

THE EVOLUTION AND SYSTEMATICS OF THE
Opuntia humifusa COMPLEX

By

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To my amazing and ever-supportive parents, Terrence and Diana Majure, my incredible wife
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Relationships among major clades of Opuntieae and the circumscription of *Opuntia* s.s. were unresolved prior to this study. *Opuntia* s.s., as currently circumscribed, comprises 120-200 species, occurring natively throughout the Americas. The *Opuntia humifusa* species complex (OHC) is taxonomically misunderstood due to high morphological variability, frequent hybridization, and polyploidy. There is no comprehensive phylogeny of either the genus or the *O. humifusa* complex. The goal of this study was to reconstruct the phylogeny of Opuntieae to elucidate major clade relationships, divergence times, and the biogeographic history of *Opuntia* s.s. Evolutionary relationships and ploidal levels of the *Humifusa* clade [HC (including the OHC)] were assessed to provide the foundation for a taxonomic revision of the OHC. Based on sequence data, *Opuntia* s.s. forms a well-supported clade, including the genus *Nopalea*, and is sister to a clade containing *Tacinga* and *Brasiliopuntia*. *Opuntia* s.s. originated in the late Miocene in southern South America and then dispersed to North American deserts. Numerous taxa originating through reticulate evolutionary processes were discovered in *Opuntia* s.s. The HC originated in northeastern Mexico/southwestern United States in the late Pliocene or early Pleistocene. *Opuntia lilae* was resolved in *Tacinga* and transferred to that genus. Although placed in synonymy with *O. triacantha*, *O. abjecta* is not closely related, and was recognized as

a separate species. *Opuntia cubensis* and *O. ochrocentra* were found to be of hybrid origin derived from different parental taxa, and were thus considered distinct from one another.

Chromosome counts of the HC revealed that 66% of 277 accessions were polyploid and displayed a much larger distribution (from the southern United States to Canada) than diploid members [restricted to the southwestern (SW) and southeastern (SE) United States in presumed Pleistocene refugia]. The SW and SE diploids each formed strongly supported clades; however, many polyploids formed as a result of the union of members of the SE and SW clades.

Phylogenetic, cytological, and morphological analyses showed that the most widespread member of the OHC, *O. humifusa* s.l., was polyphyletic and is now recognized as several distinct taxa.

The taxonomic revision presented here includes seven species of the OHC.

CHAPTER 1 GENERAL INTRODUCTION

The Cactaceae are a well-supported clade endemic to the New World and consist of between 1438 (Hunt et al. 2006) and 1850 species (Nyffeler and Eggli 2010) of mostly stem succulents that produce characteristic short shoots, embedded within the long shoot (i.e., areoles), modified leaves in the forms of spines, and ovaries deeply embedded in stem tissue or pericarpels (Mauseth 2006). Two early-diverging clades, *Pereskia* and *Rhodocactus*, retain ancestral features (e.g., large photosynthetic leaves, lack of succulent stems, cymose inflorescences, basal placentation, superior ovaries) of the family (Edwards et al. 2005). Of primary importance, in terms of species diversity, are the two major subfamilies, Opuntioideae (349 species) and Cactoideae (1498 species) (Nyffeler and Eggli 2010a), which represent the most iconic growth forms within Cactaceae, exhibiting succulent photosynthetic stems, with a drastic reduction or even loss of long-shoot leaves (although, there are exceptions to this). Subfamily Opuntioideae is unique in producing small, hair-like retrorsely-barbed spines (i.e., glochids; Mauseth 2006) and seeds with a hard funicular girdle and funicular envelope covering the seed (Stuppy 2002). Tribe Opuntieae of Opuntioideae consists mostly of species with flattened stems and sympodial growth (*Opuntia* s.s., *Tunilla*), although, *Brasiliopuntia*, *Consolea*, and *Tacinga* demonstrate indeterminate growth to some extent (Anderson 2001, Taylor et al. 2002). The Opuntieae consists of seven genera, *Brasiliopuntia*, *Consolea*, *Miqueliopuntia*, *Opuntia* s.s., *Salmiopuntia*, *Tacinga*, and *Tunilla* (Majure et al. 2012a). Although Nyffeler and Eggli (2010) concluded that *Consolea* should be considered a synonym of *Opuntia* s.s., Majure et al. (2012a) demonstrated that the genus forms a well-supported clade and is evolutionarily divergent from *Opuntia* s.s. Evolutionary relationships among the genera of

Opuntieae are still unresolved and the actual circumscription of the culturally, economically, and medicinally important genus, *Opuntia* s.s., is undetermined.

Opuntia s.s. is one of the largest genera in Cactaceae, with around 180-200 species (Anderson 2001; Nyffeler and Eggli 2010a), and exhibits the widest distribution of any genus in Cactaceae, as it occurs from Canada to Argentina (Anderson 2001) in habitats ranging from tropical to subtropical dry forests, moderate deserts, and even temperate forests (Benson 1982). *Opuntia* also has been introduced throughout the world for use as a foodstuff for humans and animals and as ornamentals (Anderson 2001; Inglese et al. 2002; Nefzaoui and Salem 2002).

Opuntia is renowned for hybridization and polyploidy (Benson 1982; Pinkava 2002; Majure et al. 2012a,b; Majure et al. in review) and also for its morphological variability, wherein certain morphological characters expressed within an individual are closely linked to environmental factors (Benson 1982; Rebman and Pinkava 2001; Majure 2007). Species of *Opuntia* also are notoriously difficult to work with from herbarium specimens, as methods used to collect specimens are typically inefficient, leading to poor specimen preservation, and the complete loss of most taxonomically useful characters as a result of the succulent nature of the plants (Reyes-Agüero et al. 2007). *Opuntia* species also are poorly collected, as a consequence of the difficulties in specimen preparation (Rebman and Pinkava 2001), and their highly bothersome glochids and spines (Reyes-Agüero et al. 2007).

One poorly understood group within *Opuntia* is the *O. humifusa* complex of the eastern United States. This group is distributed over a wide range, from Ontario, Canada, south to the Florida Keys, and west to Wisconsin, Iowa, Missouri, Arkansas, and Texas (Benson 1982; Pinkava 2003; Majure et al. 2012b). Species within the *O. humifusa* complex are known to hybridize (Benson 1982), contain numerous polyploid entities (Majure et al. 2012b), and are

poorly represented in herbaria (Majure and Ervin 2008), which has provided for a taxonomically complex history and nebulous species limits. Thus, the *O. humifusa* complex presents an opportunity to explore species boundaries, consequences of polyploidization and hybridization, and evolutionary history.

The primary goals of this study were:

- to determine the circumscription, date of origin, and biogeographic history of *Opuntia* s.s., as well as the limits of the *Humifusa* clade (including the *O. humifusa* complex);
- to clarify the phylogenetic placement of *O. lilae* and the morphological synapomorphies of the genus *Tacinga*;
- to clarify the phylogenetic placement and taxonomic status of *O. abjecta* and *O. triacantha*, and determine the origin of putative hybrids, *O. cubensis* and *O. ochrocentra*;
- to carry out chromosome counts for members of the *O. humifusa* complex and relate ploidy to historical biogeography and the formation of polyploid taxa;
- to reconstruct the phylogeny of the *Humifusa* clade, with an emphasis on determining the origins of the many polyploid taxa in the group;
- to produce a taxonomic revision of the *O. humifusa* species complex.

These goals are discussed in the following seven chapters. In Chapter 2, I reconstruct the phylogeny of Opuntieae, determine relationships among the genera of Opuntieae, provide a circumscription of *Opuntia* s.s. and the *Humifusa* clade, and use the phylogeny to test the origin of the many polyploids in the genus, as well as the biogeographic history and divergence dates of *Opuntia* s.s.

In Chapter 3, I use previously gathered data to build a phylogeny of members of the Opuntieae and determine the evolutionary placement of *O. lilae*. I then use the phylogeny to determine which morphological characters may be synapomorphic for the genus *Tacinga*, and formally transfer *O. lilae* to *Tacinga*.

In Chapter 4, I reconstruct the phylogeny of several clades of *Opuntia* s.s. This work shows that *O. triacantha* is not monophyletic, as currently circumscribed, and consists of several taxa: *O. abjecta*, *O. militaris*, and *O. triacantha*. *Opuntia cubensis* and *O. ochrocentra* are shown to be of hybrid origin, but they are derived from different parental species and should not be considered synonymous.

In Chapter 5, I report chromosome numbers for 277 accessions of members of the *Humifusa* clade and determine that the origin of many polyploids in the group is most likely the result of hybridization between the two diploid clades of the *Humifusa* clade at the end of the Pleistocene.

In Chapter 6, I reconstruct the phylogeny of the *Humifusa* clade and use the diploid topology to discover hybrid, polyploid derivatives from the union of diploid members of the two subclades, SE and SW, of the *Humifusa* clade. The widespread *O. humifusa* s.l. also is determined to be polyphyletic and should be recognized as several taxa.

In Chapter 7, I present a taxonomic revision of the *O. humifusa* complex of eastern North America, in which I recognize seven species (i.e., *O. abjecta*, *O. austrina*, *O. cespitosa*, *O. drummondii*, *O. humifusa*, *O. nemoralis*, and *O. ochrocentra*) and three infraspecific taxa within *O. humifusa* (var. *humifusa*, var. *lata*, and var. *pollardii*).

In Chapter 8, I provide general conclusions about the phylogenetic structure of Opuntieae, *Opuntia* s.s., the *Humifusa* clade, and the *O. humifusa* complex, as well as information discovered (through this study) about reticulate evolution and polyploidy in these groups.

CHAPTER 2

PHYLOGENY OF *Opuntia* S.S. (CACTACEAE): CLADE DELINEATIONS, GEOGRAPHIC ORIGINS, AND RETICULATE EVOLUTION

Background

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 2010b), 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 CO₂ 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

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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). 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 (platyopuntiods) 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 platyopuntiods 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 numerous 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. 2012b; 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. 2012b; 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. 2012b).

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. 2012b), 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.

Material 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 A.

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*, *psbJpetA*, 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 2-1). A sequence of *matK* for *Tacinga funalis* Britton & Rose was downloaded from GenBank (Appendix A). Mixtures for 25- μ L amplification reactions were as follows: 0.5 – 1 μ L of template DNA, 9.4 μ L H₂O, 5 μ L of 5 \times buffer, 2.5 μ L of 25 mmol/L MgCl₂, 1 μ L of 2.5 mmol/L dNTPs, 2 μ L betaine, 2 μ L each 5 μ mol/L primer, and 0.1 μ L *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-AI v2.0 (Rambaut 2007). All gaps introduced during alignment were coded as missing data.

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 $\geq 70\%$ 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 (\pm 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 (\pm 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 2-2), 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.

Combining the diploid data sets resulted in well-supported clades in the diploids-only analysis (Fig. 2-1). 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; Fig. 2-5 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. 2-2). 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] $\geq 70\%$) and agrees with the diploid topology, but species relationships within subclades are generally poorly supported (bs $\leq 50\%$; Fig. 2-3). 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 Fig. 2-5. However, plastid data resolved *Consolea* outside of *Opuntia* s.s. (bs = 53%), and ITS data placed *Consolea* within *Opuntia* s.s. (bs = 75%; Fig. 2-5), 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 (Fig. 2-6). *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. 2-1, 2-3), 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. 2-1). 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 deceptively 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. 2-1).

Interclade Allopolyploids and Hybrids

We recovered 24 interclade-derived taxa. Of these, 20 are inferred to be allopolyploids (4x, 5x, 6x, 8x, and 9x), and one is an interclade homoploid hybrid (Table 2-3). 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 2-3; Fig. 2-2).

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. 2-2). 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 2-3).

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* 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. 2-3) and *O. pinkavae* as sister to *O. erinacea* (Fig. 2-3). 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

(Fig. 2-5, 2-6), 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. 2-4). 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. 2-4). 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. 2012b).

Our divergence time estimates suggest that the North American clade originated 5.12 (\pm 1.6) Ma (Fig. 2-7), 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 (Fig. 2-7).

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 (Fig. 2-5). *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 (Fig. 2-5). 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 (Fig. 2-6), 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. 2-1). 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 disjct. 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 2010a), its sister taxon in our analyses.

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. caracasana*, *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 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. 2-1); 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. 2-4). 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 disjct. 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 disjct.s 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).

There are other well-known examples of similar floristic disjuncts 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 disjuncts 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 disjuncts 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 (\pm 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-Agüero 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. 2-2). 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. 2-2). This was unexpected, as several South American taxa (e.g., *O. bisetosa*, *O. boldinghii*, *O. pittieri*, *O. schumannii*) 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 woolly 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. 2012b); samples of different cytotypes are sometimes morphologically similar and form clades (e.g., *O. pusilla*; Fig. 2-3), 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.

Summary

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.

Table 2-1. DNA regions and associated primers used in this study.

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

Table 2-2. 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
<i>atpB-rbcL</i>	861	20	HKY
<i>matK</i>	905	27	F81+I+G
<i>ndhF-rpl32</i>	1699	43	GTR+I+G
<i>psbJ-petA</i>	1169	72	K91uf+l
<i>trnL-F</i>	441	14	K81uf
<i>ycf1</i>	873	51	K81uf+l+G
ITS	599	39	TVM+G
<i>ppc</i>	469	37	HKY+G
cpDNA combined	5948	227	—
nuclear combined	1068	76	—
All Combined	7016	303	—

Table 2-3. Interclade derived taxa recovered in our analyses. Ploidy is given for each species where known based on Majure et al. (submitted).

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

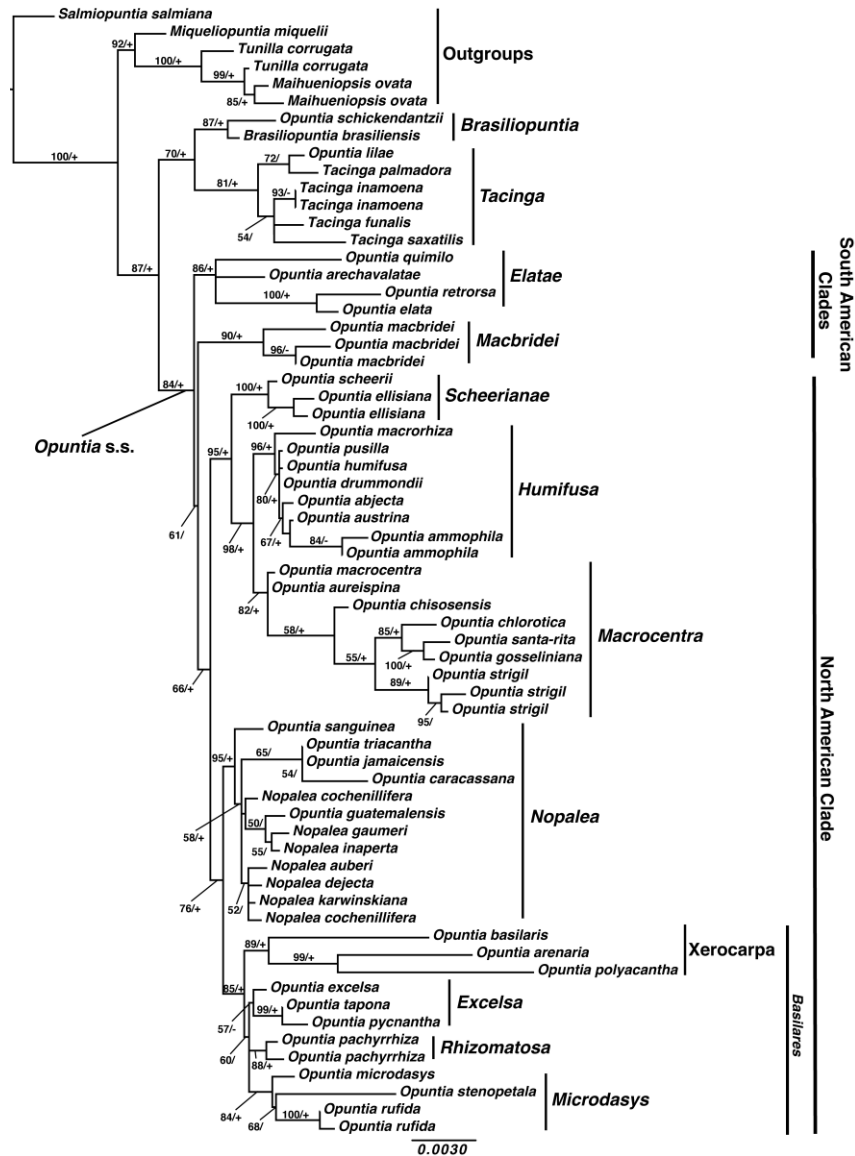


Figure 2-1. Diploid phylogeny of *Opuntia* s.s. Most likely topology from our RAxML run with 10 000 bootstrap pseudoreplicates using our combined nuclear (ITS and *ppc*) and plastid data set for diploid taxa only (i.e., all presumably polyphyletic taxa excluded). *Opuntia schickendantzii* is resolved 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.

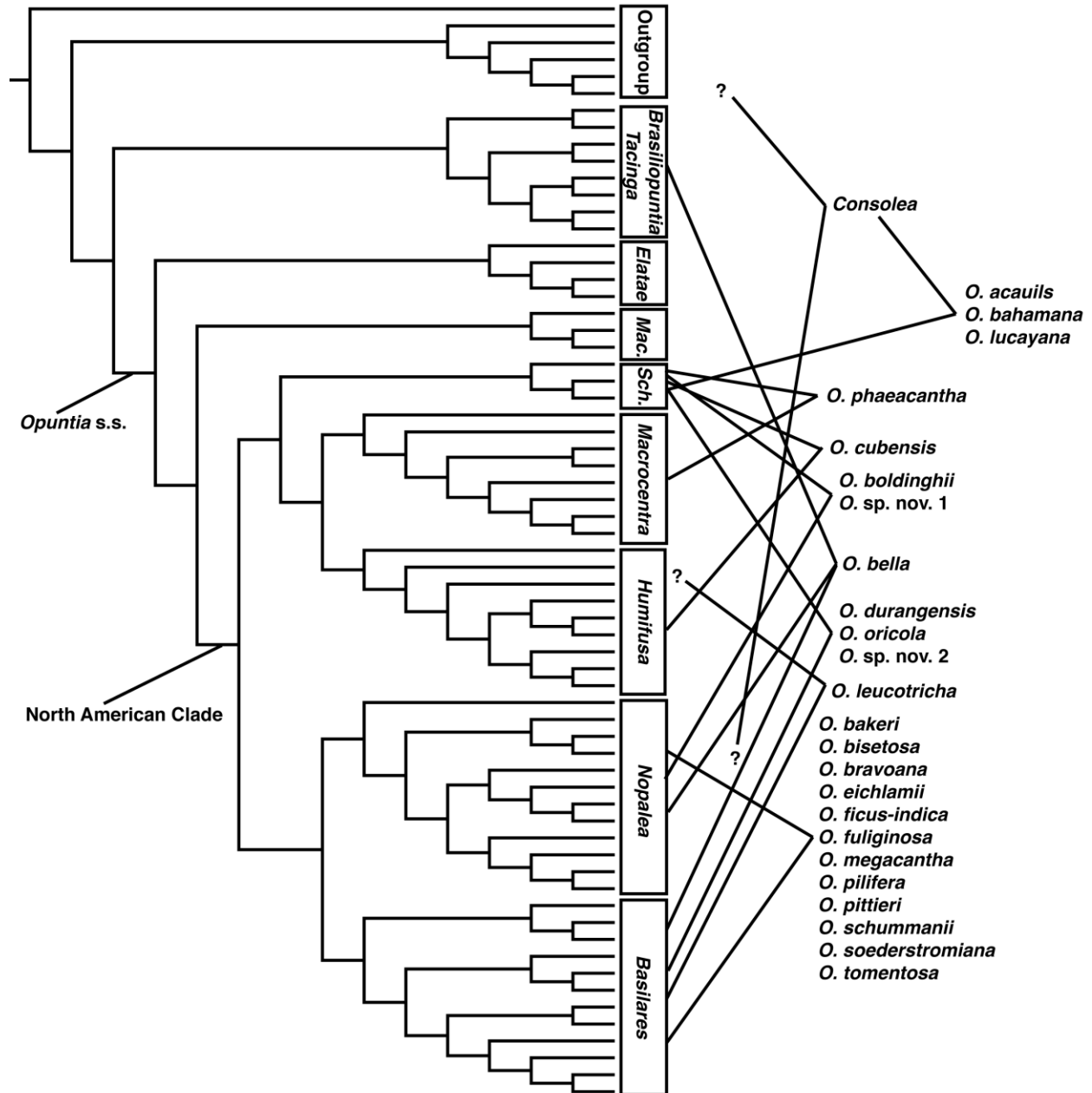


Figure 2-2. Diploid phylogeny of *Opuntia* s.s. (adapted from Fig. 2-1) with interclade reticulate taxa mapped on their putative diploid progenitor clades. We did not discover any interclade taxa derived from the South American *Elatae* or *Macbridei* clades. Instances where putative progenitors of inferred 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.

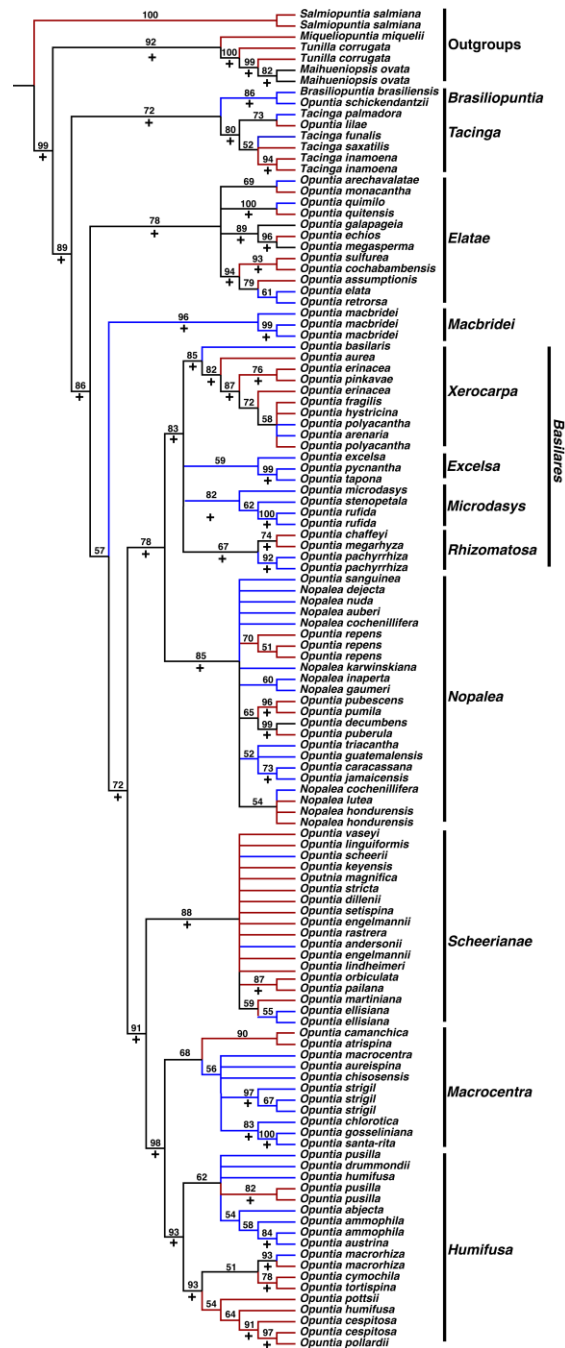


Figure 2-3. Intraclade phylogeny of *Opuntia* s.s. (total evidence phylogeny excluding interclade derived taxa). The 50% majority rule consensus tree from a RAxML analysis of 10 000 rapid bootstrap pseudoreplicates using our combined nuclear and plastid data set for all diploid taxa (blue) and intraclade 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 (+).

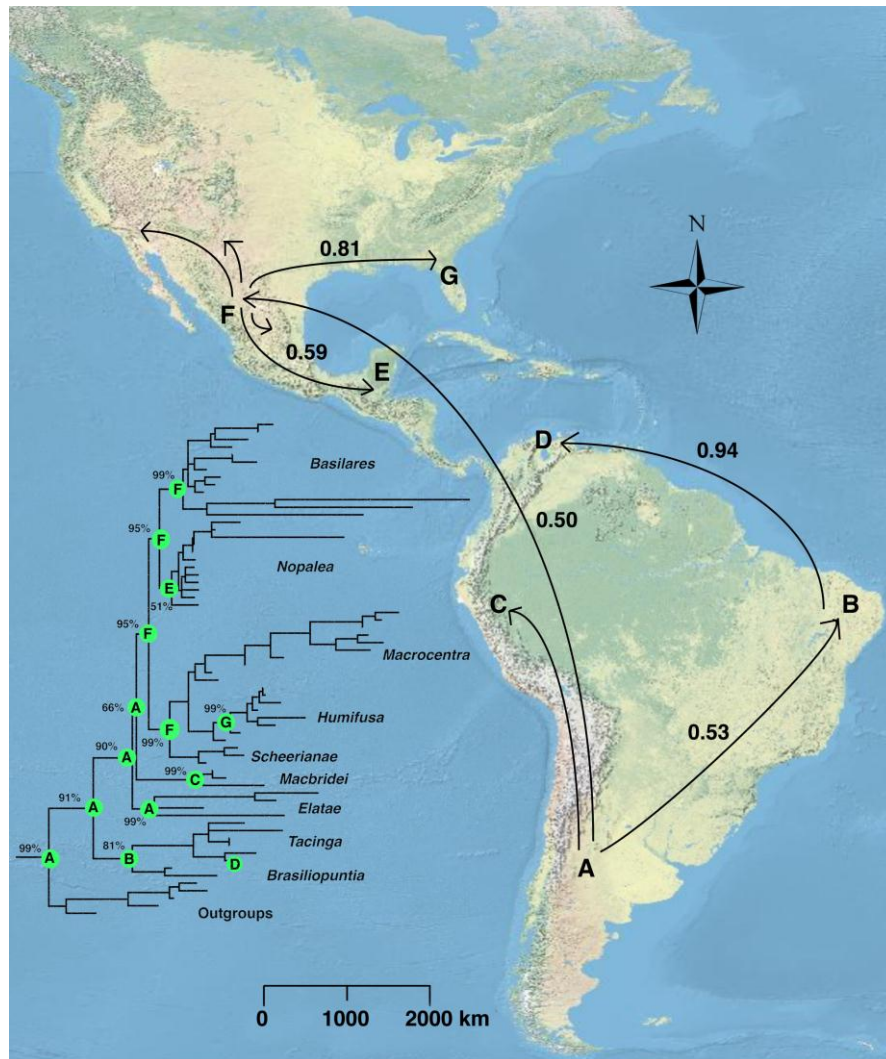
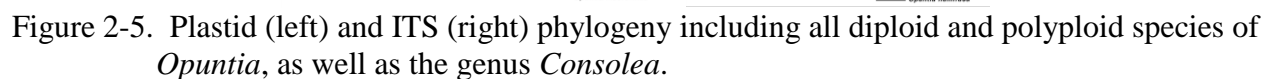


Figure 2-4. Ancestral area reconstruction and putative dispersal pathways of *Opuntia* clades. Ancestral reconstructions are represented as (A) southwestern South America, (B) Caatinga, (C) Central Andean valleys, (D) northern South America (Caribbean Region), (E) tropical dry forests (Mexico, Central America, Caribbean), (F) western North American desert region, and (G) southeastern United States. Proportional likelihoods are given for each node in the phylogeny. Dispersal probabilities 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).



