggi, HBG 18366, RBG 2000-1099, JF787432-33, JF787578-79, JF787278-79, JF712815-16, JF786842-43, JN387188-89, JN387253-54, JF786998-99; ***Tacinga funalis*** Britton & Rose, —, —, —, —, AY042660, —; ***Tacinga inamoena*** (K.Schumann) Stuppy & Taylor, *L.C. Majure 3849*, United States, FL, cult., (FLAS); DBG 1997 0017, JF787467/JF787459, JF787619/—, JF787305-06/JF712843-44, JF786870-71, JN387201-02, — JN387201-02, —, JF787026-27; ***Tacinga palmadora*** (Britton & Rose) Stuppy & Taylor, DBG 1997 0392 01 Brazil, JF787460, JF787603, JF787307, JF712845, JF786872, JN387203, JN387267, JF787028; ***Tacinga saxatilis*** (F.Ritter) Stuppy & Taylor, *C. Hamann s.n.,* cult, DBG, JF787468, JF787620, JF787308, JF712846, JF786873, JN387204, JN387268, JF787029; ***Tunilla corrugata*** (Salm-Dyck) Hunt & Illiff, DBG 2001 0005; *Hunt 66371* (DES), JF787461-62,, JF787604-05, —, JF712847-48, JF786874-75, JN387205-06, JN387269-70, JF787030-31.