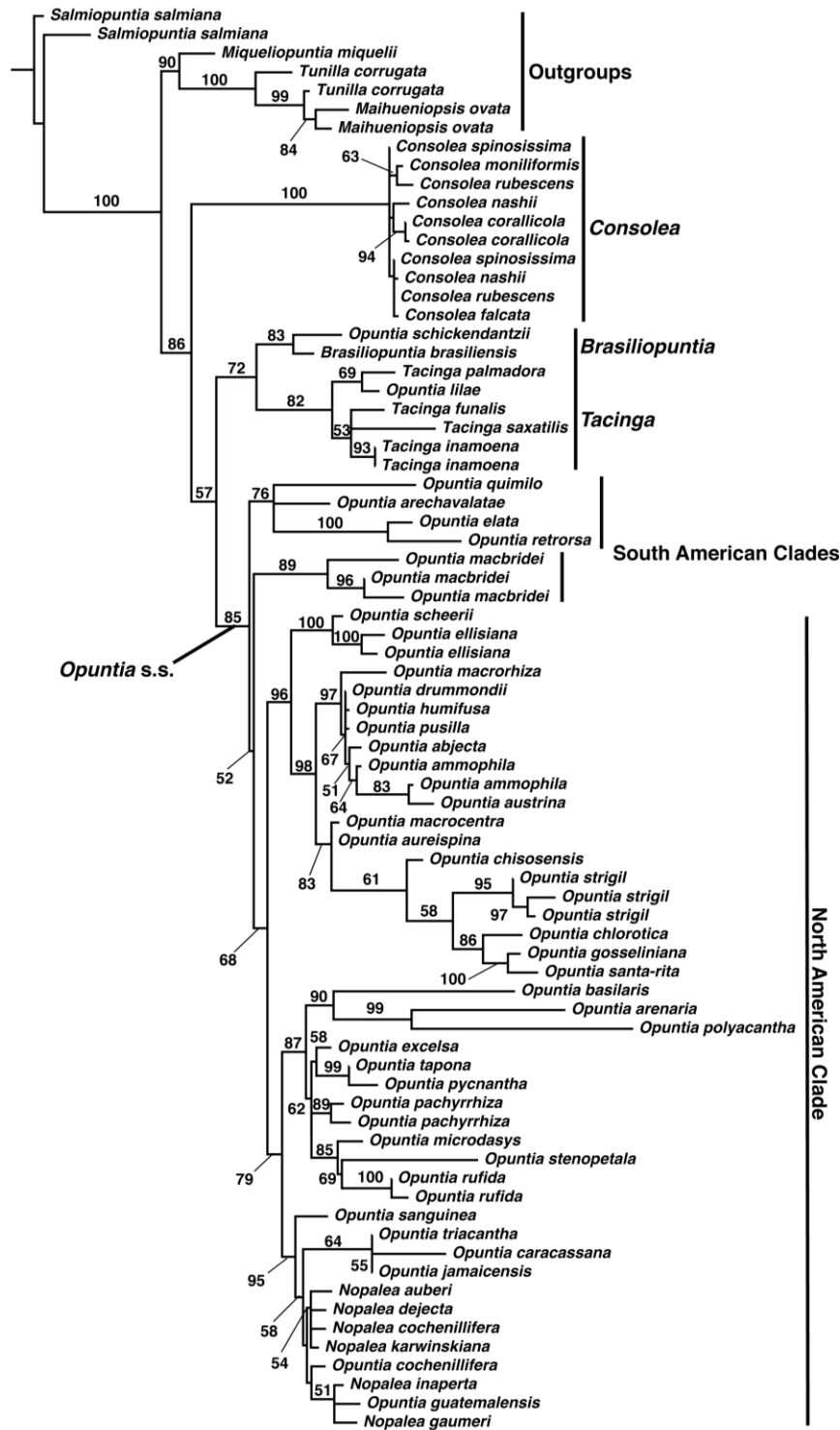


Figure 2-6. Diploid phylogeny including the genus *Consolea*. *Consolea* is resolved as sister to the *Tacinga*, *Brasiliopuntia*, *Opuntia* s.s. clade.

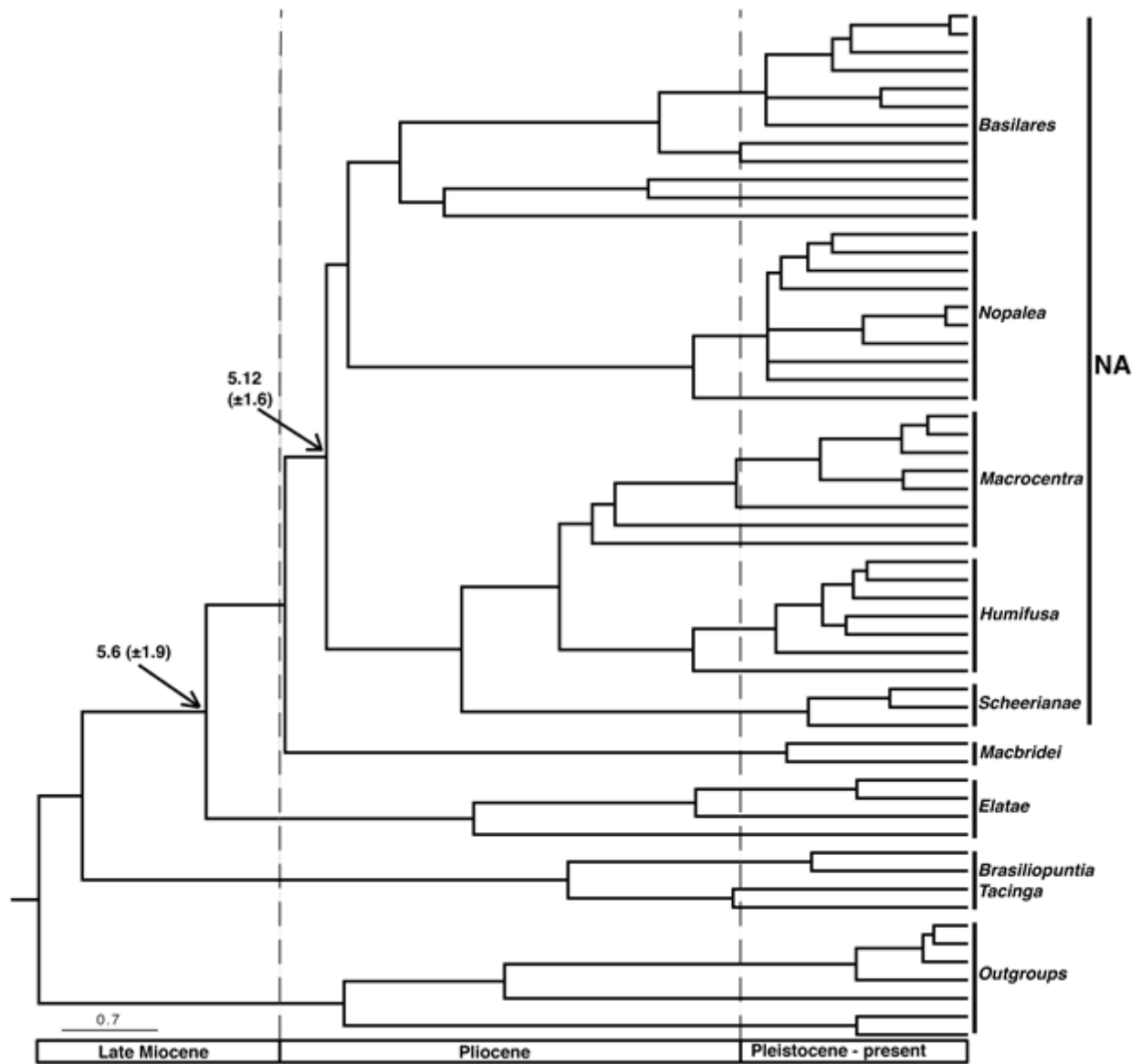


Figure 2-7. Chronogram from r8s analysis showing an early Pliocene origin of the North American clade of *Opuntia*.

CHAPTER 3
Opuntia lilae, ANOTHER *Tacinga* HIDDEN IN *Opuntia* S.L. (CACTACEAE)

Background

Tribe Opuntieae within subfamily Opuntioideae of Cactaceae consists of *Brasiliopuntia* A. Berger, *Consolea* Lem., *Miqueliopuntia* Frič ex F. Ritter, *Opuntia schickendantzii* F. A. C. Weber, *Opuntia* Mill. s.s., *Salmiopuntia* Frič ex Guiggi, *Tacinga* Britton & Rose, and *Tunilla* D. R. Hunt & Iliff (Wallace and Dickie 2002; Griffith and Porter 2009; Majure et al. 2012a). Most of the aforementioned genera were historically included in *Opuntia* s.l. (Britton and Rose 1920), but have recently been segregated based on morphological and molecular data (Stuppy 2002; Wallace and Dickie 2002). Britton and Rose (1920), however, separated the distinctive genus *Tacinga* from their broadly circumscribed *Opuntia* s.l.

At the time of its description by Britton and Rose (1920) *Tacinga* included only one species, *T. funalis* Britton & Rose, from Bahía, Brazil. This species was discovered in the characteristic dry caatinga (spelled catinga in Britton and Rose 1920) vegetation and was thus named for it using the anagram *Tacinga* (Britton and Rose 1920). Molecular phylogenetic analyses have increased the size of the genus, showing that species previously considered part of *Opuntia* s.l. are more closely related to members of *Tacinga* than to other species of *Tacinga* (e.g., *T. inamoena* (K. Schum) N. P. Taylor & Stuppy, *T. palmadora* (Britton & Rose) N. P. Taylor & Stuppy, *T. saxatilis* (F. Ritter) N. P. Taylor & Stuppy, *T. weneri* (Eggli) N. P. Taylor & Stuppy; Wallace and Dickie, 2002). Taylor et al. (2002) subsequently transferred those species to *Tacinga*, which now includes seven species (including *T. braunii* Esteves and *T. subcylindrica* (M. Machado & N. P. Taylor) M. Machado & N. P. Taylor; see Lambert 2009 and Menezes et al. 2011) and one hybrid taxon, *T. x quipa* (F. A. C. Weber) Taylor & Stuppy (Taylor et al. 2002). Morphological analyses of those taxa have also illuminated potential synapomorphies for

members of *Tacinga* (Stuppy 2002), which distinguish members of this clade from *Opuntia* s.s. and other genera in Opuntieae.

As found in some other members of Opuntieae (e.g., *Consolea*, *Nopalea* of *Opuntia* s.s.), species of *Tacinga* produce hummingbird-pollinated flowers with tepals varying in color from green (greenish-white) to red or orange. The tepals are spreading to recurved in some species (e.g., *T. funalis*, *T. inamoena*), but may be erect and form a tube in other members of the clade (e.g., *T. palmadora*, *T. weneri*). Stamens are athigmonastic, and in some taxa one or two rows of staminodia are produced (Stuppy 2002; Lambert 2009). The fruits of *Tacinga* have a characteristic deep and narrow umbilicus (Stuppy 2002). Members of *Tacinga* also develop a hook-shaped embryo, unlike the coiled embryo of *Opuntia* s.s., and have relatively reduced perisperm formation relative to the small embryo size, as compared to the very reduced perisperm and large embryo size in *Opuntia* s.s. (Anderson 2001; Stuppy 2002). Species of *Tacinga* produce seeds with a hairy funicular envelope, and some species display indeterminate growth, two characters shared by other members of Opuntieae (*Brasiliopuntia*, *Consolea*).

Trujillo and Ponce (1990) and Fernández (1995) considered *O. lilae* Trujillo & Ponce to represent part of *Opuntia* series *Tunae* of Britton and Rose (1920), although Trujillo and Ponce (1990) mention that the species does not have the easily disarticulating cladodes of the other members of that series. The geographic location of *O. lilae* in the northeastern portion of Venezuela and the proximity to the Caribbean, where other members of series *Tunae* occur (e.g., *O. caracassana* Salm-Dyck, *O. triacantha* (Willd.) Sweet), presumably were influential in decisions regarding relationships to other taxa. *Opuntia bella* Britton & Rose from Colombia, which was placed in series *Tunae* by Britton and Rose (1920) and shares characters with *O. lilae* (erect tepals, included stamens at anthesis), is suggested to be an intergeneric hybrid between

Opuntia s.s. and *Tacinga* (Majure et al. 2012a). The morphological similarity of *O. bella* and *O. lilae* may also have encouraged Trujillo and Ponce to include *O. lilae* in the series *Tunae*, as no other members of the series share obvious morphological characters with *O. lilae*.

Recent molecular analyses of *Opuntia* s.s. using plastid and nuclear sequence data revealed that *O. lilae* is most closely related to members of the *Tacinga* clade (Majure et al. 2012a). Here we reconstruct the phylogeny showing the relationship of *O. lilae* with *Tacinga* and use the phylogeny to search for putative synapomorphies of the *Tacinga* clade. We then describe morphological apomorphies of *O. lilae* (based on the type collection of *O. lilae*), which are shared with other members of the *Tacinga* clade.

Materials and Methods

Taxon Sampling and Phylogenetic Analysis

We performed a phylogenetic analysis including four species of *Tacinga* (*T. funalis*, *T. inamoena*, *T. palmadora*, *T. saxatilis*), *Brasiliopuntia*, *Opuntia lilae*, *O. schickendantzii*, and three species of South American *Opuntia* s.s., *O. arechevalatae* Spegazzini, *O. macbridei* Britton & Rose, and *O. quimilo* K. Schum. *Miqueliopuntia miquelii* (Monv.) F. Ritter, *Tunilla corrugata* (Salm-Dyck) D. R. Hunt & Iliff, and *Salmiopuntia salmiana* (J. Parm. ex Pfeiff.) Guiggi were used as outgroups. Our molecular data and morphological observations of *O. lilae* were based upon the holotype (see also Appendix B). We used previously gathered data from Majure et al. (2012a); the plastid intergenic spacers, *atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, *trnL-F*, plastid genes, *matK* and *ycf1*, the nuclear gene *ppc*, and the nuclear ribosomal internal transcribed spacers (ITS). A sequence of *matK* for *T. funalis* was downloaded from GenBank (see Appendix B). We performed a maximum likelihood analysis of combined plastid and nuclear data sets (see Majure et al. 2012a), however, with the reduced taxon data set described above, in RAxML (Stamatakis

2006) implementing 10000 rapid bootstrap (bs) pseudoreplicates under 25 rate categories using the GTR+ Γ model of molecular evolution.

Ancestral State Reconstruction

We coded the following 11 morphological characters for those taxa used in our phylogenetic analysis: 1) growth determinate vs. indeterminate, 2) pollination syndrome, i.e., insect vs. hummingbird, 3) embryo shape coiled vs. hooked, 4) funicular envelope with or without hairs, 5) stomata placement at the epidermal surface or \pm sunken vs. raised above the surface, 6) pollen exine condition, bullate, reticulate, punctate, or tectate-punctate, 7) perisperm production in relation to embryo size, greatly reduced vs. reduced, 8) umbilicus shallow and broad vs. deep and narrow, 9) bud shape acute vs. compressed, 10) stamen movement absent vs. present, and 11) staminodia absent or present.

We performed an ancestral state reconstruction in Mesquite v. 2.73 (Madison and Madison 2010) using maximum likelihood (ML) and maximum parsimony (MP) methods with unordered states to determine which characters exhibited by *O. lilae* may represent likely synapomorphies of the *Tacinga* clade. Under ML we implemented the Mk1 model of evolution, 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). We also cross-compared morphological characters of the type collection of *O. lilae* with other members of the *Tacinga* clade to support likely species relationships with regard to the phylogeny.

Results

Phylogenetic and Morphological Analysis

Opuntia lilae is well supported (bs = 92) as a member of the *Tacinga* clade. The phylogeny suggests a close relationship of *O. lilae* with *T. palmadora* (Fig. 3-1). Unlike members of the hummingbird-pollinated *Opuntia* clade, *Nopalea*, which develop acute flower

buds (Fig. 3-2A), *O. lilae* has the typical compressed flower bud apex (Fig. 3-2B) of most *Tacinga* species (e.g., *T. inamoena*, *T. palmadora*, *T. saxatilis*, *T. weneri*). However, *Tacinga braunii* and *T. funalis* have relatively long, acute flower buds (see photos in Taylor et al. 2002; Lambert 2009). The flowers of *O. lilae* have a tubular, orange-red corolla (Fig. 3-2B-D), much like that of *T. palmadora* or *T. weneri* (Anderson 2001; Taylor et al. 2002; Lambert 2009). Analysis of the fruit shows the characteristic deep, narrow, umbilicus of other *Tacinga* species (Fig. 3-2F), hairy seeds, a strongly hooked embryo, and reduced perisperm development (Fig. 3-2H), of other *Tacinga* species, as compared to *Opuntia* s.s. (Fig. 3-2G). *Opuntia lilae* also shows the characteristic tectate-punctate pollen (not shown) of other *Tacinga* species (Taylor et al. 2002).

Ancestral State Reconstruction

Of the 11 characters used in our analysis, four of those appear to represent synapomorphies for the *Tacinga* clade under both ML and MP methods (Fig. 3-2). Species of the *Tacinga* clade have: 1) hook-shaped embryos with 2) reduced perisperm, 3) raised stomata (as evident in *Opuntia lilae*, *Tacinga inamoena*, *T. palmadora*, and *T. saxatilis*), and 4) a deep, narrow umbilicus. These features contrast with the coiled embryos with greatly reduced perisperm, and superficial or sunken stomata of *Opuntia* s.s., *Brasiliopuntia*, and *Opuntia schickendanztii*. In general, other members of Opuntieae have relatively shallow and broader umbilici than those of *Tacinga* (Fig. 3-3).

Although, all species of *Tacinga* studied thus far have tectate-punctate pollen, members of *Opuntia* s.s. also have punctate pollen, which may either be finely tectate-punctate, as in members of the *Nopalea* clade (sensu Majure et al. 2012a), or punctate with large slit-like apertures, as in *O. macbridei* and *O. stenopetala* (see Leuenberger 1976). The switch from reticulate to punctate pollen in *Opuntia* s.s. appears to be related to shifts to hummingbird

pollination, as those *Opuntia* exhibiting punctate pollen are generally hummingbird-pollinated (e.g., *O. stenopetala*, members of the *Nopalea* clade). Interestingly, the finely tectate-punctate pollen of some species of *Tacinga* (e.g., *T. funalis*, among those studied) and of the *Nopalea* clade of *Opuntia* s.s. is strongly associated with hummingbird pollination and the possession of flowers with greatly exerted stamens and stigmas. However, other species of *Tacinga* (*O. lilae*, *T. inamoena*, *T. palmadora*, *T. weneri*) and *Opuntia* s.s. (e.g., *O. macbridei*, *O. stenopetala*) exhibit coarsely tectate-punctate pollen and have flowers that may be more commonly visited by both hummingbirds and insects. This variational pattern highlights the fact that pollen characters in Opuntieae are homoplasious and likely dependent on pollination syndrome.

Seeds with a hairy funicular envelope are plesiomorphic in *Tacinga* as, according to our analyses, the most recent common ancestor of Opuntieae is reconstructed as having hairy seeds. Hairy seeds have subsequently been lost in other groups (certain *Opuntia* s.s., *Tunilla*) but were retained in *Tacinga*. Bud shape, growth form, pollination syndrome, the presence of staminodia, and athigmonastic stamens are apparently homoplasious characters, as they have been derived separately in other lineages of Opuntieae. Even the *Tacinga* clade shows a shift in bud shape: from generally compressed in most members to acute in *T. funalis* and *T. braunii*. *Tacinga* also exhibits variation in growth form, with some species showing determinate growth (e.g., *O. lilae*, *T. inamoena*, *T. saxatilis*) and others indeterminate growth (e.g., *T. braunii*, *T. funalis*, *T. palmadora*), and staminodia are lacking in most members of the genus, but have been acquired in *T. braunii*, *T. funalis* and *T. weneri* (Taylor et al. 2002). Considering our phylogeny, indeterminate growth and staminodia evolved twice in *Tacinga*. However, a more complete taxon sampling (i.e., including *T. braunii*, *T. subcylindrica*, and *T. weneri*) and a well-resolved phylogeny are necessary to fully explore character shifts in the clade.

Discussion

It is clear not only from our molecular phylogenetic analyses, but also from our morphological investigation that *O. lilae* from northeastern Venezuela, is a member of the *Tacinga* clade. *Opuntia lilae* has a hook-shaped ovary (Fig. 3-2A, Fig. 3-3H) with reduced perisperm relative to embryo size (Fig. 3-2B, Fig. 3-3H) and a very deep umbilicus (Fig. 3-2D, Fig. 3-3F), as compared to most species of *Opuntia* s.s.; all of these are synapomorphic for the *Tacinga* clade. *Opuntia lilae* has a characteristic epidermis, which feels like sand paper to the touch. Fernández (1995) showed this to be the result of raised stomata (see Figs. 1-5 in Fernández 1995). *Tacinga inamoena*, *T. palmadora*, and *T. saxatilis* also have raised stomata, but we have not examined this character in other members of the *Tacinga* clade (Fig. 3-2C). Stomata are superficial or slightly sunken in *Brasiliopuntia*, *Opuntia schickendanztii*, *Salmiopuntia*, and species of *Opuntia* s.s. that have been surveyed (Eggli 1984, Majure unpubl. data). Thus, at least based on the taxon sampling of our analysis, this distinctive anatomical condition is also an apomorphy of *Tacinga*.

Opuntia lilae also possesses erect, orange-red tepals forming a tube, included stamens (unlike hummingbird-pollinated *Nopalea* of *Opuntia* s.s., which has exerted stamens), and a hairy funicular envelope, as do other species of *Tacinga* (Stuppy 2002; Taylor et al. 2002). *Brasiliopuntia*, *Consolea*, and some species of *Opuntia* s.s. also have a hairy funicular envelope (Stuppy 2002), so although characteristic of *Tacinga*, this character is not synapomorphic for the genus (see Results).

Opuntia lilae appears to be most closely related to *T. palmadora*, and likely also *T. weneri*, considering vegetative morphology, floral characters, and the close relationship of *T. palmadora* and *O. lilae* in our phylogeny. The yellowish spines of *O. lilae* (Fig. 3-2I) suggest a closer relationship with *T. palmadora* than to *T. weneri*. The hexaploid chromosome count of *O.*

lilae (Majure et al. in review), however, differs from the morphologically similar but diploid *T. palmadora* (de Castro 2008), suggesting that *O. lilae* and *T. palmadora* are likely not conspecific. Also, *O. lilae* does not show the indeterminate growth displayed by *T. palmadora* (Taylor et al. 2002). Ploidy of *T. wernerii* also needs to be determined, and detailed morphological comparisons of all three taxa need to be undertaken. Whether or not *O. lilae* is derived from hybridization and subsequent genome duplication or merely intraspecific genome duplication also requires further study.

Tacinga had been considered to be endemic to Brazil (Taylor et al. 2002; Lambert 2009); however, the distribution of this clade must now be extended to northern Venezuela, a disjunction of ca. 3,285 km from the northwesternmost population of *Tacinga* (*T. palmadora*) in Pernambuco, Brazil, based on distributions given by Taylor et al. (2002). Divergence time estimates and ancestral area reconstructions suggest that *Tacinga* originated in Brazil during the Pliocene and subsequently dispersed to northern South America (Majure et al. 2012a). *Tacinga* may have once possessed a contiguous distribution from eastern Brazil to at least the western cordillera of Colombia, where the inter-clade hybrid between *Opuntia* s.s. and *Tacinga*, *O. bella*, is found (Britton and Rose 1920; Majure et al. 2012a). Alternatively, *Tacinga* dispersed to Venezuela and Colombia, becoming an apocendemic taxon (sensu Stebbins 1971) in Venezuela and hybridizing with *Opuntia* in Colombia (Majure et al. 2012a). Sarmiento (1975) noted numerous floristic similarities (i.e., shared genera) between the caatinga and Caribbean dry region of Venezuela and suggested that these two areas may have once been closely linked by a broader dry region spanning the two areas or that the vegetation of both areas may be derived from a common origin. The presence of *O. lilae* in Venezuela would suggest a common origin scenario for certain vegetational elements in these two areas, although most *Opuntia* s.s. species

found in Venezuela (e.g., *O. boldinghii*, *O. caracassana*) are derived from North American clades (Majure et al. 2012a) and not those from South America.

Trujillo and Ponce (1990) stated that *O. lilae* is naturally rare in Venezuela and is known from only two states, Lara and Sucre. *Opuntia lilae* is known to produce vegetative propagules (i.e., to demonstrate proliferous growth) even from mature fruit (Trujillo and Ponce 1990) that contain apparently viable seeds, so the species regularly produces vegetative clones from parent plants. The role of sexual reproduction, however, is not known, but is suggested to be low (Trujillo and Ponce 1990). More research is warranted to determine whether or not apomixis may play a role in seed formation in this uncommon, highly clonal, hexaploid species. Also, better taxon sampling will be necessary to determine how closely related *O. lilae* is to *T. palmadora*, *T. weneri*, or other members of the *Tacinga* clade. Phylogenetically based morphological analyses, including all *Tacinga* species, will be necessary to fully evaluate morphological character shifts within the clade.

Because, as discussed above, our molecular and morphological phylogenetic analyses strongly support the transfer of *Opuntia lilae* to the genus *Tacinga*, we provide the following new combination. *Tacinga lilae* (Trujillo and M. Ponce) Majure and R. Puente comb. nov. Basionym: *Opuntia lilae* Trujillo and M. Ponce, *Ernstia* 58-60: 1 1990.

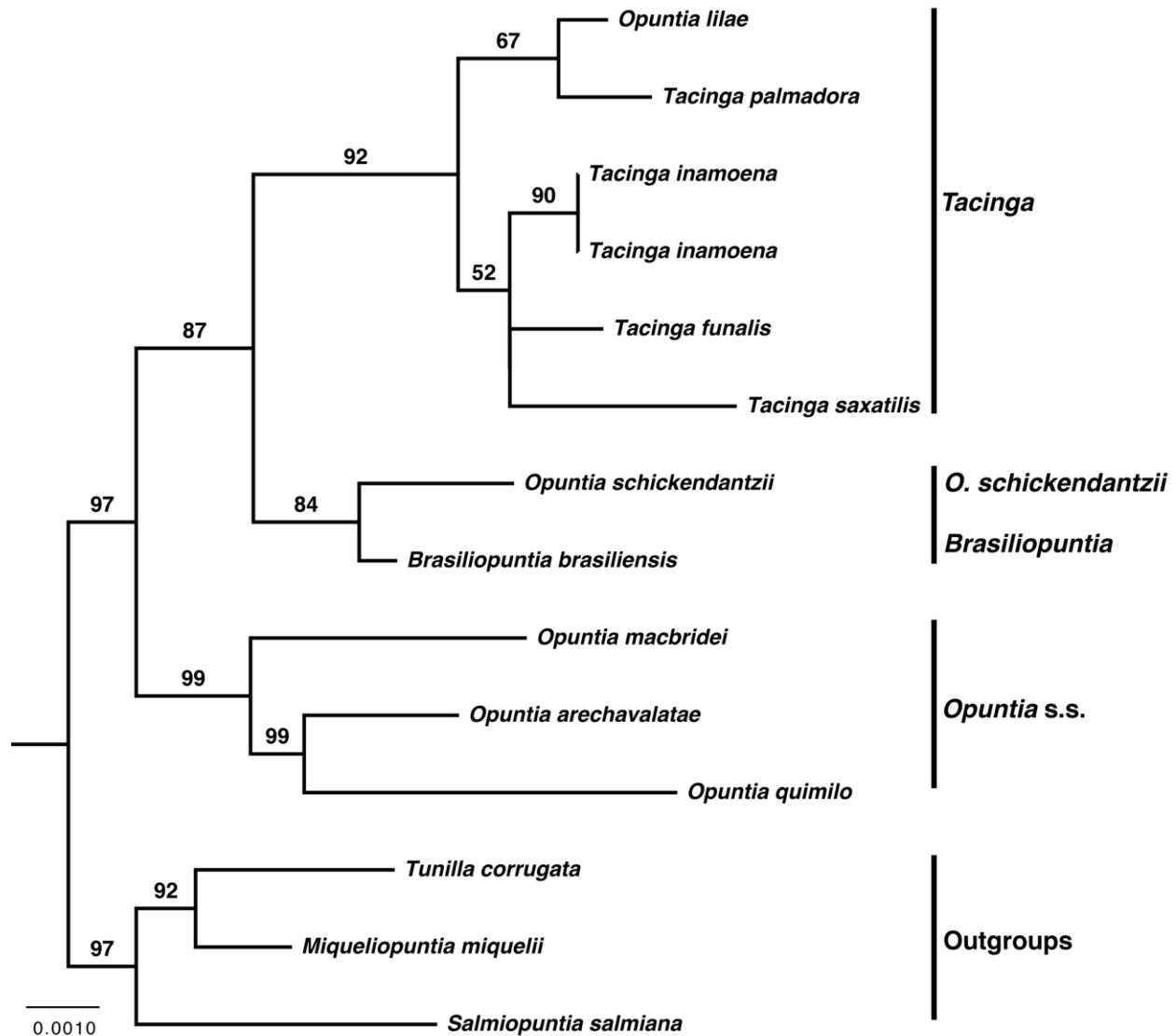


Figure 3-1. Phylogram of *Tacinga* and other members of Opuntieae from a combined analysis of nuclear and plastid loci in RAxML carrying out 10000 bootstrap pseudoreplicates under 25 rate categories and implementing the GTR+ Γ model of molecular evolution. Bootstrap values are given above the branches. *Opuntia lilae* is resolved as sister to *Tacinga palmadora* in the well supported *Tacinga* clade. Low bootstrap support and resolution for the subclade of *Tacinga* containing *T. funalis* likely is the result of lack of data (i.e., only *matK* was available for *T. funalis*) and taxon sampling.

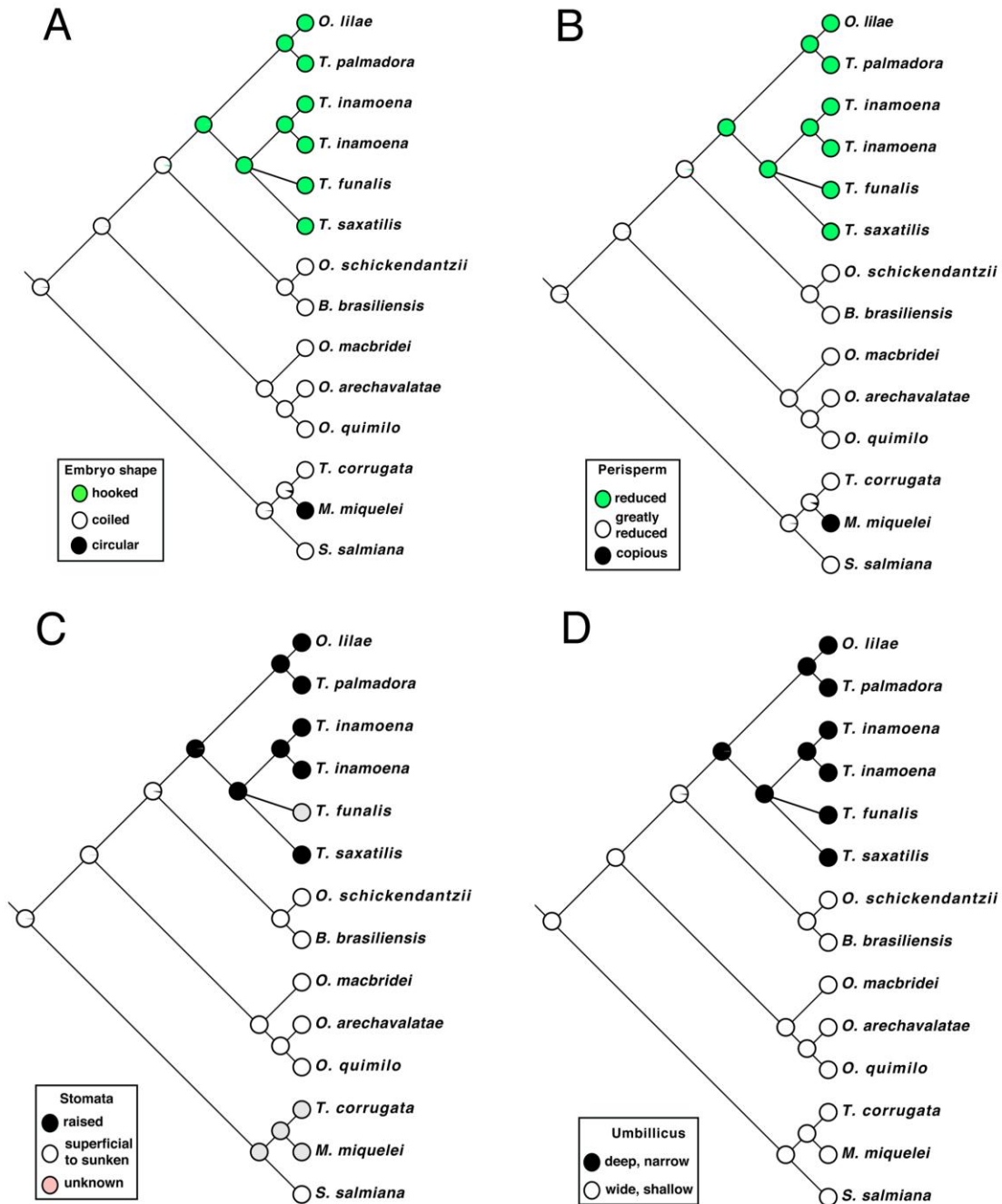


Figure 3-2. ML character-state reconstructions for A) embryo shape, B) perisperm development, C) stomatal placement, and D) umbilicus structure. Hook shaped embryos, reduced perisperm development, raised stomata, and deep, narrow umbilici are putative synapomorphies of the *Tacinga* clade as shown in our reconstructions.

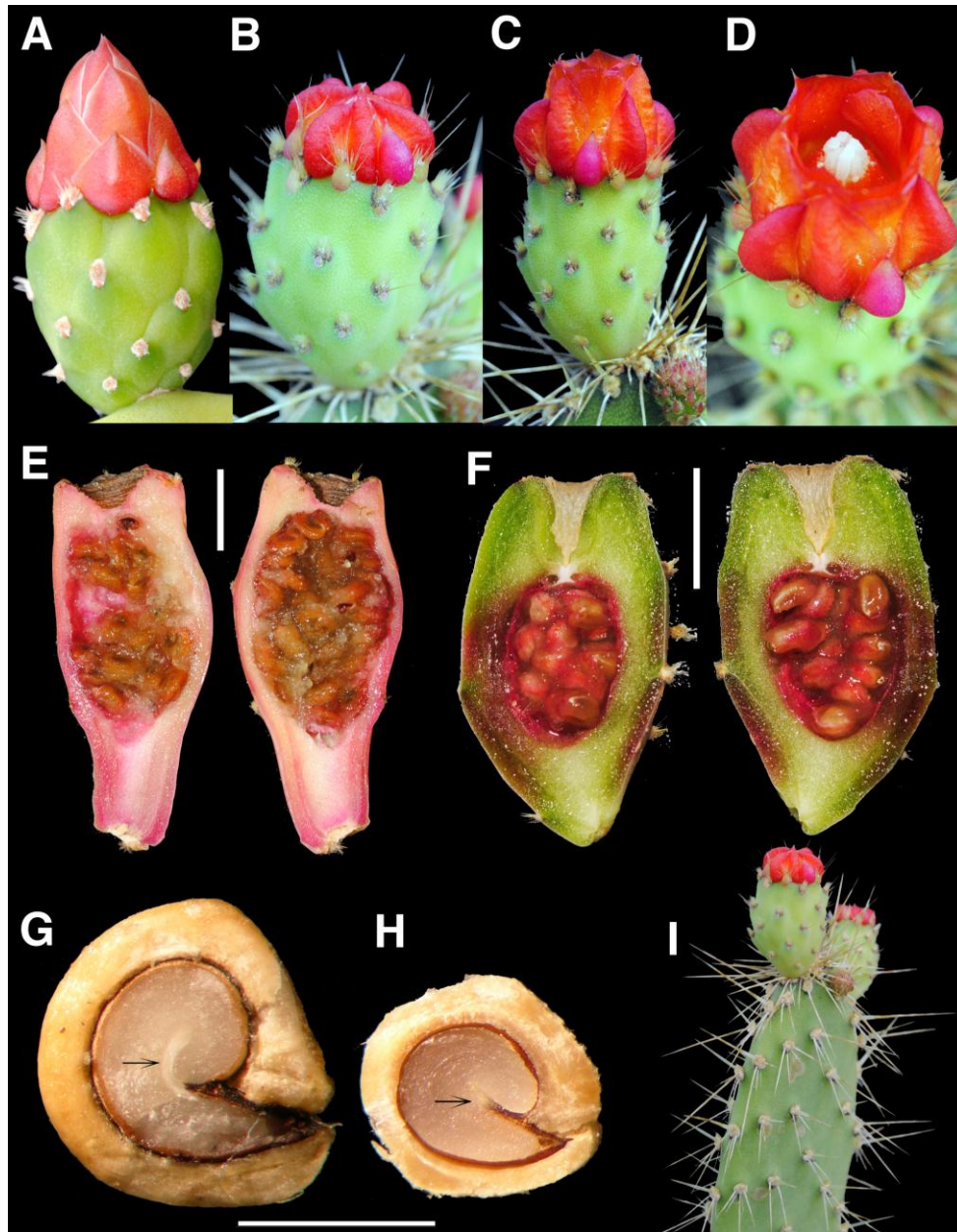


Figure 3-3. Morphological characters of *Tacinga lilae* from the type collection, Trujillo & Ponce 18643, A) acute flower bud of the hummingbird-pollinated *Opuntia cochenillifera*, contrasting with B) the dorsally compressed flower bud of *O. lilae*, C-D) red flowers with erect tepals forming a tube and included stamens typical of certain members of *Tacinga* (i.e., *T. palmadora*, *T. wernerii*), E) shallow umbilicus of *Opuntia macrorhiza* (LCM 3510) as compared to F) the deep, narrow umbilicus of *O. lilae* (scale = 1cm), G) cross section of a seed of *O. macrorhiza* showing greatly reduced perisperm development (arrow) with relation to embryo size and a coiled embryo compared to H) *O. lilae* with moderately reduced perisperm development (arrow) with relation to embryo size and a hook-shaped ovary, (scale = 3mm), and I) cladode of *O. lilae* showing spine production resembling *T. palmadora* and *T. wernerii*.

CHAPTER 4
A CASE OF MISTAKEN IDENTITY, *Opuntia abjecta*, LONG-LOST IN SYNONYMY
UNDER THE CARIBBEAN SPECIES, *O. triacantha*, AND REASSESSMENT OF THE
ENIGMATIC *O. cubensis*

Background

John Kunkell Small, a plant systematist and Curator of the New York Botanical Garden from 1898-1934, wrote a flora for the Southeastern United States for his Ph.D. dissertation (http://sciweb.nybg.org/science2/libr/finding_guide/small.asp). He produced three editions of his treatment of the Southeastern Flora from 1903-1933 (Small 1903, 1913, 1933) in which he paid special attention to the cacti of the southeastern United States. Small described 16 species of *Opuntia* from Florida alone. Two species, *Opuntia abjecta* Small and *O. ochrocentra* Small, were described from Big Pine Key, Florida (in Britton and Rose 1920). The population of *O. abjecta* at Big Pine Key was the only population that Small mentioned when he described the species and was the only population of the taxon known until recently (K. Bradley, Institute for Regional Conservation, pers. comm.). *Opuntia ochrocentra* apparently was known from the type locality and farther north (135 km) at Cape Romano (Small 1933), although only specimens from Big Pine Key and Big Munson Island have ever been seen (Benson 1982; Majure et al. 2012b).

Lyman Benson (1982) produced the beautifully illustrated and detailed, “The Cacti of the United States and Canada,” in which he placed *O. abjecta* and the Cuban *O. militaris* Britton and Rose (Britton and Rose 1919) in synonymy with the Greater and Lesser Antillean species, *O. triacantha* (Willd.) Sweet. Since that publication, the name *O. triacantha* has been used, mostly without question, for material from the Florida Keys (Doyle 1990; Pinkava 2003; Wunderlin and Hansen 2003, 2011). Interestingly, Anderson (2001) treated *O. abjecta* as a synonym of *O. triacantha*, but did not include the Florida Keys within the geographic distribution of that species. *Opuntia abjecta* (under *O. triacantha*) is considered an endangered species in Florida

(Coile and Garland 2003) and thought to be the northernmost population of *O. triacantha* in North America, occurring as a northern disjunct from the nearest population of *O. triacantha* in southeastern Cuba (i.e., *O. militaris*; Benson 1982).

Opuntia cubensis Britton and Rose was originally described from the Guantanamo Bay area of Cuba (Britton and Rose 1912), as a putative hybrid between *O. militaris* and *O. dillenii* (Ker-Gawl.) Haw. (Britton and Rose 1920). Benson (1982) later determined that another species, *O. ochrocentra* Small, also described by J.K. Small from Big Pine Key, Florida, was synonymous with *O. cubensis*, although Britton and Rose (1920) had considered *O. ochrocentra* to be a close relative of *O. dillenii*. Most authors have followed Benson's work and also included *O. ochrocentra* within *O. cubensis* (Anderson 2001; Pinkava 2003; Wunderlin and Hansen 2003, 2011).

Phylogenetic analyses of *Opuntia* (Majure et al. 2012a) and morphological studies of *Opuntia* for the monograph of the *Humifusa* clade (Chapter 7) suggest that *O. abjecta* is a different species and evolutionarily divergent from *O. triacantha* and another of its synonyms, *O. militaris*. Majure et al. (2012b) determined that material of "*O. cubensis*" from the Florida Keys was likely derived from hybridization between *O. abjecta* and most probably *O. dillenii*, instead of *O. militaris* from Cuba. We expand upon those previous analyses here with the inclusion in our phylogeny of *O. cubensis* and *O. militaris* from Cuba. We also present a detailed morphological examination of *O. abjecta*, *O. cubensis*, *O. ochrocentra*, and *O. triacantha* to provide a clear understanding of why *O. abjecta*, *O. triacantha*, *O. cubensis*, and *O. ochrocentra* should not be considered conspecific. We also discuss the relationship of *O. militaris* to *O. triacantha* from a morphological and phylogenetic perspective.

Materials and Methods

Previously gathered data from the plastid intergenic spacers *atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, *trnL-F*, the plastid genes *ycf1* and *matK*, the nuclear ribosomal internal transcribed spacers (ITS; White et al. 1990) and the nuclear gene *ppc* (Majure et al. 2012a) were used for our phylogenetic analyses. However, we enhanced our sampling to include *O. cubensis* and *O. militaris* from Cuba. Live material of *Opuntia cubensis* was obtained from field-collected (Cuba; *Areces s.n.*) material now grown at Gemini Botanical Garden, Florida. Although no recent specimens of *O. militaris* exist, to our knowledge, we were able to extract and amplify DNA from a specimen collected in 1951 (*R.N. Jervis 1033*; MICH) from the Guantánamo Bay area (see Appendix C). Both tepal and epidermal tissue produced usable DNA, although tepal tissue was superior in quality (i.e., DNA was less degraded) to the epidermal tissue used. *Opuntia triacantha* also was sampled from an herbarium sample (*Mori et al. 22693*; NY), as we did not have live material of that species. We cloned ITS and *ppc* PCR products of *O. cubensis* and *O. ochrocentra* using the Stratagene cloning kit (Stratagene, La Jolla, CA) and sequenced eight clones of each using bacterial primers (T3-T7) from the kits. We sampled the type (diploid) population of *O. abjecta* (Majure et al. 2012b) and *O. ochrocentra* (pentaploid) from Big Pine Key, as well as available herbarium material for morphological work, including the type specimens.

We also included diploid members of the *Humifusa* clade, the closely related *Macrocentra* and *Scheerianae* clades, and members of the *Nopalea* clade (sensu Majure et al. 2012a), to which *O. triacantha* is morphologically most similar (e.g., *O. caracassana*, *O. guatemalensis*, *O. jamaicensis*). South American species of *Opuntia*, *O. retrorsa* and *O. macbridei*, were used as outgroups based on results from Majure et al. (2012b) (Table 1).

For reaction specifications for each DNA region used, see Majure et al. (2012a). Sequences were edited either in Sequencher 4.2.2TM (Gene Codes, Inc., Ann Arbor, MI, USA) or Geneious ProTM 5.1 (Biomatters Ltd., Auckland, NZ), and the alignment was adjusted manually in Se-Al v2.0 (Rambaut, 2007). All gaps introduced during alignment were coded as missing data.

Combined nuclear and plastid regions were analyzed for all putative diploid taxa (see Majure et al. 2012a) using maximum likelihood (ML) in RAxML (Stamatakis 2006) carrying out 10000 nonparametric rapid bootstrap pseudoreplicates under 25 rate categories and implementing the GTR model of molecular evolution. Separate plastid and nuclear data sets containing *O. cubensis* and *O. ochrocentra* then were analyzed using the same methods.

Morphological characters (e.g., cladode shape, flower color, glochid color, growth form, spine color/development pattern) were observed, and measurements were taken from herbarium specimens of *O. abjecta*, *O. cubensis*, *O. militaris*, *O. ochrocentra*, and *O. triacantha*, and live material of *O. abjecta*, *O. cubensis*, and *O. ochrocentra*. As mentioned above, no live material of *O. militaris* or *O. triacantha* was available for study. We also compared *O. militaris* and *O. triacantha* to herbarium specimens of a closely related Caribbean species, *O. repens* (see Majure et al. 2012a).

Results

Phylogeny

Opuntia abjecta and *O. triacantha* are resolved in disparate clades. *Opuntia abjecta* is nested in the southeastern United States subclade of the *Humifusa* clade, and *O. triacantha* is closely related to the Caribbean and Central American taxa, *O. caracassana*, *O. jamaicensis*, and *O. guatemalensis* of the *Nopalea* clade (Fig. 4-1; see also Majure et al. 2012a). *Opuntia militaris*

also is nested within the *Nopalea* clade and is not closely related to *O. triacantha* but is resolved as closely related to *O. caracassana*.

Opuntia cubensis s.l. is recovered in three places; *O. ochrocentra* s.s. (from the Florida Keys) is nested within the *Humifusa* clade using plastid data. The sample of *O. cubensis* s.s. is nested in the *Nopalea* clade using plastid data (Fig. 4-2A). This suggests that the maternal parent of *O. ochrocentra* is a member of the *Humifusa* clade, and the maternal parent of *O. cubensis* is a member of the *Nopalea* clade. Two ITS copy types were discovered for both *O. cubensis* and *O. ochrocentra*, after excluding putative recombinants. One ITS haplotype of *O. ochrocentra* was recovered in the *Humifusa* clade, and another is unresolved in a grade containing members of the *Scheerianae* clade, which contains one of the putative parents of *O. ochrocentra* (based on morphology), *O. dillenii*. One ITS haplotype of *O. cubensis* is resolved within the *Nopalea* clade, as closely related to *O. militaris* (i.e., one haplotype is nearly identical to *O. militaris*), and the other haplotype is unresolved within a grade containing members of the *Scheerianae* clade (Fig. 4-2B). Two copy types also were found in *ppc* clones of *O. ochrocentra*, which were placed in the *Humifusa* clade and in a grade of other taxa (*ppc* data provide very poor resolution at the clade level), respectively; however, only one copy type of *ppc* was found from *O. cubensis*, which shared synapomorphies only with the *Nopalea* clade (Fig. 4-2C).

Morphology — *O. abjecta* vs. *O. triacantha*

Opuntia abjecta is strikingly different from *O. triacantha* in growth form, spine color and production, flower bud shape, flower color, and color of areolar trichomes and glochids.

Opuntia abjecta is a small, spreading-ascending shrub with basally disposed, radiating branches that reach up to 30 cm in height. *Opuntia triacantha* is a small erect to semi-erect shrub generally with a central, semi-cylindrical trunk much like that of its close relative *O. repens* Bello, and reaches heights of up to 40 cm or more. The spines of *O. abjecta* are strongly

retrorsely barbed like those of *O. triacantha*, but they are a lustrous, dark reddish-brown during development, instead of dull yellow as in *O. triacantha* (Fig. 4-3), and they mature to a bright white instead of pale white color. The spines of both taxa become dark gray in age. Up to 3 spines are produced from the areoles of terminal cladodes of *O. abjecta*, and these are usually all in the same plane of symmetry (e.g., all spreading, all reflexed, etc.). Up to 6 spines are produced from the areoles of *O. triacantha*, and they are in two planes of symmetry with the central spine typically divergent (porrect at $\geq 70^\circ$ angle) from the lower spines produced (Fig. 4-3), as in the closely related species, *O. repens* and *O. caracasana*. The spines of *O. triacantha* are also shorter on average than those of *O. abjecta* (3.7 cm vs. 4.4 cm). *Opuntia abjecta* has a rounded flower bud apex, while *O. triacantha* has an acute flower bud. *Opuntia abjecta* has completely dark yellow inner tepals, while *O. triacantha* has sulfur-yellow inner tepals that are often tinged pink along the midrib. Tepals are obovate in *O. abjecta* with a rounded to flat apex with a mucronate tip, and oblong to obovate in *O. triacantha* with a rounded apex, often without a mucro. The areolar trichomes of *O. triacantha*, *O. militaris*, and *O. repens* are yellowish, while the areolar trichomes of *O. abjecta* are white. *Opuntia abjecta* has stramineous-colored glochids on younger cladodes, while *O. triacantha* has bright yellow glochids on younger cladodes. In general, *O. abjecta* may be differentiated from *O. militaris* by the same features as used to distinguish it from *O. triacantha*, because, as indicated in the next section, *O. militaris* and *O. triacantha* are morphologically very similar.

Morphology — *O. militaris* vs. *O. triacantha*

Opuntia militaris is strikingly similar to *O. triacantha*, although in general *O. militaris* is smaller than *O. triacantha*. Like *O. triacantha*, *O. militaris* grows erect with one central trunk, eventually producing a small, branching shrub to 30 cm high (Britton and Rose 1920). Flower color of *O. militaris* and *O. triacantha* is similar, with both having sulfur-yellow inner tepals that

may be tinged pink along the midvein and do not have a strong mucronate tip. Flower buds in both species are acute, as in other species of the *Nopalea* clade. The average cladode length and width of *O. militaris* contrasts with *O. triacantha* (6.2 x 2.8 cm for *O. militaris* and 7.8 x 3.9 cm for *O. triacantha*). Spine lengths and diameters also are smaller in *O. militaris*, as compared to *O. triacantha* (2.5 cm long x 0.5 mm in diameter vs. 3.7 cm long x 0.76 mm in diameter).

Opuntia triacantha may have up to 6 spines per areole, and *O. militaris* may have up to 4 spines per areole, although difference in spine number needs to be explored further in the field, as it can be a highly variable character. *Opuntia militaris* also exhibits the porrect spines of *O. caracasana*, *O. repens*, and *O. triacantha*.

Morphology — *O. cubensis* vs. *O. ochrocentra*

Opuntia ochrocentra from the Florida Keys and *O. cubensis* from Cuba share morphological features suggesting that *O. dillenii* could be one of the parents of both. This similarity likely led Britton and Rose (1920) to include these taxa in the same series as *O. dillenii*, i.e., *Opuntia* series *Dillenianae*. In both taxa the spines are produced in a star-pattern from the areoles (Fig. 4-3); they also produce radial spines that are basally flattened, as in *O. dillenii*. Most developing radial spines of *O. ochrocentra* are lustrous yellow as in *O. dillenii*, but central spines are produced that are mottled to banded red-brown, as in the developing spines of *O. abjecta*. Although the spines of *O. cubensis* are produced from the areole as in *O. dillenii* (i.e., in a star-pattern), the young developing spines are dull yellow to creamy white, as in *O. militaris* and *O. triacantha*. Cladodes of *O. ochrocentra* are on average larger than those of *O. cubensis* (15.6 x 7.5 cm vs. 12.3 x 4.8 cm) and produce longer central spines (5.3 vs. 3.1 cm long). Average spine diameters are nearly the same for both taxa (1.05 vs. 1.01 mm). The central spines of both *O. ochrocentra* and *O. cubensis* are generally round in cross section and may or may not be twisted at the base, as in *O. abjecta* and *O. militaris*. Mature spines of *O.*

ochrocentra turn dark gray in age and become strongly deflexed, while mature spines of *O. cubensis* apparently turn light brown in age and do not deflex. Both species also have easily disarticulating cladodes, like those of their putative parental species, *O. abjecta* and *O. militaris*.

Below we provide a key to distinguish *O. abjecta*, *O. militaris*, *O. triacantha*, *O. cubensis*, and *O. ochrocentra*. We also include the widespread species *O. repens*, a close relative of and morphologically similar to *O. triacantha*, as shared characters of those two taxa often lead to misidentifications. This artificial key is based on both live material and herbarium specimens.

Key to the Species

1. Spines disposed from the areoles in a star-like pattern, radiating in all directions, radial spines strongly flattened dorsiventrally, central spines round in cross section.....2
1. Spines disposed from the areoles in the same plane, or with some spines prostrate, spines (including radials and centrals, if both present) round in cross section or merely twisted at the base, but not flattened.....3
2. Developing spines dull yellow to cream or dull light brown in color, spines stout to 3.1 (2.2-4.2) cm long, with one central spine (round in cross section) ***O. cubensis***
2. Developing spines bright, lustrous yellow to orange-red or mottled yellow and reddish-brown banded in color, spines delicate to 5.3 (4.7-5.8) cm long, with multiple central spines (round or twisted in cross section) ***O. ochrocentra***
3. Mature plants caespitose (with numerous branches arising from the base), inner tepals entirely yellow, developing spines dark red-brown to mottled red-brown and white, spines mostly spreading from the areoles in one plane (at $\leq 45^\circ$), flower buds rounded at the apex, cladodes rotund to obovate in outline, glochids stramineous,..... ***O. abjecta***

3. Mature plants with solitary stems (although these may form dense patches from the disarticulation of terminal cladodes), inner tepals yellow to yellow-green, often tinged with pink along the tepal midvein, developing spines dull to lustrous-yellow or creamy-white, usually spreading with one to two large porrect spines and 1 to numerous deflexed spines (at $\geq 70^\circ$), flower buds with an acute apex, cladodes narrowly elliptic, oblong, to obovate, glochids yellow..... 4
4. Cladodes sub-cylindrical to flat, narrow, on average 5 cm long, 1.8 cm wide, developing spines lustrous-yellow, spines flexible (delicate), on average 0.44 mm in diameter, 3.3 cm long, plants delicate..... *O. repens*
4. Cladodes flat, wider, on average 6.2-7.8 cm long, 2.8-3.8 cm wide, developing spines dull-yellow, spines stout, on average 0.5-0.76 mm in diameter, 2.5-3.7 cm long, plants robust..... 5
5. Cladodes 7.8 (5-10.9) cm long, 3.8 (2.4-5.8) wide, spines 3.7 (2.3-6.2) cm long and averaging 0.76 mm wide..... *O. triacantha*
5. Cladodes 6.2 (4.7-8.5) cm long, 2.8 (2.3-3.5) wide, spines 2.5 (1.4-3.1) cm long and averaging 0.50 mm wide..... *O. militaris*

Discussion

Opuntia abjecta vs. *O. triacantha*

Chromosome counts reveal that the type population of *O. abjecta* is diploid, while other material from the Florida Keys is tetraploid (Majure et al. 2012b). Also, material cultivated at Big Pine Key in a resident's yard obtained from Montgomery Botanical Center was tetraploid (LCM 3318; Majure et al. 2012b), suggesting that another population of *O. abjecta* may exist somewhere in the lower keys. The population of *O. abjecta* on Long Key is morphologically identical to that of other tetraploid material and thus is most likely tetraploid as well. *Opuntia*

ochrocentra has been recorded as pentaploid ($2n = 55$) from three accessions (Majure et al. 2012b), and *O. cubensis* was tetraploid ($2n = 44$) from one count (*Areces s.n.*) made by Majure et al. (in review). Spencer (1955) reported a diploid count for *O. triacantha* from Puerto Rico; however, we have not been able to confirm this count. No chromosome counts are available for *O. militaris*.

Benson (1982) likely placed *O. abjecta* in synonymy with *O. triacantha*, because these taxa share several morphological features, such as disarticulating cladodes, and terminal cladodes that often exhibit 2-3 spines per areole. Spines of both species overlap in length and diameter, and cladode shapes and sizes slightly overlap, as does the height of both species. *Opuntia abjecta* is only found in the Florida Keys and was considered merely a northern extension of the Caribbean *O. triacantha* (Benson 1982; Pinkava 2003). This southern Florida/Caribbean disjunction is very common for a wide variety of taxa (Wunderlin and Hansen 2003). In Cactaceae alone, *Acanthocereus*, *Consolea*, *Harrisia*, and *Pilosocereus* are shared with neighboring Caribbean Islands (Acevedo-Rodriguez 1996). *Opuntia triacantha* is commonly found on “coastal rocks” (Britton and Rose 1920). *Opuntia abjecta* likewise is found on limestone outcrops (Key Largo Limestone) within 0.5 km or less of the ocean (Benson 1982; Majure pers. obsv.). Additionally, the misidentification of the interspecific hybrid presumably involving *O. triacantha*, *O. ochrocentra* (as *O. cubensis*), added further evidence for the northern disjunct distribution of *O. triacantha* in the Florida Keys (Benson 1982).

Coincidentally, *O. austrina* Small, another endemic species to Florida, is much more similar morphologically to true *O. triacantha* than is *O. abjecta*. *Opuntia austrina* forms treelets (i.e., the *ammophila* entity) to large shrubs and generally is characterized by a single, cylindrical stem, which may be copiously spiny as in *O. triacantha* (Fig. 4-3). *Opuntia austrina* also is a

member of the *Humifusa* clade, so these morphologically similar characters are merely convergent between *O. austrina* and *O. triacantha*. *Opuntia austrina* is morphologically similar to *O. abjecta* as well, having similar spine characters, glochid and flower colors, and cladode shapes. Consequently, Benson (1982) also misidentified some material of *O. abjecta* from the Florida Keys as *O. austrina*. Although Anderson (2001) included *O. abjecta* in synonymy with *O. triacantha*, as mentioned above, he did not include the Florida Keys within the distribution of the species, although his photo of *O. triacantha* is actually of *O. abjecta* from the Florida Keys!

O. militaris* vs. *O. triacantha

Considering the limited data here and poor resolution in the diploid phylogeny (Fig. 4-1), it is still premature to determine whether or not *O. militaris* and *O. triacantha* should be considered the same species. *Opuntia militaris* shares numerous morphological features with *O. triacantha*, although it is generally less robust and has fewer spines, characters that may be influenced as a result of different environmental constraints across the distribution of the two taxa. However, even with the limited data presented here, it is obvious that *O. triacantha* and *O. militaris* are not genetically identical (e.g., *O. militaris* is more closely related to *O. caracassana* in our diploid phylogeny; Fig. 4-1). Thus, our phylogenetic data suggest that *O. triacantha* and *O. militaris* represent distinct lineages. *Opuntia militaris* is also disjunct from the nearest population of *O. triacantha* on Desecheo Island, Puerto Rico, by ca. 765 km. It will be necessary to study morphological characters and ploidal levels of living material of *O. militaris*, *O. triacantha*, and other closely related species within the Greater and Lesser Antilles (e.g., *O. caracassana*, *O. jamaicensis*, *O. repens*, *O. taylori* Britton and Rose) to determine species limits within this group. However, *O. militaris* is tentatively considered specifically distinct and is included in the above key.

The *Opuntia cubensis* Enigma

Benson (1982) considered *O. ochrocentra* Small described from Big Pine Key to be a synonym of *O. cubensis* described from Guantánamo, Cuba (Britton and Rose 1912). *Opuntia cubensis* has generally been considered a hybrid between *O. dillenii* and *O. militaris* (Britton and Rose 1920), and molecular data support the hybrid origin of *O. cubensis* from the Florida Keys (i.e., *O. ochrocentra*) between a member of the *Humifusa* clade and *O. dillenii* (Majure et al. 2012a; see Fig. 4-2A-B in this study). However, this study suggests that *O. militaris* is not conspecific with *O. abjecta* and may be more closely related to, although likely not conspecific with, *O. triacantha*. Therefore, *O. cubensis* in Cuba is derived from different putative progenitors, *O. militaris* and *O. dillenii*, than that of the Florida Keys material, which is derived from a member of the *Humifusa* clade (*O. abjecta*) and a member of the *Scheerianae* clade (*O. dillenii*). Thus, the interclade origin of “*O. cubensis*” as identified to by Majure et al. (2012a) should be referred to as *O. ochrocentra*, given that *O. cubensis* and *O. ochrocentra* are not synonymous, as shown here.

Characters of *O. abjecta* and *O. militaris* exhibited by “*O. cubensis* s.l.” could easily be mistaken for any of those putative progenitors, because those characters differentiating *O. cubensis* from *O. dillenii* are spine color and shape, the smaller growth form, and cladode disarticulation, all characters shared to some degree by the other putative maternal progenitors (*O. abjecta* and *O. militaris*). Identifying these species is made more difficult when using only herbarium material, as most identifying characters of these stem succulents, other than spine characters, are mostly lost during the drying process (Reyes-Agüero 2007).

Opuntia cubensis and *O. ochrocentra*, however, are morphologically separable. *Opuntia ochrocentra* shares the mottled yellow to reddish-brown colored young spines of *O. abjecta*, and *O. cubensis* has dull yellow young spines, as does *O. triacantha* and *O. militaris*. The spine

patterns of *O. ochrocentra* and *O. cubensis* are slightly different, with *O. cubensis* always having one strong, porrect, cylindrical central spine and *O. ochrocentra* with several weaker, cylindrical or basally twisted central spines. The spines in *O. ochrocentra* deflex and become appressed along the stem in age, a character apparently not demonstrated by *O. cubensis*. *Opuntia cubensis* generally has shorter spines and smaller cladodes than *O. ochrocentra*, as mentioned above. The cladodes of *O. cubensis* are typically elliptical in outline, while cladodes of *O. ochrocentra* may be either elliptical or obovate.

Summary

The true identities of the Floridian species, *O. abjecta* and *O. ochrocentra* were long obscured as a result of incorrect assumptions made regarding phytogeographic relationships of *Opuntia* from the Florida Keys and the Caribbean region (Benson 1982). *Opuntia abjecta* and *O. triacantha* are distinct species morphologically and phylogenetically. Thus, material from Florida should not be referred to as *O. triacantha*, but rather represents a species endemic to the state, which should be recognized as *O. abjecta*. *Opuntia cubensis* is a Cuban taxon that does not occur in Florida and which originated via hybridization between *O. militaris* of the *Nopalea* clade and likely *O. dillenii* of the *Scheerianae* clade, as suggested by Britton and Rose (1920). Material of “*O. cubensis* s.l.” from the Florida Keys should be treated as *O. ochrocentra*, which is of hybrid origin, most likely between *O. abjecta* of the *Humifusa* clade and *O. dillenii*. More research will be necessary to determine whether or not *O. militaris* is distinct from *O. triacantha*, but given the limited morphological and phylogenetic data presented here, I suggest that *O. militaris* should be regarded as a separate species.

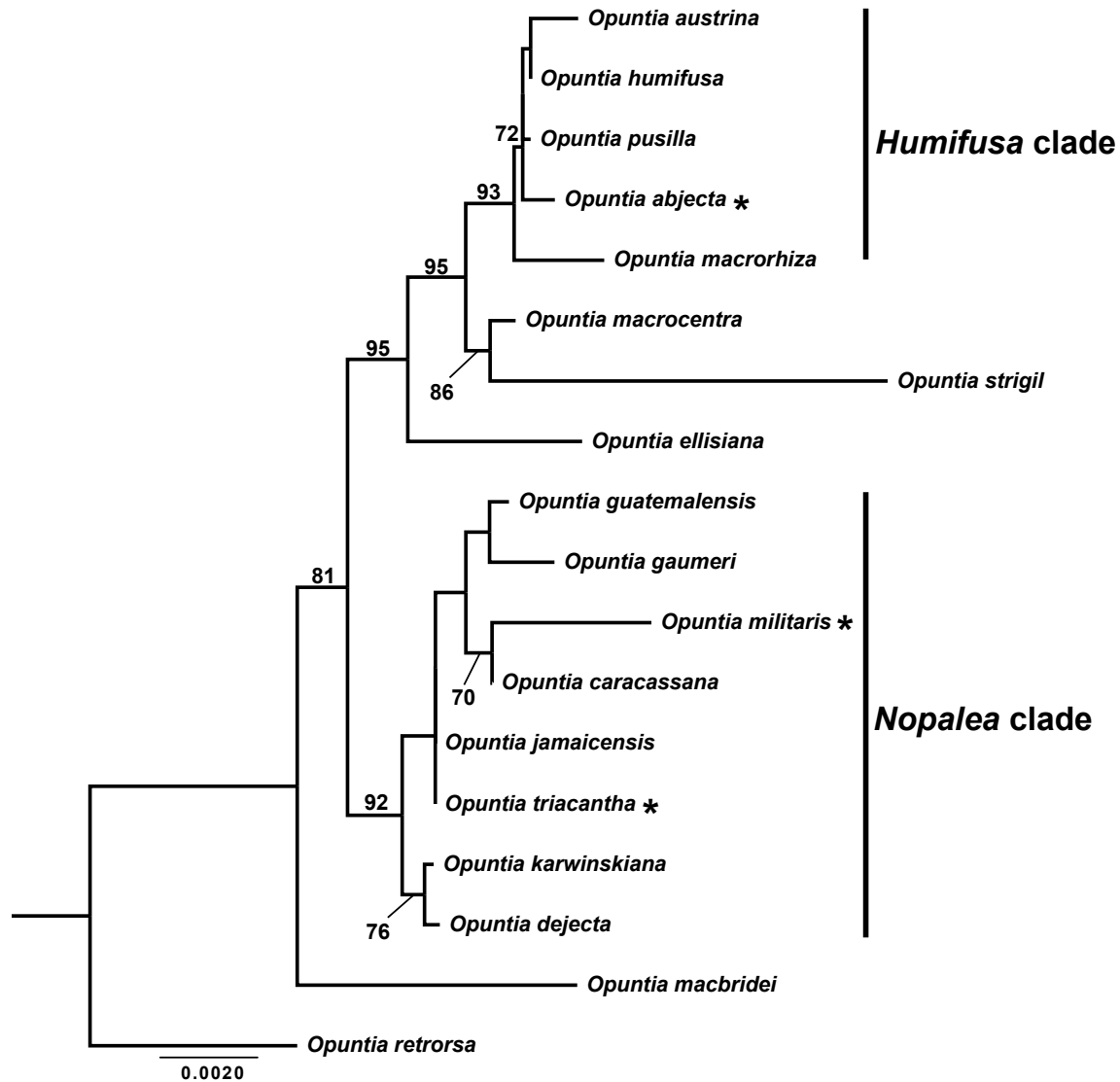


Figure 4-1. Putative diploid ML phylogeny (most likely topology) of *Opuntia* s.s. using South American species (*O. macbridei*, *O. retrorsa*) as outgroups. It is well-supported that *O. triacantha* is in a different clade (i.e., the *Nopalea* clade) than *O. abjecta* (*Humifusa* clade). *Opuntia militaris*, although nested within the *Nopalea* clade, is not resolved as sister to *O. triacantha*, with which it is currently placed in synonymy. *Opuntia abjecta*, *O. militaris*, and *O. triacantha* are denoted by asterisks. Bootstrap values are indicated above branches.

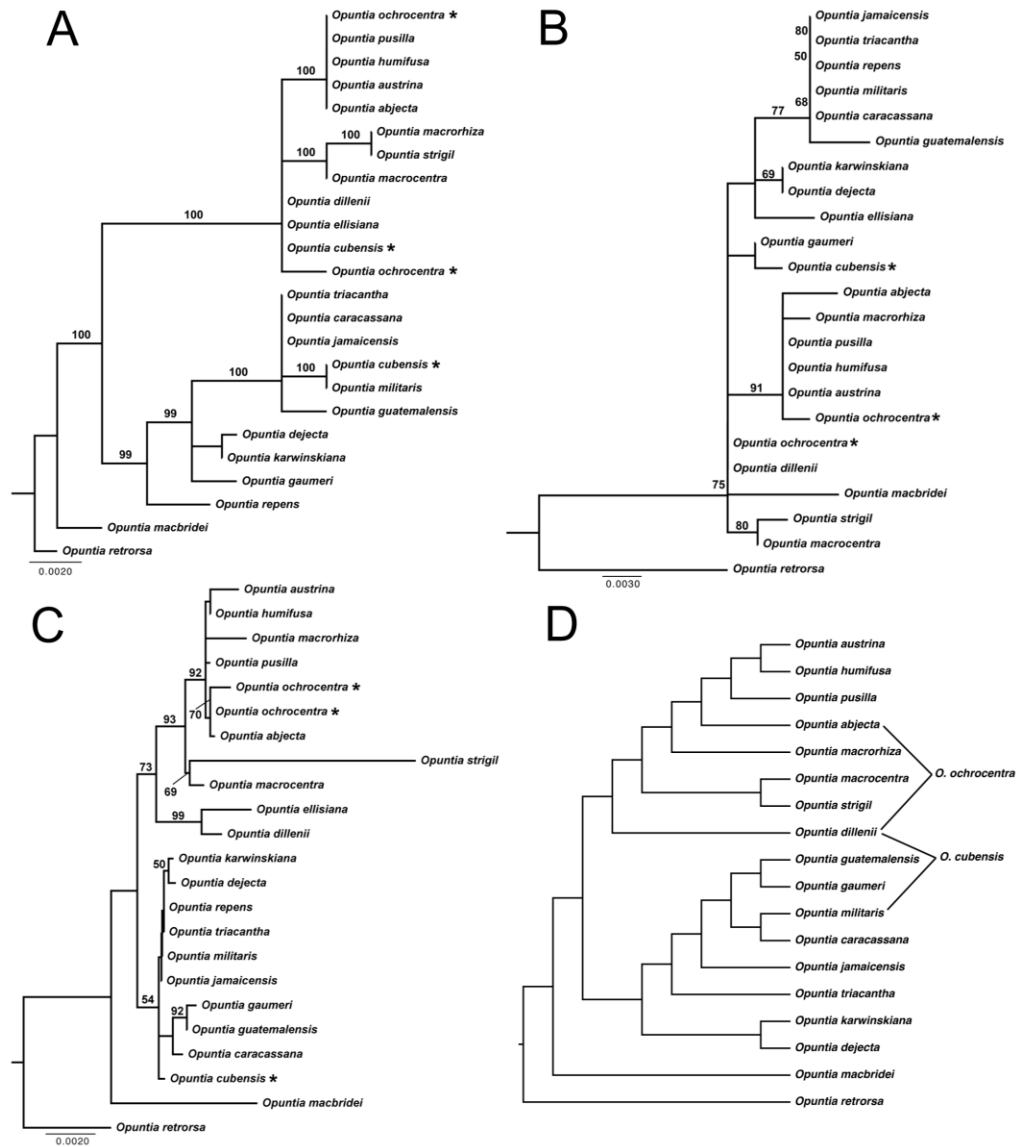


Figure 4-2. Most likely topologies (from RAxML) from ITS (A), *ppc* (B), and (C) plastid phylogenies including the putative hybrid taxa *O. cubensis* and *O. ochrocentra* (indicated with asterisks). ITS clones of *O. ochrocentra* are resolved in the *Humifusa* clade and unresolved with *O. dillenii* and *O. ellisiana* of the Scheerianae clade, while ITS clones of *O. cubensis* are resolved in the *Nopalea* clade, as well as unresolved with *O. dillenii* and *O. ellisiana*. *ppc* clones of *O. ochrocentra* are also resolved with the *Humifusa* clade and unresolved with members of the *Scheerianae* clade, while only one copy type of *ppc* was discovered for *O. cubensis* that is unresolved with a member of the *Nopalea* clade. *Opuntia ochrocentra* is resolved in the *Humifusa* clade, and *O. cubensis* is resolved in the *Nopalea* clade in the plastid phylogeny. D) represents the putative hybrid scenario, where *O. ochrocentra* was derived from *O. abjecta* (maternal lineage) and *O. dillenii* (paternal lineage), while *O. cubensis* was derived from *O. militaris* (maternal lineage) and *O. dillenii* (paternal lineage). Bootstrap values are given above branches in A-C.

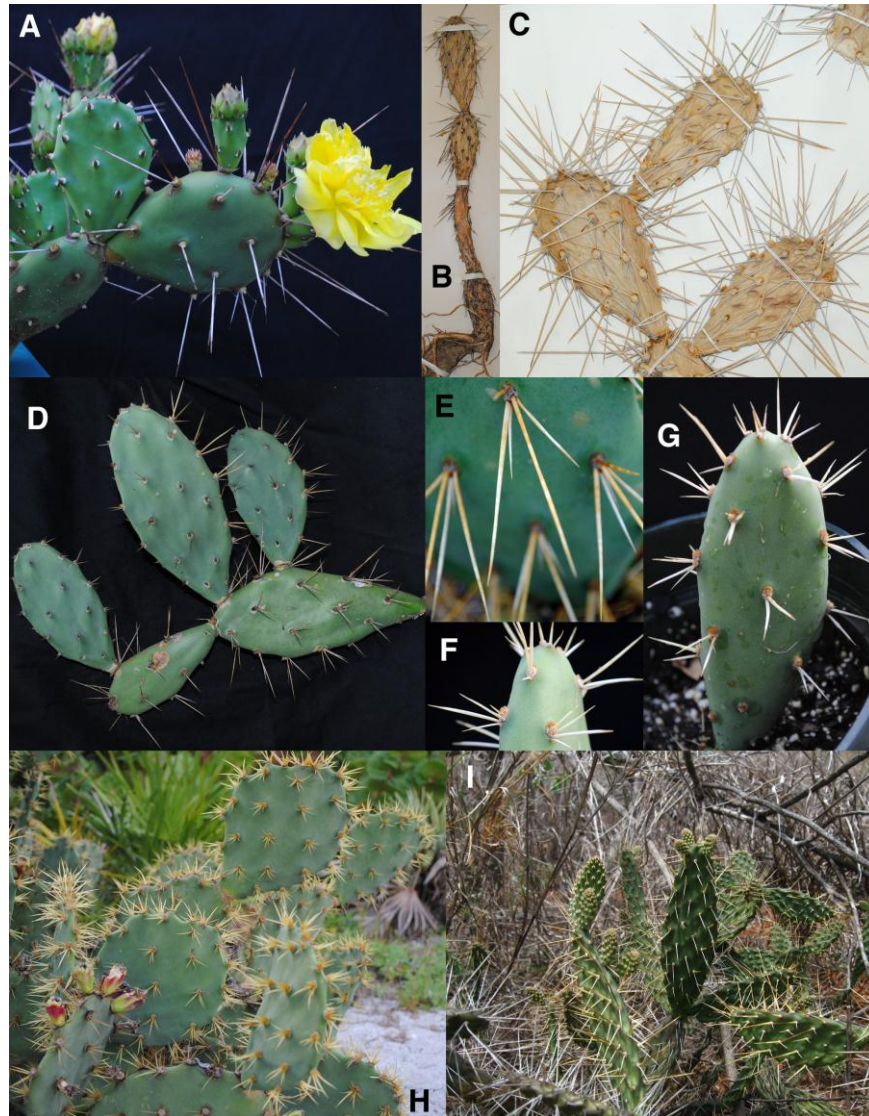


Figure 4-3. Morphological characters of *O. abjecta*, *O. triacantha*, *O. cubensis*, *O. ochrocentra*, *O. dillenii*, and *O. repens*. A) *O. abjecta* (LCM 3908) showing reddish-brown developing spines, white mature spines and stramineous glochids, B) *O. triacantha* (P. Duss 3071) showing solitary, mostly erect trunk C) *O. triacantha* (A.C. Smith 10442) showing dull yellow developing spines, pale white mature spines, and yellow glochids, D-E) *O. ochrocentra* (LCM 3907) showing one and two year old cladodes with the younger cladodes showing yellow spines as in *O. dillenii*, and the older cladodes showing spines turning white in age; in E) young spines of ca. 6 mo. in age showing mottled color (banding) typical of *O. abjecta*, F-G) *O. cubensis* (Areces s.n.) showing yellow glochids and young developing spines that are pale yellow initially aging to white; both *O. ochrocentra* and *O. cubensis* have spine growth patterns similar to *O. dillenii*, H) *O. dillenii* (Buckaneer State Park, FL), and I) *O. repens* (LCM 3839), a close relative of and morphologically similar to *O. triacantha* showing porrect central spines, as described above for certain Caribbean members of the *Nopalea* clade.

CHAPTER 5
CYTOGEOGRAPHY OF THE *Humifusa* CLADE OF *Opuntia* S.S. MILL. 1754
(CACTACEAE, OPUNTIOIDEAE, OPUNTIEAE): CORRELATIONS WITH PLEISTOCENE
REFUGIA AND MORPHOLOGICAL TRAITS IN A POLYPLOID COMPLEX

Background

Ploidy has a long tradition of utility for illuminating species boundaries, hybrid zones, and interspecific relationships among plants (e.g., Stace 2000). Knowing the ploidal levels of taxa used in phylogenetic analyses can also aid in detecting potential hybridization events through incongruence in reconstructions using biparentally inherited nuclear loci (Ionta et al. 2007; Soltis et al. 2008). Researchers have frequently used cytological data to help understand species evolution and delimitations in the nopales or prickly pear cacti, i.e., the genus *Opuntia* (Pinkava and McLeod 1971; Pinkava et al. 1973, 1977, 1985; Weedin and Powell 1978; Pinkava and Parfitt 1982; Weedin et al. 1989; Pinkava et al. 1992; Powell and Weedin 2001, 2004). Subfamily Opuntioideae (*Opuntia* s.l., as previously recognized; Benson 1982) is known to have the highest number of polyploids in Cactaceae (Cota and Philbrick 1994; Pinkava 2002), and *Opuntia* s.s. is well known for interspecific hybridization (e.g., Grant and Grant 1982; Griffith 2003) and subsequent genome duplication (Pinkava 2002; L.C. Majure (LCM), R. Puente (RP), P. Griffith (PG), W.S. Judd (WSJ), P.S. Soltis (PSS), D.E. Soltis (DES) unpubl. data).

The significance of polyploidy in plant evolution and speciation has long been recognized (Stebbins 1940, 1950, 1971; Swanson 1957; DeWet 1971; Harlan and DeWet 1975; Grant 1981; Leitch and Bennett 1997; Ramsey and Schemske 1998; Adams and Wendel 2005; Tate et al. 2005; Doyle et al. 2008; Soltis and Soltis 2009; Jiao et al. 2011). As stated by Stebbins (1950), p.

Reprinted with permission from the authors. Original publication: Majure, L.C., D.E. Soltis, P.S. Soltis, and W.S. Judd. 2012b. Cyto geography of the *Humifusa* clade of *Opuntia* s.s. (Cactaceae: Opuntioideae): Correlations with geographic distributions and morphological differentiation of a polyploid complex. *Comparative Cytogenetics* 6: 53-77.

369), “polyploidy ... is one of the most rapid methods known of producing radically different, but nevertheless vigorous and well-adapted genotypes.” Polyploidy also is considered one of the unequivocal means of true sympatric speciation (Futuyma 1998; Otto and Whitton 2000) and is considered to be common in plants (Stebbins 1940; DeWet 1971; Ramsey and Schemske 1998; Tate et al. 2005). For example, virtually all major clades of angiosperms have undergone one or more episodes of genome duplication (Soltis and Soltis 2009). Likewise, polyploidy is very important throughout Cactaceae (Pinkava 2002), and within *Opuntia* s.s., polyploids previously have been recorded in *Opuntia humifusa* (Raf.) Raf., 1820, and relatives (Bowden 1945a, b; Pinkava et al. 1985; Powell and Weedin 2004; Baker et al. 2009a, b; Majure and Ribbens in press) of the *Humifusa* clade (sensu LCM, RP, PG, WSJ, PSS, DES unpubl. data). There are currently six species recognized in the *Humifusa* clade, *O. abjecta* Small, 1923, *O. humifusa*, *O. macrorhiza* Engelm., 1850, *O. pottsii* Salm-Dyck, 1849, *O. pusilla* (Haw.) Haw., 1812, and *O. tortispina* Engelm. & J.M. Bigelow, 1856 (Pinkava 2003; LCM unpubl. data). The *Humifusa* clade is distributed widely from the western U.S. and northern Mexico (represented by *O. macrorhiza* s.l., *O. pottsii*, and *O. tortispina*) and throughout the eastern U.S. including the upper Midwest (e.g., Michigan, Minnesota, Wisconsin) and southern Ontario (Benson 1982; represented by *O. abjecta*, *O. humifusa* s.l., *O. macrorhiza* s.l., and *O. pusilla*).

Opuntia humifusa s.l. is composed of numerous morphological entities that have been recognized in certain taxonomic treatments as different species (see Small 1933). Throughout its range, *O. humifusa* s.l. has been divided into as many as 14 taxa (Britton and Rose 1920; Small 1933; Benson 1982; Majure and Ervin 2008). Thus, *O. humifusa* s.l. is occasionally referred to as a species complex (Doyle 1990). Currently, two taxa are recognized in *O. humifusa* s.l. (*O. humifusa* var. *ammophila* (Small) L.D. Benson and *O. humifusa* var. *humifusa*; Pinkava 2003).

Likewise, *Opuntia macrorhiza* has been divided into as many as 11 taxa (see Benson 1982). *Opuntia macrorhiza* was previously considered a variety of *O. humifusa* (see Benson 1962; see Table 5-1 for synonyms of *O. humifusa* s.l. and *O. macrorhiza* s.l. sampled in this study), *O. pottsii* was considered a variety of *O. macrorhiza*, and *O. tortispina* was placed in synonymy with *O. macrorhiza* (Benson 1982). *Opuntia pusilla* has been divided into several species: *O. drummondii* Graham, 1841, *O. frustulenta* Gibbes, 1858, *O. impedita* Small, 1923, *O. pes-corvi* LeConte, 1857, and *O. tracyi* Britton, 1911 (Britton and Rose 1920; Small 1933); however, Benson (1982) placed them in synonymy under the name *O. pusilla*. *Opuntia triacantha* (Willd.) Sweet, 1826, also has been divided into several species, i.e., *O. abjecta* of the Florida Keys, *O. militaris* Britton & Rose, 1919, of Cuba, and *O. triacantha* from different parts of the Greater and Lesser Antilles (Britton and Rose 1920), but all of these have since been placed in synonymy within *O. triacantha* (Benson 1982). Phylogenetic and morphological studies have indicated that *O. abjecta* is not even in the same clade as *O. triacantha* (LCM, WSJ unpubl. data) and so here is treated as *O. abjecta*.

Contributing to the confusing taxonomic history of this clade is the high degree of morphological variation exhibited by most taxa, the lack of complete sampling throughout the range of the clade, the absence of cytological and phylogenetic evidence, reliance on poorly prepared and sparse herbarium collections (Majure and Ervin 2008; LCM unpubl. data), and hybridization and polyploidy (Benson 1982; Rebman and Pinkava 2001). Careful examination of morphological characters across the geographic range of the widely distributed *O. humifusa* s.l. and *O. macrorhiza* s.l. reinforces the hypothesis that hybridization may have preceded the origin of geographical morphotypes, because morphological characters displayed by certain taxa appear to be introgressive between *O. humifusa* s.l. and *O. macrorhiza* s.l. (Table 5-2). For instance, *O.*

cespitosa Raf., 1830, from the eastern U.S. and recently recognized by Majure and Ervin (2008), has yellow tepals that are basally tinged crimson- to orange-red, a characteristic typical of *O. macrorhiza* and occasionally *O. tortispina* from western North America (Benson 1982; Pinkava 2003; Powell and Weedin 2004), but the spine characters of *O. cespitosa* are typical of *O. humifusa* s.l. (see Majure and Ervin 2008).

Although chromosome counts have been reported for many of the *Opuntia* taxa from the southwestern U.S. and other areas (Stockwell 1935; Spencer 1955; Pinkava and McLeod 1971; Pinkava et al. 1973, 1977; Weedin and Powell 1978; Pinkava and Parfitt 1982; Pinkava et al. 1985; Weedin et al. 1989; Pinkava et al. 1992; Powell and Weedin 2001; Pinkava 2002; Negrón-Ortiz 2007; Segura et al. 2007; Baker et al. 2009a, b), few chromosome counts have been reported for taxa of *Opuntia* in the eastern and midwestern U.S. (Majure and Ribbens in press), and most of those taxa belong to the *Humifusa* clade. Bowden (1945a, b), Hanks and Fairbrothers (1969), Doyle (1990), and Baker et al. (2009 a, b) have all made counts of members of the *Humifusa* clade from the eastern U.S. Bowden (1945a, b), Doyle (1990), and Baker et al. (2009a) recorded diploid ($2n = 22$) and tetraploid ($2n = 44$) material of *O. humifusa* from the eastern U.S., and Bowden (1945a) recorded tetraploid ($2n = 44$) material of *O. impedita* (currently syn. of *O. pusilla*). Hanks and Fairbrothers (1969) recorded an aneuploid number for *O. humifusa* ($2n = 17, 19$) likely in error, since aneuploids are very rare in Cactaceae (Pinkava 2002). Majure and Ribbens (in press) recorded tetraploids of *O. humifusa* s.l. and *O. macrorhiza* s.l. from the Midwest, suggesting that the northernmost populations of those taxa are polyploid. *Opuntia macrorhiza*, *O. pottsii*, and *O. tortispina* have all been counted extensively in the southwestern U.S. (Pinkava and McLeod 1971; Pinkava et al. 1973; Pinkava et al. 1977; Pinkava et al. 1992; Pinkava et al. 1998; Powell and Weedin 2001; Powell and Weedin 2004), where *O.*

macrorhiza and *O. pottsii* have been recorded exclusively as tetraploids, and *O. tortispina* has been recorded as either tetra- or hexaploid.

Chromosome counts reported for species in the *Humifusa* clade do not encompass all of the taxa within the range of the clade nor the wide distributions exhibited by several of the more common species. To further our understanding of species complexes and the evolution of polyploids within those complexes, cytological data are needed from the entire distribution of a given species (Babcock and Stebbins 1938; Stebbins 1942; Stebbins 1950). Thus, an in-depth study of the distribution of cytotypes and correlations between cytotypes and morphology is desperately needed in order to aid in the delimitation of potentially unrecognized and cryptic species and to elucidate relationships in the *Humifusa* clade.

Here we present chromosome counts for all taxa considered to be part of the *O. humifusa* complex and all taxa of the *Humifusa* clade (LCM, WSJ, PSS, DES, unpubl. data) and provide counts throughout most of the known ranges of all taxa to determine the geographic structure of ploidy and differences in ploidy among morphologically distinct taxa. We also reconstruct a phylogeny of diploid and polyploid members of the *Humifusa* clade based on nrITS data to investigate the relationship between geographic distribution and evolutionary relationships. We provide counts for another common species in the southeastern U.S., *O. stricta* (Haw.) Haw., 1812, because it has been hypothesized to hybridize with members of the *Humifusa* clade (Benson 1982). In addition, ploidy of the putative hybrid between *O. abjecta* and *O. stricta*, i.e., *O. ochrocentra* Small, 1923, was analyzed. Ploidy determinations of the *Humifusa* clade, coupled with morphological character analysis and further molecular phylogenetics, will aid in the delimitation of species in the group and in determining the origin and evolutionary significance of polyploidy in this clade.

Material and Methods

Chromosome Counts

Methods follow those of Majure and Ribbens (in press). Briefly, root tips were collected from early morning throughout early afternoon and placed in 2mM 8-hydroxyquinoline (Soltis 1980) for up to 8 hours at 4°C or in N₂O (Kato 1999) for 1 hour and then fixed in a 3:1 solution of absolute ethanol: glacial acetic acid for 2 to 24 hours. Root tips then were placed in 70% ethanol for at least 2 hours and digested in 40% HCl for 5-10 minutes (depending on the size of the root) at room temperature. Squashes were performed in 60% acetic acid and stained with 1% aceto-orcein dye and viewed on a Zeiss Photomicroscope III (Carl Zeiss, Oberkochen, Germany). To confirm each count, at least three to five metaphase cells were counted per specimen. These multiple counts per sample alleviated concerns regarding endomitosis, which has been reported in the allopolyploid (4x), *Opuntia spinosibacca* M.S. Anthony, 1956, (Weedin and Powell 1978), tetraploid *O. pusilla* (Bowden 1945b), as well as in many other angiosperms (e.g., Barrow and Meister 2003, Tate et al. 2009, I. Jordan-Thaden, pers. comm.). We counted chromosomes of 277 individuals of the *Humifusa* clade, 14 individuals of *O. stricta* s.l., three samples of the putative hybrid *O. ochrocentra*, and two individuals of the putative hybrid *O. alta* Griffiths, 1910. Generally, only one accession per population was counted.

Taxonomy

Taxa used for ploidy analysis are listed in Appendix D. Species delimitations within *O. humifusa* s.l. and *O. macrorhiza* s.l. are problematic, so we recognize both *O. humifusa* and *O. macrorhiza* as broadly circumscribed (Table 5-1). Thus, we have arranged our counts of plants within these two species (see Appendix D) according to their various segregates to determine whether the morphological variation of these segregate entities (Table 5-2) is correlated with cytotype and/or geographical and phylogenetic patterns.

Cytogeographic Analysis

We mapped the localities for all of the individuals for which we determined ploidy (277 in number) and incorporated previous counts ($n = 41$) (Bowden 1945a; Pinkava and McLeod 1971; Pinkava et al. 1973; Weedon and Powell 1978; Pinkava and Parfitt 1982; Pinkava et al. 1985; Weedon et al. 1989; Doyle 1990; Pinkava et al. 1992; Pinkava et al. 1998; Powell and Weedon 2001; Baker et al. 2009a, b; Majure and Ribbens in press) to cover the majority of the geographic distribution of each taxon. This allowed us to explore the geographic boundaries of the different ploidal levels encountered in this clade and construct hypotheses regarding polyploid formation and speciation.

Phylogenetic Analysis

We generated sequences from the nuclear ribosomal internal transcribed spacer (nrITS: White et al. 1990) for a sample of diploid ($n = 6$) and polyploid taxa ($n = 8$) of the *Humifusa* clade from the eastern and western U.S. (Table 5-3). *Opuntia basilaris* Engelm. & J.M. Bigelow, 1856, was used as an outgroup based on previous analyses of *Opuntia* (LCM unpubl. data). A phylogenetic analysis of these data was carried out to determine whether the geographic distribution of ploidy (as determined here) was correlated with the evolutionary history of the clade. We carried out a Maximum Likelihood analysis using RAxML (Stamatakis 2006) running 10000 bootstrap pseudoreplicates under 25 rate categories and the GTR+ Γ model of molecular evolution.

Results

The base chromosome number for Cactaceae has been well established as $x = 11$ (Remski 1954; Pinkava and McLeod 1971; Lewis 1980; Pinkava et al. 1985; Pinkava 2002), and we saw no deviation from this in our counts (Appendix D). Out of 318 counts of the *Humifusa* clade, including 41 from the literature, 210 (66%) were polyploid and 108 (34%) were diploid. Diploid

($2n = 2x = 22$) and tetraploid ($2n = 4x = 44$) *O. humifusa* s.l. and *O. macrorhiza* s.l. were discovered (Fig. 5-1A-D, I-J, L). Diploid *O. humifusa* s.l. is restricted entirely to the southeastern U.S., whereas diploid *O. macrorhiza* s.l. is restricted entirely to the southwestern U.S. (eastern Texas (see Appendix D) and southeastern New Mexico (M. Baker and D.J. Pinkava pers. comm.)). Tetraploid members of *O. humifusa* s.l. and *O. macrorhiza* s.l. are much more widely distributed throughout the U.S. than are their diploid relatives (Fig. 5-2). Tetraploids of *O. humifusa* s.l. are found from Massachusetts south to the southeastern U.S. where they abut the distribution of diploid taxa and throughout the eastern and midwestern U.S. Tetraploid *O. macrorhiza* s.l. is distributed throughout parts of the Great Plains through the midwestern U.S., most of the southwestern U.S., parts of the Rocky Mountains, and the upper Sierra Madre Occidental in Sonora, Mexico (Fig. 5-2).

Diploid, triploid, and tetraploid populations of *O. pusilla* were discovered (Fig. 5-1E-G) throughout its restricted range in the southeastern U.S. (Fig. 5-3). Interestingly, with the exception of two populations, polyploid individuals ($3x$ and $4x$) were mostly confined to the coastline, although diploid populations were much more widespread throughout the interior part of the distribution of the species (Fig. 5-3). Of the three examples of *O. abjecta* sampled from the Florida Keys, one was diploid (Fig. 5-1H), and two were tetraploid. *Opuntia tortispina* (southwestern U.S.) was hexaploid in six and tetraploid in one of the populations examined (see Fig. 5-2 for hexaploid distribution).

Individuals of *O. stricta* sampled from the southeastern U.S. were all hexaploid. Samples included members of the taxa considered by some (Anderson 2001) to be *O. dillenii* (Ker-Gawl.) Haw., 1819, and *O. stricta*. Three individuals of the putative hybrid *O. ochrocentra* from two

localities in the Florida Keys were pentaploid (Fig. 5-1K), and the putative hybrid *O. alta* was hexaploid.

Maximum likelihood analysis of ITS data reveals that the *Humifusa* clade is made up of two well-supported subclades. One is restricted to the southeastern U.S. and includes polyploid members of *O. pusilla* and *O. abjecta*, and the other includes southwestern diploid *O. macrorhiza* and all other polyploids pertaining to *O. humifusa* s.l., *O. macrorhiza* s.l., and *O. tortispina*. There is no further resolution within the tree at the species level using ITS (Fig. 5-4). Species relationships within these two clades are further resolved with the addition of other loci (LCM unpubl. data), however, that is beyond the scope of this study.

Discussion

Opuntia macrorhiza has only been recorded previously as tetraploid (Pinkava et al. 1971; 1973, 1977, 1992, 1998; Powell and Weedin 2001; 2004; Pinkava 2003). These are the first reports of diploid *O. macrorhiza* and likely represent descendants of those progenitors from which tetraploid *O. macrorhiza* s.l. and other polyploids arose. Likewise, this is the first report of diploid and triploid *O. pusilla*, which was formerly known only from tetraploid counts (Bowden 1945a).

Diploid members of *O. humifusa* s.l. (e.g., represented by the segregate taxa *O. ammophila* Small, 1919, *O. austrina* Small, 1903, *O. lata* Small, 1919, in this study; see also Appendix D) exhibit high levels of morphological variability but each is diagnosable morphologically, which suggests that these segregate taxa may need to be recognized at the species level. Likewise, diploid material of *O. macrorhiza* s.l. from eastern Texas (e.g., *O. xanthoglochla* Griffiths, 1910, in this study; see also Appendix D) and southeastern New Mexico is morphologically distinct from tetraploid material of *O. macrorhiza* s.l., which may also justify the recognition of *O. xanthoglochla* and *O. macrorhiza* as separate species.

Our hexaploid counts of *O. stricta* are consistent with those of Pinkava et al. (1992) and Negrón-Ortiz (2007). In contrast, Spencer (1955) reported *O. stricta* from Puerto Rico to be diploid. Other authors have also found Spencer's counts from Puerto Rico to be inconsistent with more recent counts (e.g., Negrón-Ortiz 2007 for *Consolea* Lem., 1862). Our three pentaploid counts of *O. ochrocentra* support the proposed hybrid origin of this species between hexaploid *O. stricta* ($2n = 66$) and diploid *O. abjecta* ($2n = 22$) through unreduced gametes of *O. abjecta*. *Opuntia ochrocentra* also exhibits intermediate morphological characters (e.g., growth form, spine characters) that further support its hybrid origin (LCM unpubl. data).

Diploid Refugia and Polyploidy Formation

Polyploidy is very common within the *Humifusa* clade, occurring in 66% of the samples reported here. Most researchers that have studied *Opuntia* cytologically have found polyploid taxa (e.g., Bowden 1945a; Weedon and Powell 1978; Pinkava et al. 1985; Doyle 1990; Segura et al. 2007; Baker et al. 2009a, b; Majure and Ribbens in press; but see Spencer 1955). All diploids in our analysis were restricted to either the southeastern or southwestern (eastern Texas and southeastern New Mexico) U.S., and the polyploid individuals were found nearly everywhere in between as well as north of these two diploid "refugia." The disjunct pattern observed here in the *Humifusa* clade and in other studies between the southeastern U.S. and the southwestern U.S. is thought to have occurred as a result of the disruption of a semi-arid zone along the Gulf Coast region during the mid-Pleistocene (Webb 1990; Althoff and Pellmyr 2002). These two areas likely served as glacial refugia for a variety of animals and plants (e.g., Remington 1968; Davis and Shaw 2001; Al-Rabab'ah and Williams 2002; Althoff and Pellmyr 2002; Soltis et al. 2006; Waltari et al. 2007; Whittemore and Olsen 2011) and may have promoted current species richness and genetic diversity in southern populations (Hewitt 2000). Specifically, Swenson and Howard (2005) identified southeastern Texas and northern Florida as Pleistocene refugia for

animal and plant species. Species from these regions subsequently came into contact following the last glacial maximum and formed hybrid zones at contact areas expanding out from these refugia. Swenson and Howard (2005) also hypothesized “postglacial routes of expansion” from these proposed diploid refugia (e.g., Fig. 1, G & H in Swenson and Howard 2005). Those postglacial routes and diploid contact zones are consistent with the current distributions of polyploid taxa within *O. humifusa* s.l. and *O. macrorhiza* s.l. The restricted diploid and widespread polyploid distribution pattern has been recorded in many other plants and is a common pattern seen in polyploidy complexes (Babcock and Stebbins 1938; Stebbins 1950, 1971; DeWet 1971; Lewis 1980; Grant 1981; Parfitt 1991).

The seemingly disjunct southeastern New Mexico diploid population of *O. macrorhiza* s.l. may represent a mere extension of the eastern Texas diploid refugium, which has since been mostly replaced by polyploid taxa. Alternatively, a diploid extension may still exist but was not detected due to the lack of cytological data for populations from east Texas to southeastern New Mexico (Fig. 5-2). Diploid taxa of other clades (e.g., *O. polyacantha* Haw. var. *arenaria* (Engelm.) Parfitt, 1819) are coincidentally found near the same region (Pinkava 2002, 2003), however, suggesting that a third diploid refugium, i.e., in southeastern New Mexico-western Texas, may need to be recognized.

Pinkava (2003) suggested that an *O. humifusa*-*O. macrorhiza*-*O. pottsii* complex originated along the east coast of the U.S. and spread westward to Arizona, where it came into contact and hybridized with *O. polyacantha* and formed the mostly hexaploid *O. tortispina*. From our data, this scenario is plausible in that *O. tortispina* has morphological characters representative of both *O. polyacantha* and *O. macrorhiza* and is found where populations of diploid and tetraploid *O. macrorhiza* s.l. and diploid *O. polyacantha* come into contact.

However, considering the two diploid refugia suggested by our analyses and what is known about the historical biogeography of the southeastern U.S. (e.g., Webb 1990), it is likely that the *Humifusa* clade originated in the southwestern U.S. and adjacent northern Mexico, then dispersed eastward into the southeastern U.S. The arid habitat along the coast of the Gulf of Mexico during the mid-Pliocene to early Pleistocene would have been interrupted during the mid-Pleistocene, creating the disjunct and promoting the genetic divergence among diploid populations we see today (Fig. 5-4). Taxa from these two diploid refugia would have come back into contact and formed the widely successful polyploids of the Midwest and eastern U.S. (Fig. 5-5). This scenario is further corroborated by phylogenetic analyses, where eastern U.S. polyploids of *O. humifusa* s.l. are resolved in a clade with the southwestern diploid *O. macrorhiza* (Fig. 5-4). The lower frequency of diploids encountered in western populations of the *Humifusa* clade also suggest that those diploid populations may be older (see Stebbins 1971, p. 157) than those of the southeastern U.S.; however, this could merely be a bias resulting from more limited sampling of western populations.

The various morphotypes of tetraploid *O. macrorhiza* in the western U.S. likely arose from southwestern diploid populations but subsequently spread in all directions after formation. Tetraploid *O. macrorhiza* appears to have arisen numerous times, given that several morphotypes exist throughout its range. However, only two diploid morphotypes are known to exist (eastern Texas and southeastern New Mexico), suggesting that other ancestral diploids may have since gone extinct or have not yet been found, or that polyploid taxa exhibiting unique, derived characters were partly responsible for the origin of certain morphotypes, which have no diploid counterparts. Stebbins (1971) suggested that there are several degrees of maturation of polyploidy complex formation (i.e., initial, young, mature, declining, relictual), which may be

deduced by comparing the relative geographic distribution of polyploids versus diploids. By these criteria, *Opuntia humifusa* s.l. and *O. macrorhiza* s.l. may represent a mature polyploid complex. The diploid taxa are less common than polyploids and are largely restricted in distribution, whereas the polyploid taxa are much more widespread.

Stebbins (1971) also proposed that mature polyploid complexes are relatively young, derived during the Plio- or Pleistocene epochs. This scenario would place polyploid formation in the *Humifusa* clade at the same time as Pleistocene megafauna. Thus, frequent environmental disturbances associated with glacial and interglacial cycles could have mediated the repeated contact of divergent diploid taxa leading to polyploid formation. Migrating herbivores would have then dispersed those polyploidy products over large geographic areas (Jansen 1986). Divergence time estimation of the *Humifusa* clade places the origin of the clade in the late Pliocene to early Pleistocene (LCM, RP, PG, WSJ, PSS, DES unpubl. data), in agreement with this scenario. The occurrence of only polyploid individuals in previously glaciated areas of the U.S. provides further evidence for their subsequent spread into those available niches following the last glacial maximum.

Many polyploid populations of *O. humifusa* s.l. and *O. macrorhiza* s.l., especially in the eastern U.S., are largely isolated from one another and from diploid populations, suggesting that polyploid formation is not ongoing, at least on such a large scale as during the Pleistocene or immediately after the last glacial maximum. In contrast, polyploids in *O. pusilla* are mostly sympatric with diploids in the Gulf of Mexico region and are represented by triploids and tetraploids. Polyploids of *O. pusilla* also do not share the wide geographic distribution of those polyploids derived from *O. humifusa* s.l. and *O. macrorhiza* s.l. These observations suggest that the polyploids of *O. pusilla* may have formed only recently, do not share comparable dispersal

agents, or lack the obvious adaptive advantages of those polyploids derived from *O. humifusa* s.l. and *O. macrorhiza* s.l.

Many polyploid populations of *O. humifusa* s.l. and *O. macrorhiza* s.l. occupy northerly distributions and thus have a very high tolerance to cold temperatures. The hexaploid *Opuntia fragilis* (Nutt.) Haw., 1819 (not in the *Humifusa* clade) similarly inhabits areas of northern North America (Parfitt 1991; Loik and Nobel 1993; Ribbens 2008; Majure and Ribbens in press), with diploid relatives (e.g., *O. polyacantha* var. *arenaria*) restricted to the southwestern U.S. (Parfitt 1991; Pinkava 2002). Thus, certain polyploid taxa appear to be more cold-resistant than their southerly diploid relatives (and presumed progenitors). *Opuntia humifusa* s.l. from northern areas of its distribution can withstand temperatures of -20°C (Nobel and Bobich 2002). However, the cold tolerance of diploid taxa has not been tested. Certain polyploid taxa of the *Humifusa* clade may therefore be better adapted to adverse environmental conditions than their diploid progenitors, which may partly explain their wide distribution relative to their diploid counterparts.

Agamospermy

The tetraploid *O. cespitosa* (an entity within *O. humifusa* s.l.; see Table 5-1) produces viable seed in the absence of outcrossing (Majure pers. obsv.), so this taxon is either self-compatible, which is common in Cactaceae (Rebman and Pinkava 2001), or agamospermous. Agamospermy is commonly associated with polyploidy (Stebbins 1950; DeWet and Stalker 1974; Harlan and DeWet 1975; Lewis 1980; Grant 1981; Whitton et al. 2008) and has been reported in numerous polyploidy *Opuntia* species as well (Reyes-Agüero et al. 2006; Felker et al. 2010), including *O. humifusa* s.l. and *O. stricta* (Naumova 1993). Agamospermy would account for the high level of morphological variation observed among polyploid populations, as a result of the maintenance of a specific genotype within a given population through the lack of

recombination (DeWet and Stalker 1974). Some agamic complexes also have wider distributions than their diploid progenitors (Babcock and Stebbins 1938; Stebbins 1950), as do certain polyploid taxa in this study.

Autopolyploidy vs. Allopolyploidy

The mechanism by which *Opuntia* polyploids are formed (auto- vs. allopolyploidy) is unclear. Unreduced gametes have frequently been found in meiotic analyses of Cactaceae (e.g., Pinkava et al. 1977; Pinkava and Parfitt 1982; Pinkava et al. 1985). Unreduced gamete formation coupled with interspecific hybridization (allopolyploidy) likely is a major factor in polyploid formation within the genus, given that *Opuntia* is renowned for hybridization (Benson 1982; Grant and Grant 1982; Pinkava 2002; Griffith 2004; LCM, RP, PG, WSJ, PSS, DES unpubl. data). It is probable that unreduced gamete formation within a single species (autopolyploidy) also plays a role in the formation of polyploids. Autopolyploids have been discovered in Cactaceae (Pinkava et al. 1985; Sahley 1996; Hamrick et al. 2002) and may be more common than is suspected.

Opuntia humifusa as currently circumscribed consists of numerous morphological entities, which are either diploid or tetraploid; those populations differing in ploidy are generally geographically well separated from one another. It is evident from our phylogenetic analysis (Fig. 5-4) that *O. humifusa* is polyphyletic. Considering morphological and genetic data, it is likely that tetraploid *O. humifusa* is of allopolyploid origin. However, the pattern in *O. pusilla* is different, with populations of diploids found in close proximity to populations of triploids and tetraploids (Fig. 5-3). This evidence, plus morphological similarity among ploidal levels, suggests possible formation of autopolyploids. This same pattern is seen in other autopolyploid taxa (Lewis 1967; Nesom 1983), although there are exceptions to this pattern (Stebbins 1950; Soltis 1984; Husband and Schemske 1998). Molecular phylogenetic analysis (Fig. 5-4) and

morphological characters (LCM, RP, PG, WSJ, PSS, DES unpubl. data; see Fig. 5-1E-G) of *O. pusilla* also do not support an interspecific hybrid origin for the different ploidal levels herein observed for this species, although more variable molecular markers, cytogenetic work, and more detailed morphological analyses are needed to appropriately address this question.

Morphological Correlations with Polyploids

Some polyploid taxa in the *Humifusa* clade share morphological characters with diploids and other polyploids, suggesting that they may be derived from hybridization (Table 5-2).

Opuntia nemoralis Griffiths, 1913, (Fig. 5-1J; an entity within *O. humifusa* s.l.; see Table 5-1) shares spine color and orientation, cladode color, and glochid color of tetraploid *O. macrorhiza* (from Arkansas), although, it possesses small and easily disarticulating cladodes, retrorsely-barbed spines, and the pile forming growth form and yellow flowers of *O. pusilla* (Fig. 5-1E-G). *Opuntia cespitosa* (Table 5-1), as mentioned above, exhibits the red-centered flowers, glaucous-gray cladodes, and dark glochids (Fig. 5-1I) of tetraploid *O. macrorhiza* (Fig. 5-1D), as well as the spine characters of diploid *O. humifusa* s.l. (= *O. ammophila*, *O. austrina*, *O. lata*; Table 5-2).

Throughout the distribution of the most common polyploid taxa, there also are polyploid populations that appear to be introgressive products of hybridization with other polyploids. For instance, in Michigan, Wisconsin, and western Illinois, certain populations display characters of both *O. cespitosa* and tetraploid *O. macrorhiza* (see Majure 2010, Fig. 5-1). In Bibb County, Alabama, populations appear to be intermediate between *O. cespitosa* and *O. pollardii* Britton & Rose, 1908, (tetraploids of *O. humifusa* s.l.; see Table 5-1), with the red-centered flowers and rotund cladodes of *O. cespitosa*, but the yellowish glochids and light green cladode color of *O. pollardii*. In Fayette County, Tennessee, plants appear intermediate between *O. humifusa* s.s. (i.e., tetraploid *O. humifusa* represented by the type collection) and *O. cespitosa*, having the

yellowish glochids of tetraploid *O. humifusa* s.s. and the spine characters of *O. cespitosa*. Each one of the areas in which these intermediate plants occur appears to be a region of secondary contact, where polyploid taxa have introgressed to form new polyploidy morphotypes that exhibit characters of both of the putative parents.

In the eastern U.S., most populations are represented by only one morphotype and thus appear to be morphologically stable (except for typically variable characters such as spine number; see Rebman and Pinkava 2001), indicating that hybridization is not ongoing among genomically distinct polyploid taxa. In contrast, in central Arkansas and populations farther west, more than one species and/or morphotype may be encountered within a given population. Also, in many coastal populations throughout the southeastern U.S., more than one species may be encountered, and putative hybrid taxa are sometimes observed.

Summary

Members of the *Humifusa* clade are found throughout most of the continental U.S., with no obvious breaks or disjuncts in distribution patterns until detailed analyses of chromosome number were carried out. Our analyses indicate that diploid taxa in the *Humifusa* clade are presently confined to the southwestern and the southeastern U.S., which likely represent Pleistocene refugia for these taxa. Polyploid taxa of *O. humifusa* s.l. and *O. macrorhiza* s.l. were likely formed when diploids from these two refugia came into contact during interglacial cycles of the Pleistocene. This scenario is supported further by phylogenetic analyses, in which two clades correspond to these two diploid refugia, and polyploid taxa are found in either clade. Polyploid taxa likely also contributed to the diversity of polyploid morphotypes through secondary contact and introgression with other polyploids. After the end of the last glacial maximum, open niches would have been readily available for colonization by polyploid taxa produced towards the leading edge of the expansion and distribution of the *Humifusa* clade. These polyploids

subsequently dispersed throughout most of the continent and occupied all suitable habitats available after glacial retreat, accounting for the distribution that we see today. Distributional success was enabled by the extreme cold tolerance displayed by many of the polyploid taxa, which allowed them to colonize more northern areas presumably unsuitable for diploid taxa.

Table 5-1. Synonyms of *O. humifusa* s.l. and *O. macrorhiza* s.l. sampled during this study.

<i>Opuntia humifusa</i> s.l.	<i>Opuntia macrorhiza</i> s.l.
<i>Opuntia allarei</i>	<i>Opuntia fusco-atra</i>
<i>Opuntia ammophila</i>	<i>Opuntia grandiflora</i>
<i>Opuntia austrina</i>	<i>Opuntia xanthoglochla</i>
<i>Opuntia cespitosa</i>	<i>Opuntia cespitosa</i>
<i>Opuntia lata</i>	<i>Opuntia lata</i>
<i>Opuntia nemoralis</i>	<i>Opuntia nemoralis</i>
<i>Opuntia pollardii</i>	<i>Opuntia pollardii</i>

Table 5-2. Selected taxa of *O. humifusa* s.l. and *O. macrorhiza* s.l. with morphological characters and corresponding ploidy. Polyploids often exhibit characters from more than one diploid taxon or characters of other polyploids, although certain characters (e.g., red glochids) have not been observed in any diploids analyzed thus far.

Taxon (ploidy)	Flower color	Cladode color	Spine barbedness/ Cladode disarticulation	Glochid color
<i>O. ammophila</i> (2x)	Yellow	Dark green	Not barbed/no	Stramineous
<i>O. austrina</i> (2x)	Yellow	Dark green	Barbed/yes	Stramineous
<i>O. cespitosa</i> (4x)	Red-centered	Glaucous green	Not barbed/no	Red
<i>O. lata</i> (2x)	Yellow	Dark green	Barbed/yes	Stramineous
<i>O. humifusa</i> (4x)	Yellow	Dark green	Not barbed/no	Stramineous
<i>O. macrorhiza</i> (4x)	Red-centered	Glaucous green	Not barbed/no	Red/yellow
<i>O. nemoralis</i> (4x)	Yellow	Glaucous green	Barbed/yes	Yellow
<i>O. pollardii</i> (4x)	Yellow	Dark green	Barbed/yes	Stramineous
<i>O. xanthoglochia</i> (2x)	Red-Centered	Glaucous green	Not barbed/no	Yellow

Table 5-3. Taxa used in phylogenetic analyses of ITS sequence data given with their GenBank accession numbers.

Accession	Locality	GenBank accession #
<i>Opuntia basilaris</i> (outgroup)	Inyo Co., CA R. Altig s.n.	JF786913
<i>Opuntia abjecta</i> (2x)	Monroe Co., FL LCM 3908	JF787021
<i>Opuntia abjecta</i> (4x)	Monroe Co., FL LCM 3318	JQ245716
<i>Opuntia ammophila</i> (2x)	Marion Co., FL LCM 2826	JF786904
<i>Opuntia austrina</i> (2x)	Highlands Co., FL LCM 3450	JF786911
<i>Opuntia cespitosa</i> (4x)	Scott Co., MO LCM 2441	JQ245717
<i>Opuntia humifusa</i> (4x)	Warren Co., VA LCM 3800	JQ245718
<i>Opuntia lata</i> (2x)	Irvin Co., GA LCM 3785	JF786949
<i>Opuntia macrorhiza</i> (4x)	Kerr Co., TX LCM 3510	JF786960
<i>Opuntia nemoralis</i> (4x)	Garland Co., AR LCM 2196	JQ245720
<i>Opuntia pusilla</i> (2x)	Lowndes Co., MS LCM 843	JQ245721
<i>Opuntia pusilla</i> (3x)	Baldwin Co., AL LCM 1091	JF786985
<i>Opuntia pusilla</i> (4x)	Jackson Co., MS LCM 1920	JF786986
<i>Opuntia tortispina</i> (6x)	Hutchinson Co., TX LCM 3533	JF787020
<i>Opuntia xanthoglochla</i> (2x)	Bastrop Co., TX LCM 1982	JQ245719

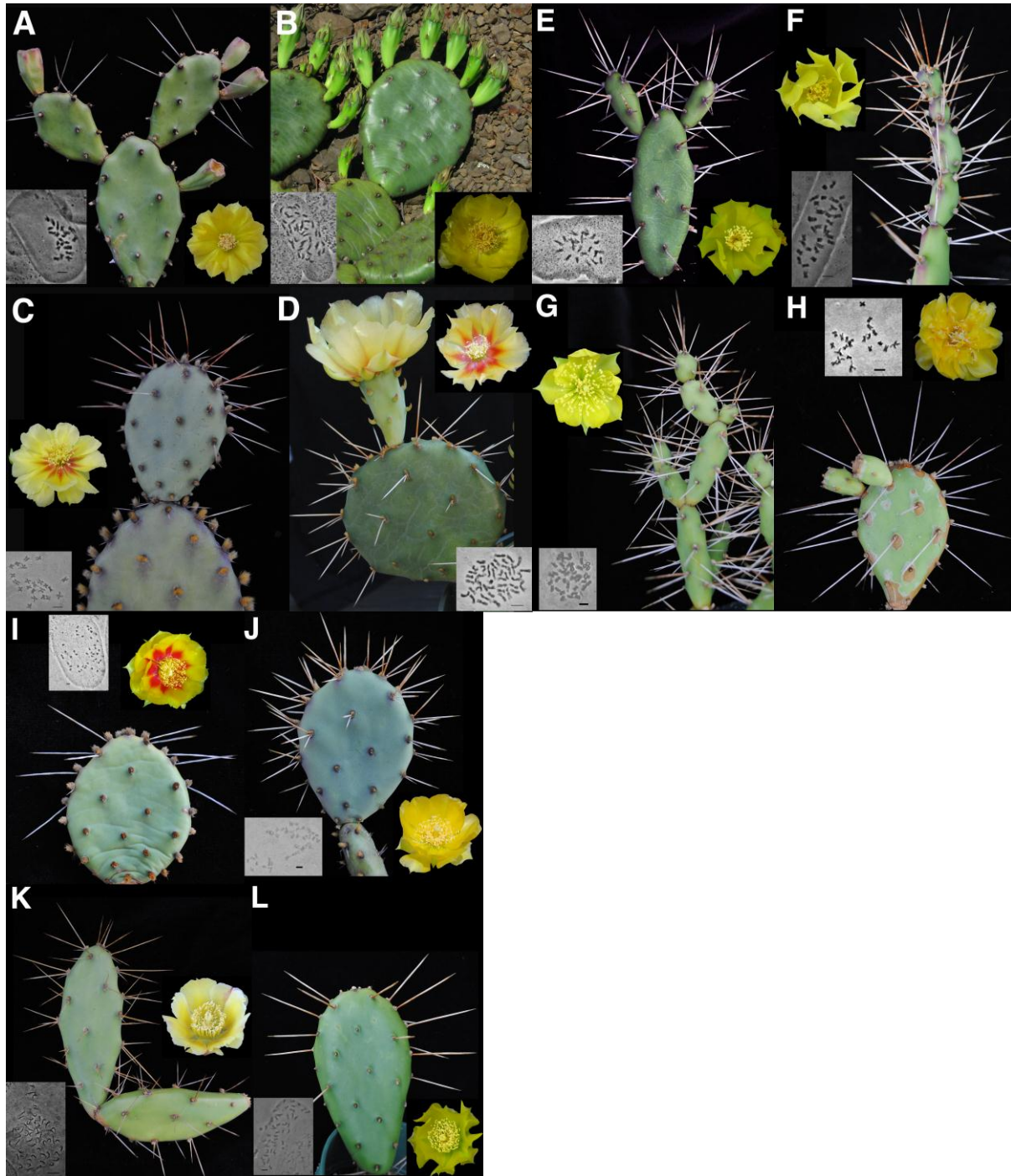


Figure 5-1. Selected taxa in the *Humifusa* clade with associated chromosome squashes A) diploid *O. humifusa* (*O. lata*) LCM 4106 B) tetraploid *O. humifusa* s.s. LCM 3810 C) diploid *O. macrorhiza* (*O. xanthoglochis*) LCM 1983 D) tetraploid *O. macrorhiza* LCM 3510 E) diploid *O. pusilla* LCM 753 F) triploid *O. pusilla* LCM1033 G) tetraploid *O. pusilla* LCM 3700 H) diploid *O. abjecta* LCM 3908 I) tetraploid *O. humifusa* (*O. cespitosa*) LCM 2610 J) tetraploid *O. humifusa* (*O. nemoralis*) LCM 4204 K) pentaploid *O. ochrocentra* LCM 3907 and L) tetraploid *O. humifusa* (*O. pollardii*) LCM 769. Bars on photomicrographs = 5 μ m.

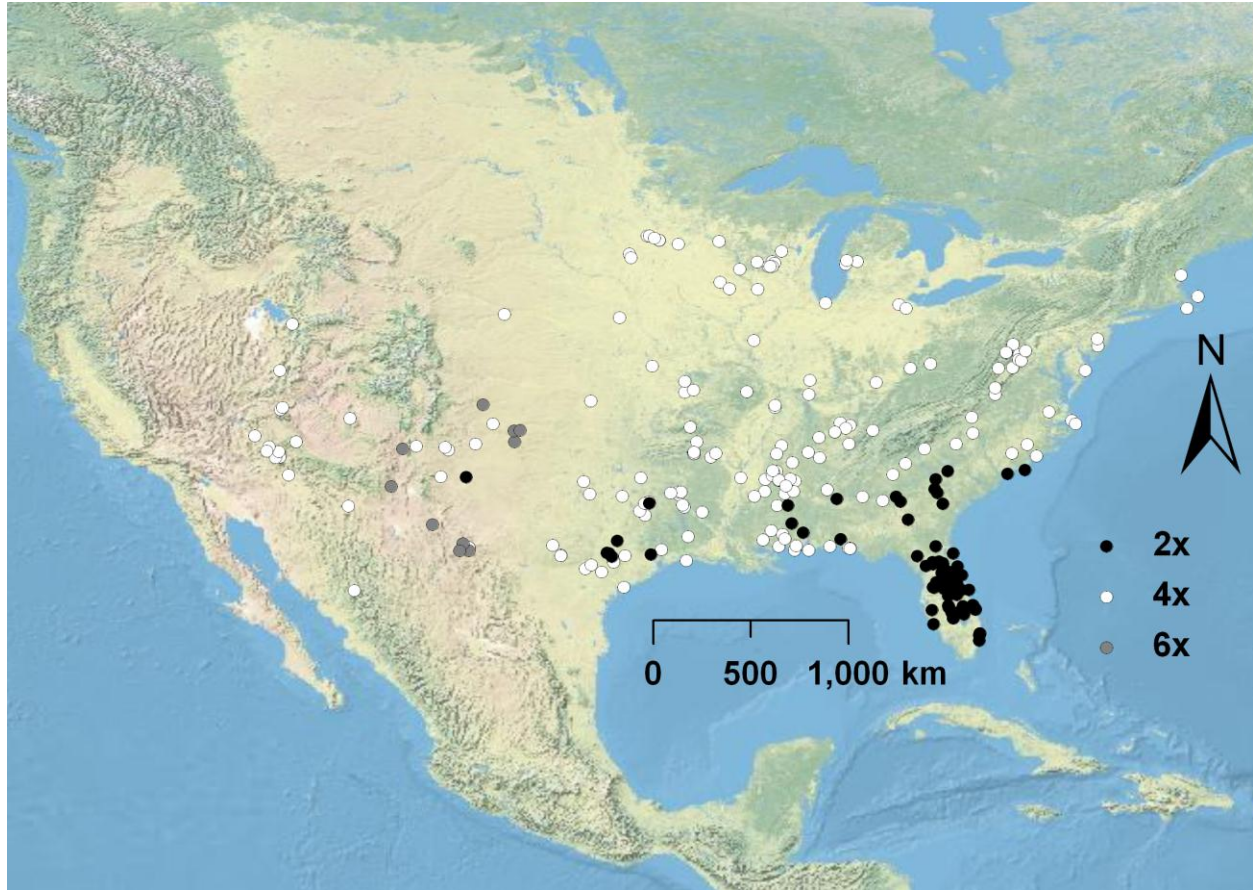


Figure 5-2. Cytogeography of *O. humifusa* s.l., *O. macrorhiza* s.l., *O. pottsii*, and *O. tortispina*. Diploids are represented with black circles, tetraploids by white circles, and hexaploids are represented by gray circles. *Opuntia humifusa* diploids are confined to the southeastern U.S., and *O. macrorhiza* diploids are located in eastern Texas and southeastern New Mexico.

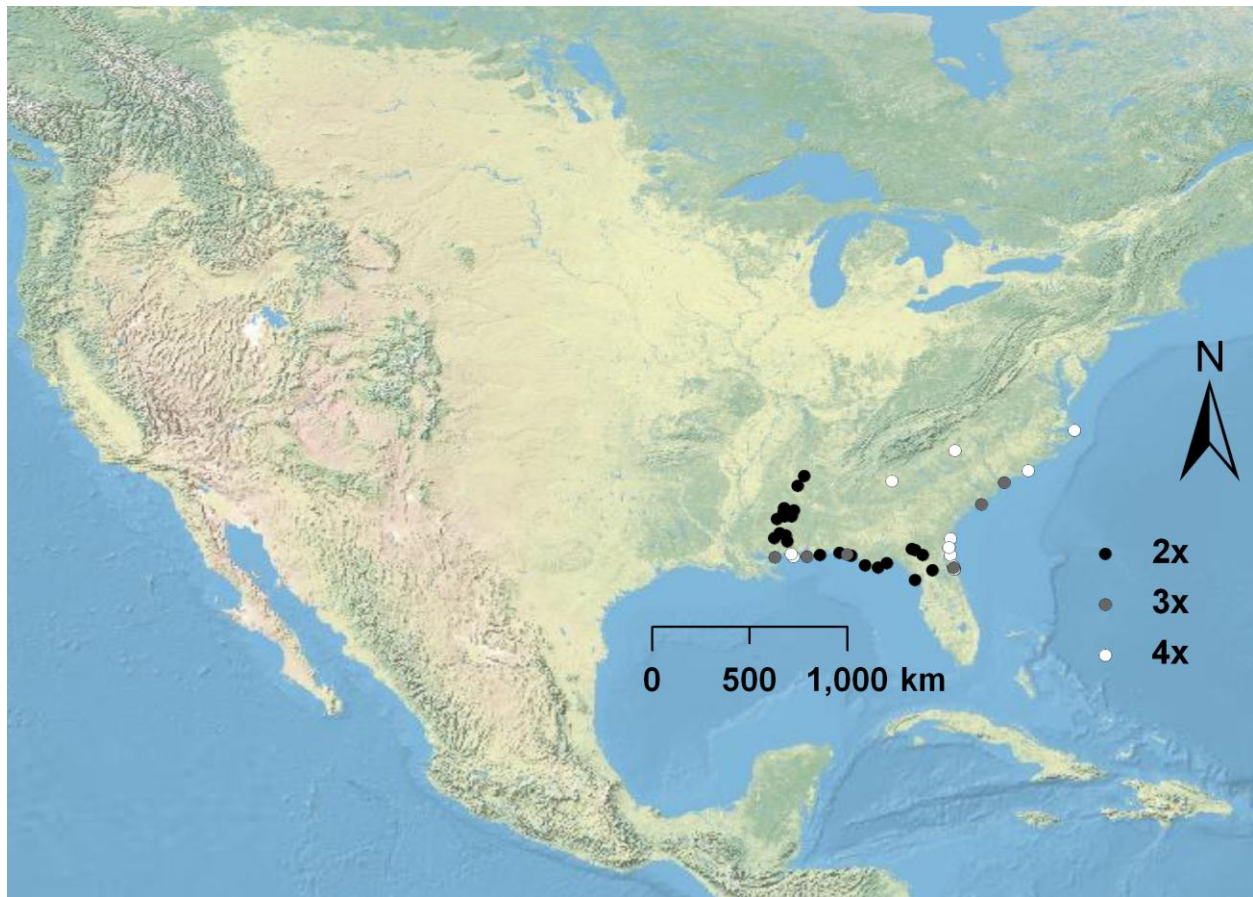


Figure 5-3. Cyto geography of *O. pusilla*. Diploids are represented by black circles, triploids by gray circles, and tetraploids by white circles. Note that most polyploids are restricted to coastal areas.

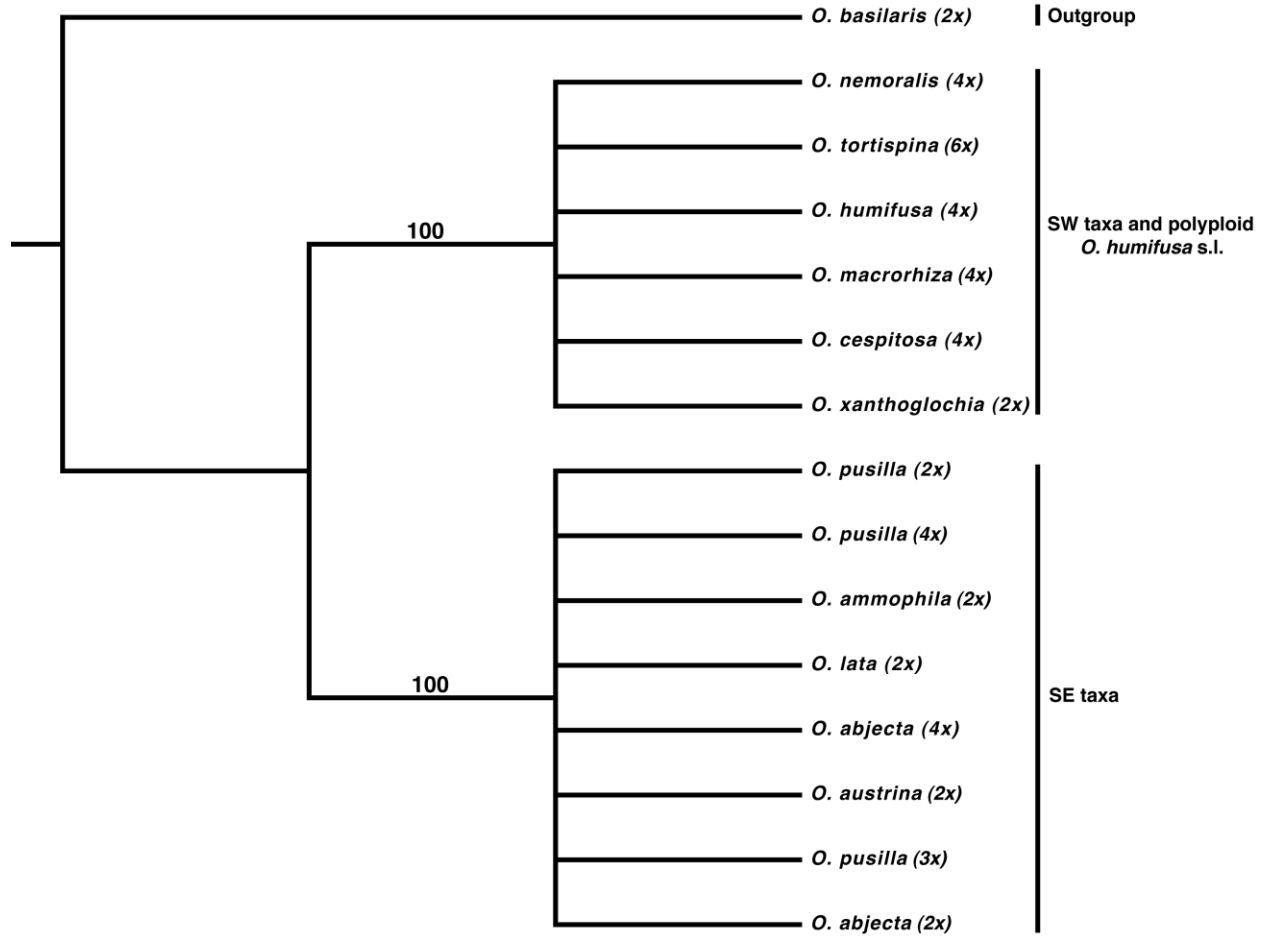


Figure 5-4. Majority-rule consensus topology from 10000 ML bootstrap pseudoreplicates using RAxML, based on the nrITS region. The western diploid *O. macrorhiza* s.l. (*O. xanthoglochia*) forms a well-supported clade with polyploid *O. macrorhiza*, *O. tortispina*, and the eastern polyploid morphotypes of *O. humifusa* s.l. (*O. cespitosa*, *O. humifusa*, and *O. nemoralis*). The southeastern diploid morphotypes of *O. humifusa* s.l. (*O. ammophila*, *O. austrina*, *O. lata*) and diploid *O. abjecta* and *O. pusilla* form a well-supported clade with polyploid members of *O. pusilla* and *O. abjecta*.

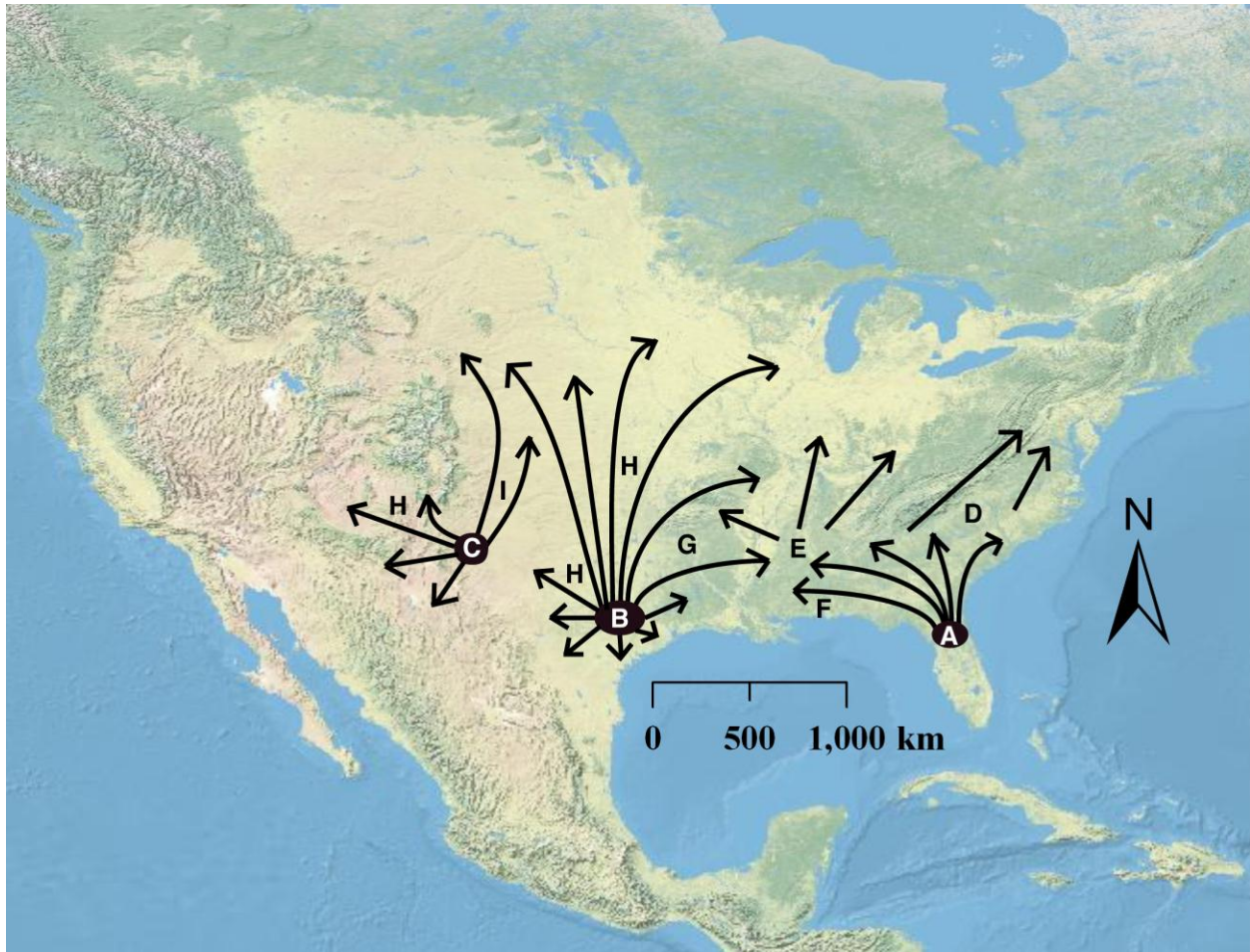


Figure 5-5. Hypothetical origin and subsequent dispersal of polyploid taxa from diploid refugia. Diploid refugia are represented by A southeastern *O. humifusa* s.l. diploids B–C eastern Texas and southeastern New Mexico *O. macrorhiza* s.l. diploids D–I represent polyploid formation where D represents *O. humifusa* E represents *O. cespitosa* F represents *O. pollardii* G represents *O. nemoralis* H represents tetraploid *O. macrorhiza* (showing likely multiple formations), and I represents tetra- and hexaploid *O. tortispina*.

CHAPTER 6
PHYLOGENY OF THE *Humifusa* CLADE (*Opuntia* S.S.): WHAT DIPLOIDS CAN TELL US
ABOUT THE EVOLUTIONARY HISTORY OF THE GROUP

Background

Opuntia s.s. (nopales, prickly pear cacti) is a well supported clade of shrubs and trees in subfamily Opuntioideae of Cactaceae (Majure et al. 2012a). Flat, succulent, photosynthetic stem segments with determinate growth characterize species within the clade (Pinkava 2003). These species may be either hummingbird or insect pollinated (Diaz and Cocucci 2003; Puente 2006; Reyes-Agüero et al. 2006; Majure et al. 2012a). The clade is suggested to have originated in southern South America with subsequent expansion into North America (Majure et al. 2012a) and is considered to have the widest geographical range of any genus within Cactaceae (Anderson 2001; Wallace and Dickie 2002).

The *Humifusa* clade is the result of a small radiation of insect pollinated species within *Opuntia* s.s., which is proposed to have originated in western North America with subsequent migration into the eastern United States at the end of the Pliocene or beginning of the Pleistocene (Majure et al. 2012a, b). The clade currently consists of six recognized species, *O. abjecta* Small, *O. humifusa* (Raf.) Raf., *O. macrorhiza* Engelm., *O. pottsii* Salm-Dyck, *O. pusilla* (Haw.) Haw., and *O. tortispina* Engelm. ex Bigelow (see Pinkava 2003; Majure et al. 2012a, b), although, the use of the name *O. tortispina* (Pinkava 2003) is mostly based on the misinterpretation of *O. cymochila* (Pinkava pers. comm.), so the name *O. cymochila* will be used throughout the rest of this study for that taxon. Morphological and cytological data suggest that the recognition of additional species in the clade may be warranted (Majure and Ervin 2008; Majure et al. 2012b).

As shown in Majure et al. (2012b) with ITS data, the *Humifusa* clade consists of two subclades, 1) the southwestern *O. macrorhiza* s.l. subclade (SW), which includes diploid and tetraploid *O. macrorhiza* s.l., tetraploid *O. humifusa* s.l., tetraploid *O. pottsii*, and tetra- and

hexaploid *O. cymochila*, and 2) the southeastern United States *O. humifusa* s.l. subclade (SE), which includes diploid *O. humifusa* s.l., diploid and tetraploid *O. abjecta*, and diploid, triploid, and tetraploid *O. pusilla*. This suggests that the widely distributed taxon *O. humifusa* s.l. is not monophyletic and may actually be composed of several morphologically cryptic species.

For example, the widespread, tetraploid taxon, *O. cespitosa*, which is currently in synonymy with *O. humifusa*, has yellow flowers with red centers, a character typical of the SW subclade, but exhibits a growth form and spine characters that are more typical of certain members of the SE subclade. Morphologically, this suggests that *O. cespitosa* may have originated from hybridization between the two subclades, but also that it constitutes a different entity that should be recognized separately from members of its putative progenitor subclades.

Although, *O. cymochila* was resolved in the SW subclade in previous analyses (Majure et al. 2012a, b), it is suggested, morphologically and cytologically, to have originated via hybridization between *O. macrorhiza* (of the SW subclade) and *O. polyacantha* (Pinkava 2003) of the *Polyacantha* clade (Majure et al. 2012a). This is supported by spine patterns, flower color, and tetra- and hexaploid chromosome counts, but has not been verified with DNA sequence data (Majure et al. 2012a, b).

Polyploidy is very common throughout *Opuntia* s.s. (Majure et al. in review) and is also quite widespread in the *Humifusa* clade. Out of 318 counts reported for the *Humifusa* clade, roughly two thirds (66%) were polyploid and 34% were diploid (Majure et al. 2012b). Polyploid taxa in this group are much more widespread than diploid members of the clade, extending from the southeastern United States, as far north as Ontario, Canada, but diploid taxa are restricted to two presumed glacial refugia in the southwestern and southeastern United States (Majure et al. 2012b). The pattern of widely distributed polyploids and geographically restricted diploids is a

common observation in polyploid complexes (Stebbins 1950; Grant 1981). The wide distributions exhibited by certain polyploid taxa may be facilitated by their higher cold tolerance, as compared to their southern diploid counterparts (Majure and Ribbens 2012; Majure et al. 2012b). For example, Nobel and Bobich (2002) report that *O. humifusa* s.l. from the northern United States (i.e., part of the polyploid distribution of the species; Majure and Ribbens 2012; Majure et al. 2012b) is able to survive temperatures as low as -25°C. Tolerance to more extreme environmental conditions by polyploid taxa in contrast to their diploid relatives is a common feature in many polyploid complexes (Stebbins 1950; 1971; Grant 1981; Levin 1983). Harsh environmental conditions have even been suggested to increase the frequency of polyploidy (Stebbins 1950; Grant 1981; see also review by Soltis and Soltis 2009).

Most polyploid taxa in the *Humifusa* clade are thought to have arisen as a result of secondary contact with divergent diploid taxa from the southwestern (SW clade) and southeastern United States (SE clade) during and after the Pleistocene (Majure et al. 2012b). Newly formed polyploids between these two clades would have subsequently occupied open, available niches northward concomitant with glacial retreat after the last glacial maximum (LGM). This scenario is supported by divergence time estimation of the *Humifusa* clade, polyploid distribution patterns, morphology, and a phylogenetic analysis using ITS sequence data (Majure et al. 2012a, b).

Species limits in the *Humifusa* clade are unresolved partly as a result of presumed hybridization among species resulting in individuals or populations demonstrating combinations of characters of putative progenitors, which may obscure clear morphological synapomorphies for species. Also, species of *Opuntia* are rarely collected, and when they are, poor collection methods of these succulents generally result in low quality specimens (Reyes-Agüero et al. 2007)

that lack much if any useful taxonomic information, as their three-dimensional structure is typically lost. Lastly, *Opuntia* are inherently morphologically variable, wherein morphological characters exhibited by an individual may depend on microclimatic conditions (e.g., numbers of spines produced, cladode sizes, etc.; Benson 1982; Rebman and Pinkava 2001; Majure 2007). Thus, species determinations may be virtually impossible from herbarium specimens unless the collector sampled morphological diversity from throughout a given population and made note of those morphological characters lost in the collection process (e.g., cladode thickness, epidermis color, flower color, growth form, etc.).

In this study we aim to reconstruct the phylogeny of the diploid members of the *Humifusa* clade to aid in the determination of species boundaries, as well as to test the origin of polyploid taxa (especially *O. humifusa* s.l.) within the clade, using maternally inherited plastid and biparentally inherited nuclear data. We further test the proposed hypothesis of the origin of polyploids via hybridization between the two diploid clades, which has been proposed based on diploid glacial refugia, polyploid distributions, and morphological characters (Majure et al. 2012b).

Material and Methods

Taxon and Marker Sampling

We sampled all six recognized species within the *Humifusa* clade (see above) from throughout their ranges, including diploids and polyploids of those species, when applicable (Majure et al. 2012b). We also sampled the different morphotypes of diploid and tetraploid *O. humifusa* s.l. (e.g., *O. ammophila* (2x), *O. austrina* (2x), *O. cespitosa* (4x), *O. humifusa* s.s. (4x), *O. lata* (2x), *O. nemoralis* (4x), *O. pollardii* (4x) and *O. macrorhiza* s.l. (e.g., *O. allairei* (4x), *O. fusco-atra* (4x), *O. grandiflora* (4x), *O. macrorhiza* s.s. (4x), *O. xanthoglochla* (2x)) (see Table 1). *Opuntia polyacantha* was used as an outgroup based on (Majure et al. 2012a) and to test the

origin of *O. cymochila*, as *O. polyacantha* is suggested to be one of the parents of *O. cymochila* (Pinkava 2003). We sampled the plastid intergenic spacers, *ndhF-rpl32*, *psbJ-petA*, *trnL-F*, the plastid genes, *ycf1* and *matK*, the low copy nuclear gene *ppc*, the nuclear ribosomal internal transcribed spacers (ITS) following Majure et al. (2012a), and the low copy nuclear gene, *isi1* (Rook et al. 2006). See Majure et al. (2012b) for primers and reaction specifications for *ndhF-rpl32*, *psbJ-petA*, *trnL-F*, *matK*, *ycf1*, *ppc*, and ITS.

After initial amplification, cloning, and sequencing of *isi1* products derived from primers designed by (Franck et al. in press), we discovered two copies of *isi1*, a “short” copy (ca. 555 bp long) and a “long” copy (ca. 1265 bp long). We designed the primers, *isi1*.Op.82F: 5’ GTC ACT ATG TAT GGT AGC CAT TGC CTG C 3’ and *isi1*.Op.1222R: 5’ GGA TGC TTT GAT TGC TTT GCT GCT GGA TTC 3’ for the long copy of *isi1*, as analysis of the long copy revealed molecular synapomorphies for both the SW and SE clades. Hence, this copy was deemed useful as a marker for uncovering potential reticulations between the two clades. Reaction specifications for *isi1* are the same for markers used in Majure et al. (2012a). PCR cycling conditions for *isi1* were as follows: 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.

We cloned a subset of polyploid taxa for ITS and *isi1* using the Stratagene cloning kit (Stratagene, La Jolla, CA) to search for multiple copies derived from the union of divergent genomes through allopolyploidy. The gene *ppc* was uninformative for this purpose and was not cloned for polyploid taxa. Cloning was focused on those polyploid taxa that were resolved in different locations using plastid and directly sequenced ITS products and the multiple polyploid taxa of *O. humifusa* s.l. (Table 1). We cloned one accession each of four tetraploid taxa of *O.*

humifusa s.l.: *O. cespitosa*, *O. humifusa* s.s., *O. nemoralis*, and *O. pollardii*. We also cloned ITS for tetraploid *O. macrorhiza* s.l., the tetraploid *O. pottsii*, and hexaploid *O. cymochila*. We cloned *isi1* products of tetraploid *O. macrorhiza* s.l. (including the taxa *O. allairei*, *O. macrorhiza* s.s., and *O. macrorhiza* from AR), and a segregate of *O. humifusa* s.l., i.e., *O. nemoralis*. (We had only marginal success amplifying *isi1* for many of the polyploid taxa). We sequenced eight clones of each accession using bacterial primers (T3-T7) from the kits. Sequences were edited either in Sequencher 4.2.2TM (Gene Codes, Inc., Ann Arbor, MI) or Geneious ProTM 5.1 (Biomatters Ltd., Auckland, NZ) and the alignment was adjusted manually in Se-Al v2.0 (Rambaut, 2007). Any obvious recombinant sequences were excluded from phylogenetic analyses.

Phylogenetic Analysis

Maximum likelihood (ML) analysis was conducted using RAxML (Stamatakis 2006) undertaking 1000 nonparametric rapid bootstrap (bs) pseudoreplicates under 25 rate categories using the GTR+ Γ model of molecular evolution for sequencing data and the BINGAMMA model of evolution for binary data (see below). We first performed a combined analysis of plastid and nuclear loci of only diploid taxa, as ploidy for all taxa under study here has been documented (Majure et al. 2012a) and the addition of allopolyploid (i.e., reticulate) taxa may lead to topological incongruence among data sets (Majure et al. 2012b), which is likely not the result of incomplete lineage sorting or other biological processes that could lead to incongruence (see Wendel and Doyle 1998). We separated our diploid dataset into 1) sequence data, and 2) sequence data plus binary data of 7 coded indels from the combined plastid and nuclear dataset. Indels coded were those that were most likely homologous among ingroup taxa based on the outgroup (i.e., basal-most taxa; Graham et al. 2000). Polyploid taxa were added to both plastid and nuclear datasets and analyzed separately, after initial analyses of diploid taxa, to test for

topological incongruence between nuclear and plastid phylogenies. Indel coding was not used for phylogenetic analyses with polyploids included. Topological incongruence for a given polyploid taxon among resultant plastid and nuclear phylogenies was taken as evidence for allopolyploidy (i.e., hybrid origin among divergent parental genomes).

Results

Phylogenetic Analysis (Diploid Taxa)

As in Majure et al. (2012b), the *Humifusa* clade was composed of two subclades, the southwestern *O. macrorhiza* clade (SW) and the southeastern *O. humifusa* clade (SE). The combined analysis of DNA sequence data along with indel codings, as well as analysis of DNA sequences alone, provide support for the two subclades (bs = 100/100 and 99/83, respectively; Fig. 6-1). Very little sequence divergence is evident in the resulting topology within the SE clade. *Opuntia pusilla* is resolved as sister to the rest of the clade, and diploid *O. humifusa* (*lata* entity) is supported by indel coding (bs = 79/), as sister to a clade containing *O. abjecta* and *O. humifusa* (*austrina* and *ammophila* entities). The three accessions of diploid *O. macrorhiza* are resolved in a well-supported clade (as noted above; bs = 100/100), however, the diploid *O. macrorhiza* entity referred to as *O. xanthoglochia* from eastern Texas does not form a clade with the other accession of *O. xanthoglochia* from eastern Texas but rather forms a well-supported clade (bs = 84/87) with diploid material from New Mexico.

Phylogenetic Analyses (Polyploid Taxa)

Plastid data resolve *O. humifusa* s.l. and *O. macrorhiza* s.l. in several places. The *O. humifusa* s.l. taxa (i.e., *O. cespitosa* (from MI, MS, and TN), *O. nemoralis* (3 accessions, AR=1 and LA=2), and *O. pollardii* (3 accessions, AL, GA, and MS)) and one unnamed taxon of *O. macrorhiza* s.l. (1 accession, AR) are resolved in a well-supported clade within a grade of SE diploid clade members. Likewise, *Opuntia humifusa* s.s. (3 accessions, MA, MD, and MS) is

unresolved with other members of the SE diploid clade. Triploid and tetraploid *O. pusilla*, and tetraploid *O. abjecta* are also resolved with diploid members of the SE clade. Members of both *O. humifusa* s.l. (i.e., *Opuntia allairei*, 1 accession, TX, *O. cespitosa*, 2 accessions, MI, WI, and *O. nemoralis*, 1 accession, LA) and *O. macrorhiza* s.l. [i.e., *O. fusco-atra*, 1 accession, TX, *O. grandiflora*, 2 accessions, MS, TX, *O. macrorhiza* s.s., 3 accessions, TX=2, NM=1, and *O. macrorhiza* (unnamed taxa), 2 accessions, AR, UT], as well as *O. pottsii* and *O. cymochila*, are resolved in the diploid SW clade (Fig. 6-2).

Directly sequenced PCR products of ITS for polyploid taxa virtually never exhibited polymorphisms in chromatograms. Directly sequenced ITS products of *Opuntia macrorhiza* s.s. and its segregate taxa, formed a well-supported clade with the SW diploids along with *O. pottsii* and *O. cymochila*. As well, most eastern taxa belonging to *O. humifusa* s.l. also were recovered in the SW clade (e.g., *O. cespitosa*, *O. humifusa* s.s., *O. nemoralis*), except for *O. pollardii* and one accession of *O. nemoralis* from LA, which were recovered within the SE clade (Fig. 6-3).

ITS clones of *O. humifusa* s.s. and *O. nemoralis* were recovered in both the SW and SE clades, while ITS clones of *O. pollardii* were only recovered in the SE clade and clones of *O. cespitosa*, *O. macrorhiza* s.l., and *O. pottsii* were only recovered in the SW clade. Clones of *O. cymochila* were recovered in the SW clade and with the outgroup, *O. polyacantha*, one of its putative progenitors (Fig. 6-3).

Only one copy type was recovered for *isi1* clones for *O. pollardii*, which was resolved again with the SE clade. Likewise, only one copy type was recovered for *O. nemoralis*, which was resolved in the SW clade. One accession each of *O. macrorhiza* s.l. (*O. macrorhiza* unnamed entity, AR) and *O. humifusa* s.l. (*O. allairei*) was resolved in the SW clade and a subclade of the SW clade with a clone of *Opuntia pottsii*. The same accession of *O. macrorhiza*

(unnamed entity) from AR also was resolved in the SE clade. Only one copy type of *isi1* was found for *O. macrorhiza* s.s., which was resolved in the SW clade (Fig. 6-4).

Discussion

The recent origin of the *Humifusa* clade (from the late Plio- to early Pleistocene; Majure et al. 2012a) most likely has not allowed sufficient time for notable sequence divergence among diploid members of the SE clade using the markers implemented in this study. However, taxon relationships among the diploid members of the SE clade are mostly resolved with DNA sequence data only, and are further supported with the addition of binary data from indel coding (see Fig. 6-1). Diploid taxa within the SE clade are morphologically diverse, ranging from small, prostrate species with disarticulating cladodes, mostly of the coastal zone of the southeast (excluding the Florida peninsula; e.g., *O. pusilla*), to large, robust shrubs or small tree-like taxa of the interior Florida peninsular scrub (e.g., *O. ammophila* and *O. austrina* entities, both elements within *O. humifusa* s.l.), and ascending to slightly erect, shrubs of the Florida Keys (e.g., *O. abjecta*). Morphological and phylogenetic data suggest that several diploid members of *O. humifusa* s.l. should be recognized as separate from tetraploid *O. humifusa* s.s., especially considering the paraphyly of *O. humifusa* s.l. in our diploid phylogeny (i.e., *O. ammophila* and *O. austrina* entities vs. the *O. lata* entity; Fig. 6-1).

Members of the diploid SW clade are not notably morphologically divergent from one another. The two accessions of the *O. xanthoglochia* entity are more similar to one another, morphologically, than with the one diploid accession from NM, however, they are not sister taxa in our phylogeny (Fig. 6-1). So, these diploid accessions likely represent one species considering morphological observations and phylogenetic data.

The wide genetic divergence between the SW and SE clades was further increased as those two clades were most likely separated during the early-mid Pleistocene by the proposed

disruption of the Gulf Coast arid zone (Webb 1990) as suggested by Majure et al. (2012b). The genetic discordance among members of both clades was thus influential in the production of allopolyploids when members of the SE and SW clades came back into contact with one another (see below *Opuntia humifusa* s.l.), although, the production of autopolyploids is likely a factor in the evolution of the diversity exhibited by both the SW and SE clades.

Opuntia abjecta* and *O. pusilla

Polyploid members of both *O. abjecta* and *O. pusilla* were always resolved in the SE clade, suggesting that those taxa were only derived from SE clade members (Table 2). Polyploids of these two species are nearly identical to diploid individuals suggesting possible autopolyploid formation (Stebbins 1950; Soltis et al. 2007). However, one tetraploid accession of *O. pusilla* and one tetraploid accession of *O. abjecta* were of different haplotypes than their putative diploid counterparts (and other polyploid accessions of both species), suggesting that they could have arisen through hybridization with another member of the SE clade, although, this will need to be tested further with population genetic level approaches. Genetic differences are also known to occur between autopolyploid taxa and their diploid progenitors (Soltis et al. 1989; Judd et al. 2007; Soltis et al. 2007).

***Opuntia humifusa* s.l.**

Our results indicate that *O. humifusa* s.l. is polyphyletic, with the polyploid taxa of *O. humifusa* s.l. being derived from separate crosses, mostly between the SW and SE clades, and the SE diploid taxa forming a paraphyletic assemblage (see above). The taxon *Opuntia humifusa* s.s. was derived from hybridization between the SW and SE clades, with the SE clade as the maternal lineage and the SW clade as the paternal lineage. The taxa referred to as *O. cespitosa* and *O. nemoralis* were each derived from two-way crosses, with the SE clade and SW clades serving as both maternal and paternal lineages. *Opuntia pollardii* appears to have been derived

solely from the SE clade (based on plastid, ITS, and *isi1* data), and *O. allairei* most likely is derived from the SW clade only (Table 6-2) and thus should not be considered synonymous with *O. humifusa* s.s., contrary to Benson's (1982) placement of the taxon.

The clade formed from the tetraploid taxon of *O. humifusa* s.l., *O. pollardii*, and close relatives in the plastid phylogeny consisted only of polyploid taxa (Fig. 6-2), so the diploid counterpart to this clade either was not sampled or simply no longer exists, although, the diploid, *O. lata* entity of *O. humifusa* s.l., is very similar morphologically to *O. pollardii*. Autopolyploid formation of *O. pollardii* cannot be ruled out. The close relationship of the putative SE-derived *O. pollardii* to those taxa derived from both the SE and SW clades (*O. cespitosa*, *O. macrorhiza*, AR, and *O. nemoralis*), suggests that *O. pollardii* is one of the putative parents of those taxa, at least in some crosses leading to those morphotypes.

***Opuntia macrorhiza* s.l.**

Opuntia macrorhiza s.s. and several other polyploid taxa (*O. allairei*, *O. fusco-atra*, *O. grandiflora*) were only resolved in the SW clade, suggesting that they originated via members of that clade only. Whether or not those polyploids were formed as the result of autopolyploidy or allopolyploidy is still to be determined. Diploid members of *O. macrorhiza* (e.g., entity *O. xanthoglochis*; Fig. 6-1), are morphologically very similar to tetraploid *O. macrorhiza* s.s., although, they have more tenuous spines and tend to be smaller plants, so the production of autopolyploids in this group is possible. Very few diploids appear to exist in the primary range of these taxa (e.g., southwestern United States), and it is likely that most putative progenitors of these polyploid taxa could be extinct or that some polyploid taxa were actually derived from crosses among other polyploid taxa. The production of fertile hybrids is most effective among taxa with the same chromosome number (Lewis 1967), and this could also account for the morphological diversity in polyploid taxa, which is not seen in the diploids.

One accession of *O. macrorhiza* from AR, however, is clearly an allopolyploid derived from the SW and SE clade. However, this individual is not typical, morphologically, for *O. macrorhiza*, as it produces flowers with completely yellow tepals. Typical flowers of *O. macrorhiza* s.s. have yellow tepals that are basally tinged red.

Opuntia pottsii

Opuntia pottsii also was resolved completely within the SW clade and exhibited plastid, ITS, and *isi1* sequences that were unique to this species. *Opuntia pottsii* is the strangest member of the SW clade, being the only species that commonly produces pink flowers and that has a single, stout trunk (although diminutive) much like the more robust taxon, *O. austrina*, of the SE clade. Determining the origin of this tetraploid will most likely require broader sampling of the species throughout its range, which extends into the Chihuahuan and Sonoran deserts (Powell and Weedin 2004). This will also require a search for putative diploid progenitors, if any still exist. It may also be possible that *O. pottsii* is of autopolyploid origin, or its putative diploid progenitors are extinct, and thus no morphologically similar taxa have been discovered for comparison with the species.

Opuntia cymochila

Although *Opuntia cymochila*, a mostly hexaploid species, has been recovered in the SW clade using ITS and plastid data (Majure et al. 2012a, 2012b), morphology has long-suggested that *O. polyacantha* of the *Xerocarpa* clade (sensu Majure et al. 2012a), may also be one of the putative progenitors (Pinkava 2003). ITS haplotypes recovered here also support a close relationship with *O. polyacantha* and the SW clade, implicating an interclade origin for this species. It is most likely that *O. cymochila* arose through hybridizations between a member of the SW clade and *O. polyacantha* at the boundary of diploid and tetraploid populations of *O. macrorhiza* s.l. and diploid populations of *O. polyacantha*, as suggested by Pinkava (2003). The

formation of *O. cymochila* likely has occurred numerous times, as both tetraploid and hexaploid individuals have been reported (Pinkava 2003; Powell and Weedin 2004; Majure et al. 2012b). Recurrent formation of polyploid species is not uncommon (Soltis and Soltis 1991, 1999; Soltis et al. 2007).

Morphological Characters of the SE and SW Clades

The SE and SW clades are morphologically distinct. Diploid members of the SE clade exhibit stramineous-colored glochids, spines that are typically retrorsely barbed to some degree, and flowers with completely yellow inner tepals. Diploid members of the SW clade, on the other hand, exhibit bright yellow glochids, smooth spines lacking noticeable retrorse barbs (at least to the touch), and flowers with yellow inner tepals that are basally tinged red, reddish-brown, red-orange, or reddish-pink. Both clades contain members that exhibit tuberous roots, a character attributed mostly to *O. macrorhiza* s.l. (Benson 1982) of the SW clade.

Although, numerous species of *Opuntia* have been recorded exhibiting more than one flower color (e.g., *O. macrorhiza*, *O. pottsii*; Pinkava 2003), it is clear from our analyses that differences in flower color are directly related to differential crosses leading to the origin of the taxon (or morphotype; see *O. macrorhiza* from AR above). This is easily exhibited in *O. humifusa* s.l., which is often reported as having yellow flowers or yellow flowers with red centers (Britton and Rose 1920; Small 1933; Kalmbacher 1976; Ferguson 1987; Doyle 1990). For example, the tetraploid taxon of *O. humifusa* s.l., *O. cespitosa*, has yellow flowers with red centers and was partially derived from the polyploid *O. pollardii* clade of the SE clade and partially derived from the SW clade (Table 6-2). Tetraploid *Opuntia humifusa* s.s., on the other hand, has completely yellow flowers and was derived from other members of the SE clade (not the polyploid *O. pollardii* clade) and the SW clade (Table 6-2). Thus, more research into

different flower colors exhibited by species of *Opuntia* may reveal that many of those morphotypes are of distinct origins from one another.

Spine characters also may be analyzed in this manner. Those spines produced by *O. cespitosa* resemble *O. pollardii* in length, diameter, and their development from the cladode, whereas *O. humifusa* s.s. is mostly spineless, as are some diploid SE populations of *O. humifusa* s.l. (*O. ammophila*, *O. austrina*, and *O. lata* entities). Spines produced by *O. nemoralis* are strikingly similar to those of *O. macrorhiza* s.l. (of the SW clade) in color and development from the areoles, while the growth form and flower color of *O. nemoralis* is suggestive of characters seen in *O. pusilla* (of the SE clade). Hence, morphological characters also often are indicative of the crosses leading to the formation of those taxa.

Summary

The *Humifusa* clade is composed of two well supported diploid subclades, the SE and SW clades, which diverged from one another most likely as a result of a break in the arid zone along the Gulf Coast of southeastern North America during the Pleistocene. Members of both clades eventually formed contact zones primarily in eastern North America, where they formed numerous allopolyploid entities, several of which appear to represent cryptic species. These allopolyploid taxa exhibit morphologically unique combinations of characters derived from their progenitor clades. Several of these polyploid taxa undoubtedly arose multiple times, as shown by bidirectional gene flow (i.e., from plastid and nuclear data), leading to the formation of those taxa (e.g., *O. cespitosa*, *O. nemoralis*; see Table 6-2).

Opuntia humifusa s.l. as currently circumscribed is highly polyphyletic, consisting of different ploidal levels, and a wide array of morphological diversity. Diploid members of *O. humifusa* s.l., according to our phylogeny, form a paraphyletic assemblage and thus should be recognized as separate taxa.

Consequently, our concept of the species that occur in the eastern United States must be reevaluated to take into account their evolutionary history, as revealed through cytological, morphological, and phylogenetic data, if we intend to incorporate the biological processes involved in species formation in this clade into an informative and predictive, phylogenetically accurate system of classification. However, if we are to regard different morphotypes of distinct origins as species, it will also require careful analysis of morphological characters and ploidy over the entire distribution of the taxon, where possible, to generate a practical system of classification based on cohesive morphological characters for a given species.

Table 6-1. Synonyms of *O. humifusa* s.l. and *O. macrorhiza* s.l. used in our analyses. Synonyms are listed on the left (based on Benson 1982, Pinkava 2003, Powell and Weedin 2004) and are given with their ploidy as reported by Majure et al. (2012a).

Synonyms	Currently Recognized
<i>Opuntia allarei</i> Griffiths (4x)	<i>Opuntia humifusa</i> s.l.
<i>Opuntia ammophila</i> Small (2x)	
<i>Opuntia austrina</i> Small (2x)	
<i>Opuntia cespitosa</i> Raf. (4x)	
<i>Opuntia lata</i> Small (2x)	
<i>Opuntia humifusa</i> (Raf.) Raf. s.s. (4x)	
<i>Opuntia nemoralis</i> Griffiths (4x)	
<i>Opuntia pollardii</i> Britton (4x)	
 <i>Opuntia fusco-atra</i> Griffiths (4x)	 <i>Opuntia macrorhiza</i> s.l.
<i>Opuntia grandiflora</i> Griffiths (4x)	
<i>Opuntia macrorhiza</i> Engelm. s.s. (4x)	
<i>Opuntia xanthoglochia</i> Griffiths (2x)	

Table 6-2. Polyploid taxa of the *Humifusa* clade sampled in our analyses of nuclear and plastid data. Taxa are listed with their inferred maternal lineage based on plastid data and inferred paternal lineage based on nuclear data.

Taxon	Maternal lineage (cp)	Paternal lineage (nuclear)
<i>O. abjecta</i>	SE Clade	SE Clade
<i>O. allairei</i>	SW Clade	SW Clade
<i>O. cespitosa</i>	SE Clade/SW Clade	SE Clade/SW Clade
<i>O. cymochila</i>	SW Clade	<i>Polyacantha</i> Clade
<i>O. fusco-atra</i>	SW Clade	SW Clade
<i>O. grandiflora</i>	SW Clade	SW Clade
<i>O. humifusa</i> s.s.	SE Clade	SW Clade
<i>O. macrorhiza</i> AR	SE Clade/SW Clade	SE Clade/SW Clade
<i>O. macrorhiza</i> s.s.	SW Clade	SW Clade
<i>O. nemoralis</i>	SE Clade/SW clade	SE Clade/SW Clade
<i>O. pollardii</i>	SE Clade	SE Clade
<i>O. pottsii</i>	SW Clade	SW Clade
<i>O. pusilla</i>	SE Clade	SE Clade

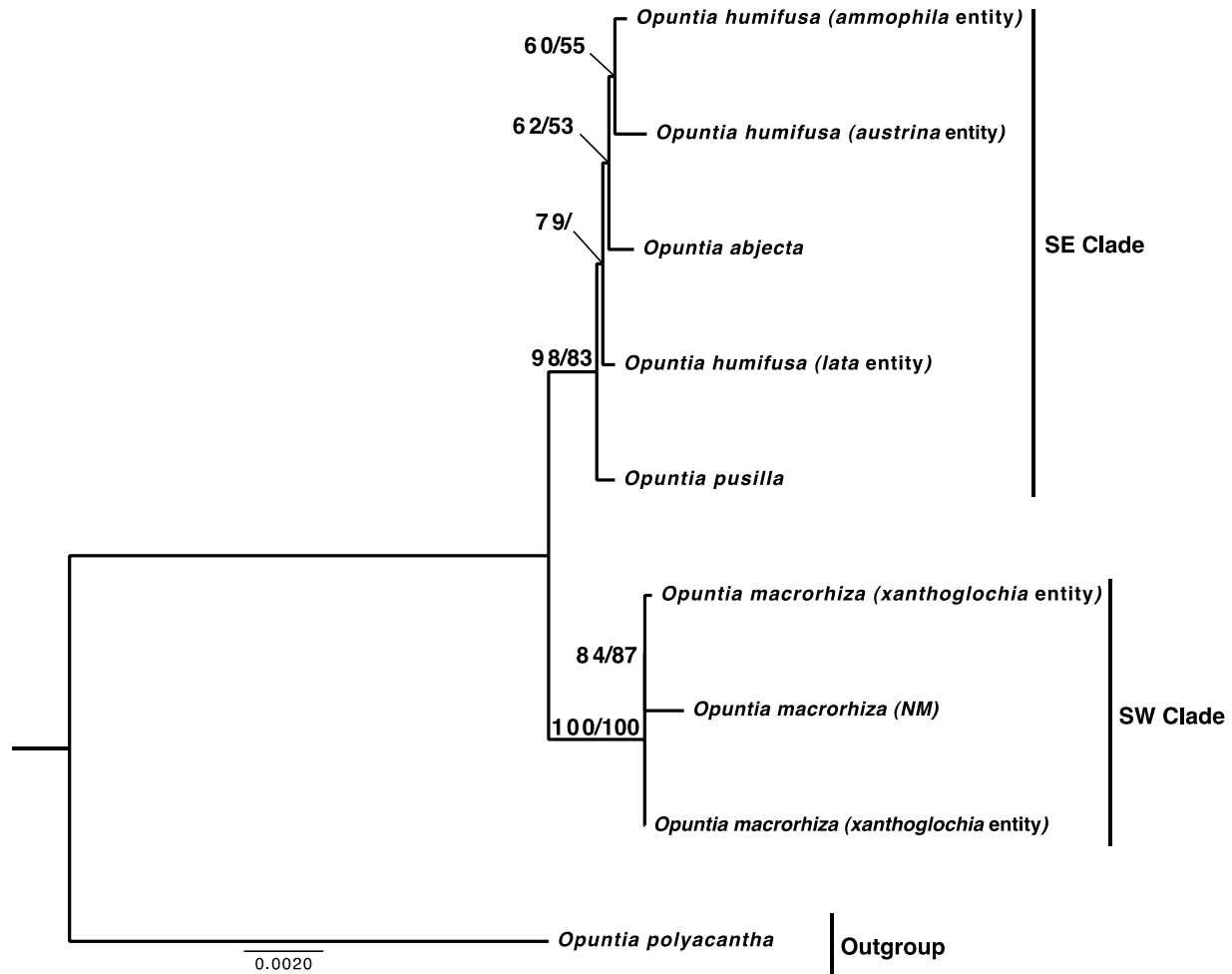


Figure 6-1. Phylogeny of diploid taxa of the *Humifusa* clade using combined plastid and nuclear data. In the SE clade, *O. pusilla* is sister to the rest of the taxa, and *O. humifusa* s.l. is made paraphyletic by *O. abjecta*. The diploid entity, *O. xanthoglochia*, of the SW clade (*O. macrorhiza* s.l.), is not sister to another accession of the same morphotype. Bootstrap values are given above branches. Bootstrap values on the right are for the sequence data plus indel coding dataset, and those on the left represent just the sequence dataset (see Materials and Methods).

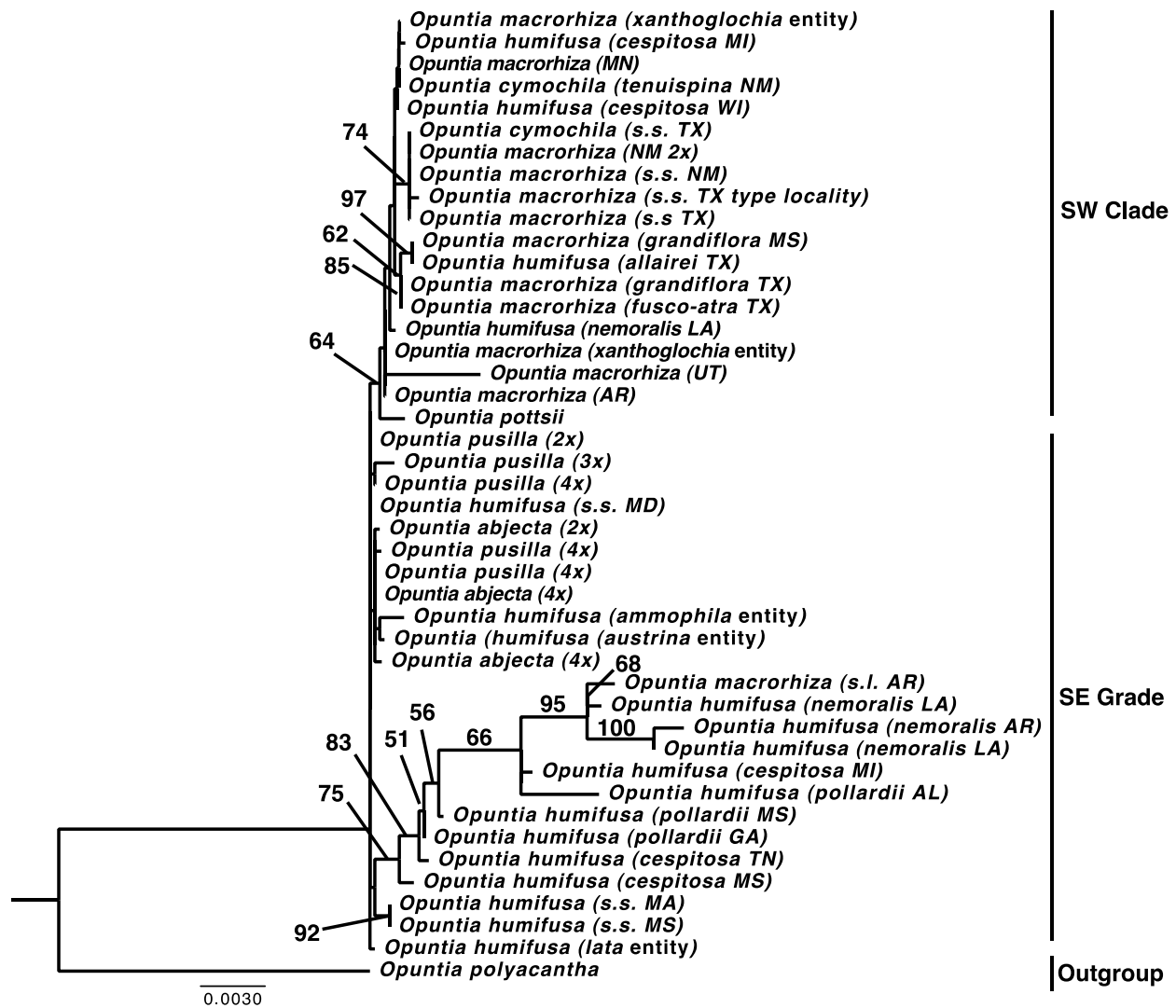


Figure 6-2. Plastid phylogeny including polyploid taxa. The SE clade of the diploid phylogeny is unresolved here as a grade, although, an entirely polyploid clade (the *pollardii* clade) is well supported (bs=75) within the SE grade. The SW clade is resolved, as in the diploid phylogeny. Members of *Opuntia humifusa* s.l. and *O. macrorhiza* s.l. are found in both the SW clade and the SE grade. Polyploid *O. pusilla* and *O. abjecta* are only recovered in the SE grade.

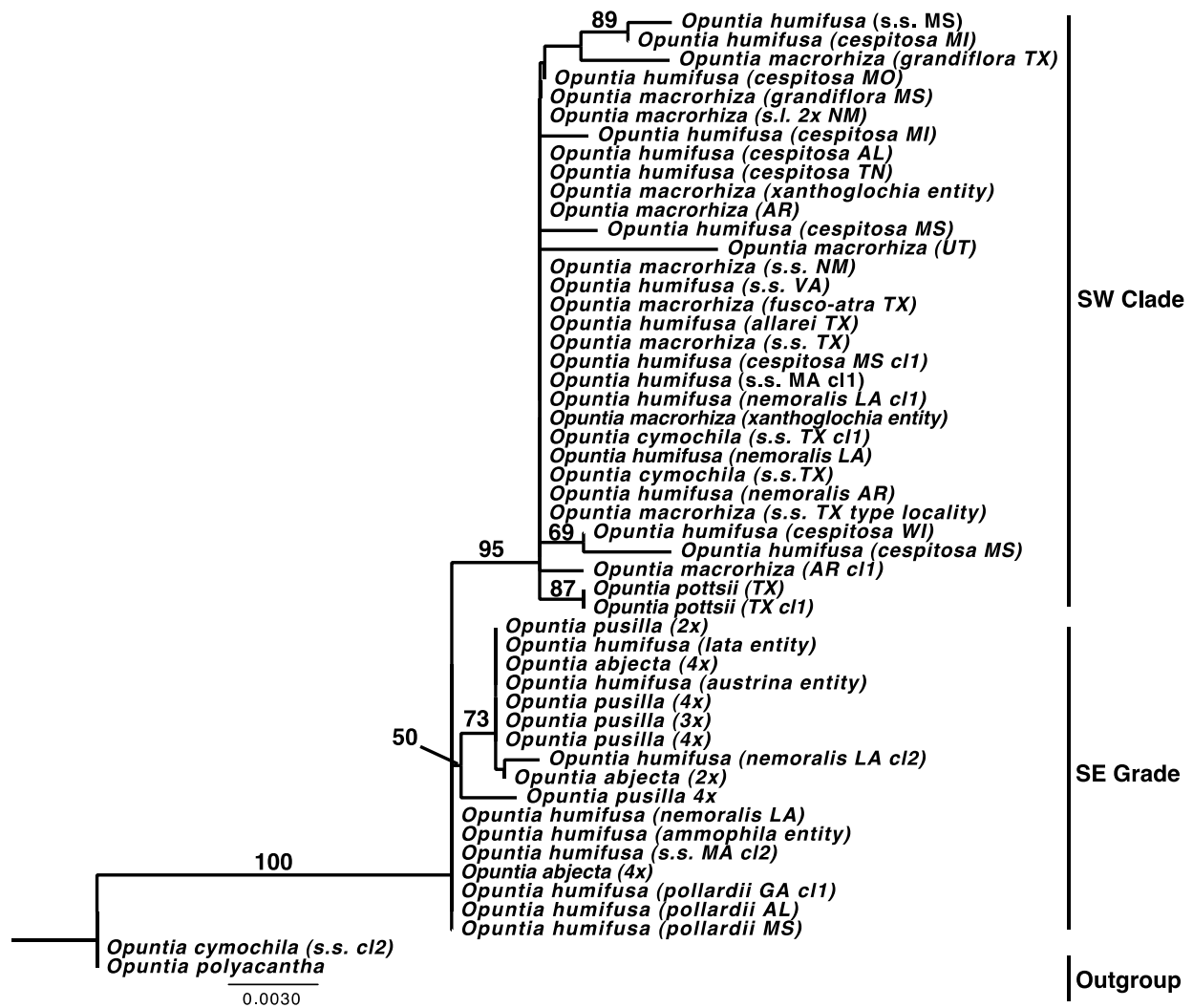


Figure 6-3. ITS phylogeny including polyploids. The SE clade of the diploid phylogeny is resolved here as a grade, as in the plastid phylogeny, although, one clade within the SE clade is resolved, albeit poorly (bs=50). The SW clade is again resolved and is well supported. Members of *O. humifusa* s.l. and *O. macrorhiza* s.l. are found in both the SW clade and SE grade, as well as the SE subclade. *Opuntia pollardii* is once again resolved with SE taxa. Clones of *Opuntia cymochila* are recovered within the SW clade and as sister to *O. polyacantha*.

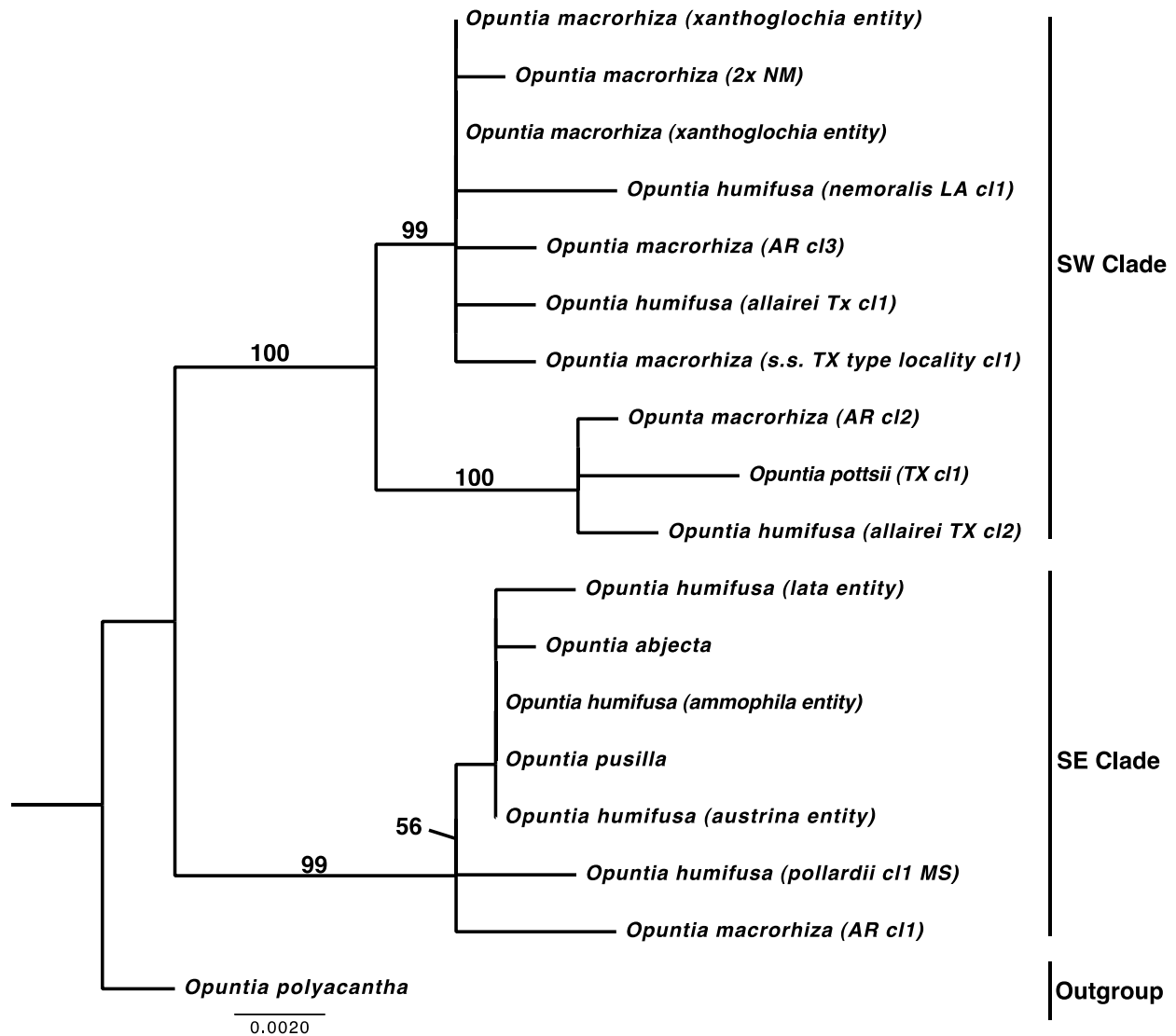


Figure 6-4. The *isi1* phylogeny including polyploid taxa. The SE and SW clades are well supported (bs=99 and 100, respectively). Once again members of both *O. humifusa* s.l. and *O. macrorrhiza* s.l. are resolved within both the SE and SW clades. *Opuntia pollardii* is resolved with SE clade members, as in the ITS and plastid phylogenies.

CHAPTER 7
TAXONOMIC REVISION OF THE *Opuntia humifusa* COMPLEX (*Opuntia*: CACTACEAE)
OF THE EASTERN UNITED STATES

Background

Opuntia Mill. is native throughout the Americas, ranging from southern Argentina to Canada (Anderson 2001); the genus occupies many habitats, from seasonally dry tropical and subtropical deciduous forests and scrub, to moderate desert environments, to temperate prairies, coastlines, and forest openings (Benson 1982). *Opuntia* is considered to be the most widespread genus in Cactaceae (Anderson 2001).

Opuntia exhibits very interesting morphological characters, which include longitudinally flattened stem segments, or cladodes, that take over the photosynthetic function of the small, ephemeral long shoot leaves that are produced as the cladode develops. Cladodes may be glabrous or pubescent and may be a number of different colors. All species of *Opuntia* have glochids, or retrorsely barbed and deciduous hair-like spines that are produced from specialized short shoots (areoles), which are mostly included within the stem tissue. These often become exerted and conspicuous as the cladode develops and form a formidable armament against herbivores. Long spines are also produced in most species. These can be strongly retrorsely barbed or smooth. Some species form one type of spine, while others may develop both central (those produced from the center of the areole) and radial spines (those produced from around the periphery of the areole). The development of spines from the areole can be a useful taxonomic character. Spine color changes through time but can also be diagnostic at the specific level. Virtually all species of *Opuntia* strongly produce betalain pigments under stressful conditions, so water or cold stressed plants often become reddish, pinkish, or purplish around the areoles. *Opuntia* can form shrubs and small or large trees. Most tree-like taxa are found in tropical or subtropical areas. In temperate areas, smaller shrubby taxa, which commonly sprawl or trail

along the ground, are more frequently found. Although not a synapomorphy of *Opuntia*, the seeds are characteristic in having a bony funicular girdle that surrounds a bony funicular envelope, which covers the embryo. The funicular envelope may be glabrous or hairy and surface features of the funicular envelope may be taxonomically useful for delimiting species.

Opuntia originated in the late Miocene in southern South America and from there dispersed north into the North American desert region (modern-day central and northern Mexico and southwestern United States), where the clade diversified and expanded through to the Caribbean Islands, and throughout the rest of the continental United States. A small clade, the *Humifusa* clade, eventually migrated to the eastern United States (Majure et al. 2012a), where it experienced an additional, small radiation there as well. The *Humifusa* clade consists of two subclades, a southwestern subclade (SW) including the widespread taxon *O. macrorhiza* s.l., and the southeastern subclade (SE), which includes the widespread taxon, *O. humifusa* s.l. and several other species (Chapter 6). The diploids of the SW subclade are characterized by procumbent species with yellow glochids, non-retrorsely barbed (smooth) spines, and yellow flowers with red centers. Red-centered flowers are also seen in diploid members of the sister clade to the *Humifusa* clade (i.e., *Macrocentra* clade), and so likely represents an ancestral state in the *Humifusa* clade. Diploids of the SE subclade are characterized by procumbent, trailing, and erect species that have stramineous glochids, retrorsely barbed spines (to some degree), and entirely yellow flowers. Numerous polyploid taxa have formed within the *Humifusa* clade. Several of those taxa were shown to be the products of hybridization between the SE and SW subclades and demonstrate characters of both of those clades (Majure et al. 2012b; Chapter 6). The SE clade and polyploid derivatives occurring in the eastern United States are here referred to as the *O. humifusa* complex.

Hybridization, Polyploidy, and Morphological Variability

Hybridization in *Opuntia* is common and plays into polyploid formation and oftentimes the origin of new species (Pinkava 2002; Majure et al. 2012a). The ability for taxa to readily hybridize and produce nearly fertile offspring would suggest the breakdown of species boundaries by those biologists following a strict biological species concept (Mayr 1942). Hybridization in *Opuntia* s.s occurs even among members of widely divergent clades and with other closely related genera, such as *Consolea* (Majure et al. 2012a). Hence, as in many plant groups, the ability to hybridize and form viable offspring is meaningless regarding species boundaries (Soltis and Soltis 2009).

In *Opuntia*, hybridization between different species is frequently associated with polyploidization (allopolyploidy; see Majure et al. 2012a), so reproductive barriers likely exist among divergent diploid species. However, polyploidization, of those hybrid derivatives, presumably aids in overcoming sterility barriers (Stebbins 1950, 1971; Grant 1981; Levin 1983). The true mechanism behind polyploidization in this group needs further study, however, unreduced gametes are commonly found in *Opuntia*, which are likely the primary cause of the formation of polyploids (Pinkava 2002) both within (i.e., autopolyploidy) and among species (i.e., allopolyploidy).

Although, allopolyploidy appears to be the most common type of polyploidy in *Opuntia*, autopolyploidy may also be relatively common. Several taxa of *Opuntia* have been suggested to produce autopolyploids [e.g., *O. abjecta*, *O. drummondii*, *O. humifusa* (subsp. *pollardii*), *O. macrocentra*, *O. strigil*; Majure et al. 2012a, b; Chapter 6], however, this needs to be investigated further.

Polyploidy serves as a bridge for species formation and, in many cases, the combination of different genomes, which may also lead to adaptations to extreme environmental conditions, as

in northern temperate members of the *Humifusa* clade (Nobel and Bobich 2002; Majure and Ribbens 2012; Majure et al. 2012b) and the *Polyacantha* clade (e.g., Nobel and Bobich 2002; Majure and Ribbens 2012).

It is very well known that *Opuntia* can be incredibly variable morphologically, in which cladode size, spine production, tuberous root production, among other features, are in many instances phenotypically plastic (Britton and Rose 1920; Benson 1982; Rebman and Pinkava 2001; Majure 2007; Majure and Ervin 2008). Thus, aside from hybridization and polyploidy, species delimitation in the group is made much more difficult, as populations of a species may show polymorphisms that result from growth under divergent environmental conditions (Majure 2007; Majure and Ervin 2008). Also, because of their succulence and spine production, opuntias are rarely collected, or the resulting specimens are improperly processed leading to scarce and very poor representation in herbaria (Reyes-Agüero et al. 2007).

Phenologically, species of *Opuntia* within the *Humifusa* complex are highly variable in flowering time, which seems to be directly related to changes in temperature regimes. For example, *O. austrina* in Florida alone may begin to flower in south Florida in mid-March but in the same year may bloom in north Florida at the end of March or beginning of April. The same individual, if moved to cooler climates, will further alter its flowering time. Material of *O. austrina* from Florida, which typically blooms around the beginning of April, blooms around the first or second week of May in central Mississippi (Majure, pers. obs.). The same phenomenon can be seen in *O. cespitosa* and *O. humfusa*. Southern populations start to flower before more northerly populations. Individuals taken from northern populations and transplanted to more southerly locations alter their flowering times within one or two growing seasons to nearly match those of the local inhabitants (Majure, pers. obs.).

Taxonomic History of the *O. humifusa* Complex

Opuntia humifusa (Raf.) Raf. was described in 1820, albeit with no type locality (Rafinesque 1820; as *Cactus humifusus*), as a low-growing, yellow-flowered, spineless (except for the glochids) species. Rafinesque described the range of the species as from New York to Kentucky and west to Missouri. The majority of the distribution given for *O. humifusa* by Rafinesque (Kentucky west to Missouri) actually is inhabited by a red-centered flowered *Opuntia* (see *O. cespitosa* below), so it is apparent that Rafinesque did not have a clear idea of the distribution of the species he was describing. Rafinesque (1820, 1830) noted the confusion of *Opuntia humifusa* with that of *Cactus opuntia* L. (basonym of *O. opuntia* (L.) Karst., nom. illeg.) of the Atlantic coast. Nonetheless, *O. humifusa* was again synonymized in later treatments under the tautonym *Opuntia opuntia* (see Britton and Rose 1920, Leuenberger 1993). Rafinesque (1830) described two more species, *O. cespitosa* Raf. from Kentucky and Tennessee (Rafinesque 1832), and *O. mesacantha* Raf. from west Kentucky to Louisiana (Rafinesque 1832), which also were subsequently placed in synonymy with *O. humifusa* (see Britton and Rose 1920).

Engelmann (1856) proposed another name, *O. rafinesquei*, apparently in honor of Rafinesque, which he used to replace all three previously described species, *O. cespitosa*, *O. humifusa*, and *O. mesacantha*. At this time, *O. vulgaris* Mill. was accepted instead of *O. opuntia* and thus two species were recognized in the eastern United States, *O. vulgaris* of the Atlantic coast and *O. rafinesquei* ranging in distribution from the Mississippi Valley from Kentucky to Missouri and north to Minnesota (Engelmann 1856). Notably, although Rafinesque (1820) gave nearly the same distribution for the species (his *O. humifusa*), he described the flowers as yellow, while Engelmann (1856) described them as being mostly yellow with red centers, demonstrating that Engelmann at least had a clear idea of the morphology of the species that grew throughout the range given with his description. *Opuntia macrarthra* Gibbes was later described by Gibbes

(1859) for low-growing, yellow-flowered material from South Carolina. Britton and Rose (1908) described yet another species of low-growing, yellow-flowered, spiny *Opuntia*, from the coast of Biloxi, Mississippi, *O. pollardii* Britton & Rose. Wherry (1926) described the yellow-flowered *O. calcicola* from West Virginia, a species apparently restricted to circumneutral soils. John Kunkel Small began his exploration of Florida in the early 1900s, where he described 10 species from the *O. humifusa* complex, *O. abjecta* Small, *O. austrina* Small (Small 1903), *O. ammophila* Small, *O. lata* Small (Small 1919), *O. eburnispina* Small, *O. impedita* Small, *O. pisciformis* Small, *O. turgida* Small (Britton and Rose 1923), *O. atrocapiensis* Small, *O. cumulicola* Small, *O. nitens* Small, and *O. polycarpa* Small (Small 1933), most of which Benson (1982) later placed in synonymy with *O. humifusa* or merely considered them hybrid derivatives of *O. stricta* (Haw.) Haw. and *O. humifusa* (except for *O. abjecta*). Benson (1982) placed *O. abjecta* of the Florida Keys in synonymy with the Caribbean species *O. triacantha* (Willd.) Sweet.

Benson (1982) recognized three varieties of *O. humifusa*, *O. humifusa* var. *ammophila* (Small) L.D. Benson, *O. humifusa* var. *austrina* (Small) Dress, and *O. humifusa* var. *humifusa*. Subsequent researchers have mostly followed Benson's treatment (Doyle 1990; Pinkava 2003), although, Pinkava (2003) did not recognize *O. humifusa* var. *austrina*, and Wunderlin and Hansen (2003, 2011) did not recognize any varieties within *O. humifusa*.

Oddly, Benson (1982) concluded that *O. humifusa* is strictly a yellow-flowered species, as further demonstrated in his key, although, his figure 438 of *O. humifusa* (Benson 1982; p. 439), is a typical specimen of what Majure and Ervin (2008) referred to as *O. cespitosa* that has yellow flowers with red centers. It is thus apparent that Dr. Benson did not have a clear idea of the delimitation of *O. humifusa*, a problem that likely developed from his use of herbarium specimens to interpret morphological variability across such a large range, and the fact that many

such specimens lose diagnostic features. Pinkava (2003) likewise suggested that *O. humifusa* has completely yellow flowers and used red-centered flowers to separate *O. macrorhiza* from *O. humifusa*. However, the majority of the distribution given for *O. humifusa* by both Benson (1982) and Pinkava (2003) is of the red-centered taxon referred to here as the tetraploid, *O. cespitosa* (see below). Leuenberger (1993) recognized that *O. humifusa*, although now widely accepted as the correct name of a widely distributed species in eastern North America, had not been formally typified. Thus, he neotypified *O. humifusa* based on material from Pennsylvania, as no type specimens for the species described by Rafinesque exist (Leuenberger 1993).

Opuntia drummondii Graham was described from Appalachicola, Florida (Maund 1846). Subsequent researchers described numerous taxa for the same type of material from the Atlantic and Gulf coasts, i.e., *O. pes-corvi* LeConte ex Engelmann (Engelmann 1856), *O. frustulenta* Gibbes (Gibbes 1859), and *O. tracyi* Britton (Britton 1911). Benson (1982) later placed all of these taxa, including *O. drummondii*, in synonymy under an ambiguous species of unknown origin and with no known type specimen, *O. pusilla* (Haw.) Haw. The name has since been accepted by subsequent researchers (Doyle 1990; Pinkava 2003; Wunderlin and Hansen 2003, 2011).

Opuntia nemoralis Griffiths was described from Longview, Texas by Griffiths (1913) and has since been placed in synonymy both with *O. drummondii* (Weniger 1967, 1970) and *O. humifusa* (Benson 1982).

More recently, Majure and Ervin (2008) suggested that *O. humifusa* is composed of several taxa and used the name *O. cespitosa* for material of *O. humifusa* s.l. with red-centered flowers. Cytological (Majure et al. 2012b) and phylogenetic (Majure et al. 2012a; Chapter 6) work has

provided further evidence, clearly indicating that *O. humifusa* is not monophyletic and actually consists of several taxa. Those taxa are treated here.

Seven species are recognized in this treatment of the *O. humifusa* complex. These are *Opuntia abjecta* Small, *O. austrina* Small, *O. cespitosa* Raf., *O. drummondii* Graham, *O. humifusa* (Raf.) Raf., *O. nemoralis* Griffiths, and *O. ochrocentra* Small. Three subspecies of *O. humifusa* are recognized: *O. humifusa* subsp. *humifusa*, subsp. *lata* (Small) Majure, and subsp. *pollardii* (Raf.) Majure. *Opuntia cespitosa*, *O. humifusa* subsp. *humifusa*, and *O. nemoralis* are allopolyploid derivatives of the southeastern (SE) and southwestern (SW) subclades of the *Humifusa* clade. *Opuntia humifusa* subsp. *pollardii* is a tetraploid, and apparently has been derived solely from the SE clade, while *O. humifusa* subsp. *lata* is a diploid member of the SE clade (Chapter 6). *Opuntia ochrocentra* is an allopolyploid derived from a member of the southeastern subclade and *O. dillenii* (Ker-Gawl) Haw. (Majure et al. 2012a; Chapter 4). Species outside of the *O. humifusa* complex that occur in the eastern United States, either as ornamentals or naturally, are not covered in this treatment (e.g., *O. engelmannii*, *O. fragilis*, *O. leucotricha*, *O. macrorhiza*, *O. monacantha*, *O. stricta*). In addition, this revision does not include members of the *Humifusa* clade that belong to the SW subclade, i.e., *O. macrorhiza*, *O. pottsii*, and relatives, which are species primarily distributed throughout the western United States and northern Mexico.

Species Concept

I apply a combined approach using phylogenetic, evolutionary, ecological, and morphological species concepts to delimit species in the *Humifusa* clade (Donoghue 1985; de Queiroz 2007). Species relationships and boundaries in *Opuntia* are obscured by the paucity of morphological characters and frequently also by the inadvertent loss of the few that exist in the process of preparing herbarium specimens (although with effort taxonomically useful specimens

can be prepared; see Reyes-Agüero et al. 2007). In addition, the succulence of these plants inhibits collectors, and the resulting lack of herbarium material, and especially those with useful habitat and morphological data, make specific and infraspecific delineation – exclusively through the use of herbarium specimens – virtually impossible in many instances. Thus the time consuming process of collecting and growing plants for use in assessing morphological variability (and correlating this variability with geography) is the only means to study the group in a relatively unbiased manner. The scarcity of detailed biological data, especially regarding variation in chromosome number, and the lack of an understanding of phylogenetic relationships also long has impeded proper species delimitation in this clade. Those data coupled with observations based on live material greatly enhance the ability to make accurate estimates of species boundaries. Undoubtedly, some researchers may find the species circumscription employed here to be too finely drawn, while others may wish that even more species had been recognized. I have taken a relatively conservative approach to species delimitation, underscoring the evolutionary history of these organisms, as well as their morphological cohesiveness and ploidy levels. The taxa here recognized are believed to be both biologically meaningful (reflecting the complex evolutionary history of the group) and diagnosable using accepted/traditional systematics methods (and thus appropriate for recognition in Floras and ecological investigations).

The following key was generated through the use of living specimens, supplemented by herbarium material, and so is most useful for identifying living individuals. In addition, knowledge of the range of morphological variation within a population is often necessary to accurately identify the species, as individuals within a population may or may not display characters essential for the identification of a given species (as the result of phenotypic plasticity,

age of the plant, or other factors). As a result, this dichotomous key is best used to identify a species when there is information about morphological variation within a given population and also when the entire plant, in living condition, is available for observation.

Description of the *Opuntia humifusa* Complex

Small to large shrubs or treelets, erect, decumbent, or trailing, 0.1-2 m tall, branching profusely or sparingly; with tuberous or fibrous roots. Cladodes elliptical, rotund, oblong, or obovate, 0.8-29.5 cm long, 0.6-11.3 cm wide, 4-19.9 mm thick, dark or yellow-green, or glaucous, gray-green, margins smooth or scalloped, remaining turgid or cross-wrinkling during the winter. Leaves green or glaucous, gray-green, 2.2-13.8 mm long, ascending parallel to the cladode or spreading, tips reflexed or not. Glochids conspicuous, exserted, or inconspicuous, included within the areole, red, reddish-brown, yellow, or stramineous when young, aging dark brown, light brown, or amber. Spines absent or 1-18 per areole, 0.9-10.3 cm long, 0.2-1.3 mm in diameter, dark brown, reddish brown, yellow, brown and white or brown, white, and yellow mottled during development, turning white with age and later gray, cylindrical, flattened, or twisted at the base, only central spines present or radial and central spines present, retrorsely-barbed or smooth to the touch. Flowers: outer tepals green, yellow green or red with light green margins, ovate, triangular, or triangular subulate, inner tepals yellow or yellow with red bases, 7-10, obovate, or obtriangular to emarginate, 2.2- 5.5 cm long, generally with a mucronate apex, staminal filaments yellow or yellow with yellow-green, or red bases. stigmas white, cream, or green, 3-10 lobed. Berries clavate or barrel-shaped, 1.8-5.0 cm long, pink, purple, red, orange-red, or green at maturity. Seeds 3.1-5.9 mm long, with the funicular envelope smooth, or only moderately elevated by the cotyledons and hypocotyl of the embryo, or bumpy, greatly elevated by the cotyledons and hypocotyl of the embryo, funicular girdle 0.4-1.3 mm wide, regular, smooth, or irregular, bumpy.

Key to the Members of the *Humifusa* Complex

1. Radial spines numerous, flattened at base.....*O. ochrocentra*
1. Radial spines 0-1, cylindrical or flattened at base.....2
2. Plants forming small trees, large shrubs, or sub-shrubs; stems ascending or erect, 0.3-2 m tall;
inner tepals entirely yellow.....3
2. Plants forming small shrubs in clumps or mats; stems ascending, decumbent or trailing; 0.1 to
0.5 m tall; inner tepals entirely yellow or yellow with red bases.....4
3. Plants developing from a single flat or terete stem (or trunk), usually erect or strongly
ascending, cladodes not easily disarticulating, spines \pm barbed to the touch, outer tepals
ascending, incurved, or recurved, in bud, Peninsular FL..... *O. austrina*
3. Plants branching from the base, thus forming clumps, stems strongly ascending, cladodes
easily disarticulating, spines strongly retrorsely barbed to the touch, outer tepals incurved
in bud, Florida Key.....*O. abjecta*
4. Cladodes easily disarticulating, flat or cylindrical, spines strongly retrorsely barbed to the
touch.....5
4. Cladodes not easily disarticulating, flat, spines \pm retrorsely barbed.....6
5. Cladodes glaucous, gray-green, developing spines yellow or bright white, glochids yellow or
dull brown, inner tepals yellow or rarely yellow with pinkish bases.....*O. nemoralis*
5. Cladodes not glaucous, dark green, developing spines dark reddish-brown, or brown and white
mottled, glochids stramineous, inner tepals entirely yellow, never with colored bases.....7
6. Cladodes not noticeably glaucous, dark green, inner tepals entirely yellow
.....*O. humifusa*
6. Cladodes glaucous, gray-green or lead-green, inner tepals entirely yellow, or yellow with
colored bases.....8

7. Plants small, sometimes even diminutive, cladodes 3.6 (0.8-11.1) cm long, 1.8 (0.6-3.4) cm wide, 10.4 (5.3-14.8) mm thick, elliptical, oblong, or rounded in shape, terminal cladodes mostly cylindrical in cross section, with 1-2 areoles per diagonal row at midstem, coastal southeastern United States.....*O. drummondii*
7. Plants larger, not diminutive, cladodes 7.6 (3.2-13.5) cm long, 4.5 (2.4-6.7) cm wide, and 10.2 (6.5-15.8) mm thick, elliptical, or rotund, terminal cladodes not cylindrical in cross section, with 2-3 areoles per diagonal row at midstem, Florida Keys
.....*O. abjecta*
8. Plants small, cladodes 6.3 (4.5-8.4) cm long, 3.9 (2.8-5.8) cm wide, 11.2 (8.1-14.2) mm wide, oblong, elliptical, or obovate, \pm easily disarticulating, spines \pm barbed to the touch, 2.3 (1.4-3.0) cm long, inner tepals almost always yellow, rarely faintly pink at the base, W of the Mississippi River*O. nemoralis*
8. Plants larger, cladodes 10.5 (3.8-18.7) cm long, 8.0 (3.2-11.3) cm wide, 10 (4-19.2) mm thick, mostly elliptical, obovate, or more commonly rotund, not disarticulating, spines smooth to the touch, 2.9 (1.5-4.3) cm long, inner tepals basally tinged crimson red, orange-red, reddish brown, or pinkish-red, widespread, eastern United States.....*O. cespitosa*

1. *Opuntia abjecta* Small in Britton and Rose, The Cactaceae, pp. 102 & 226c. 1923.—TYPE:

United States. Florida, Monroe Co.: hammock, southeastern tip of Big Pine Key, 12 Apr 1921, *J.K. Small s.n. with G.K. Small, P. Matthews* (holotype: NY!; see Fig. 7-2A).

Shrubs to 0.3 m tall, usually with multiple stems arising from the base, stems strongly ascending and rigid; roots commonly forming tubers in older individuals. Cladodes disposed mostly with margins parallel to the soil surface, thus the cladode disposed with the broad (flat) side perpendicular to soil surface, not becoming cross-wrinkled during the winter (as in other

non-erect species, such as *O. drummondii* and *O. humifusa*). Cladodes easily disarticulating from the nodes, generally dark green, not glaucous, and with slightly raised podaria, cladodes round to obovate (or more typically elliptical in tetraploids) in outline with 2-3 areoles per diagonal row at midsection of cladode, cladodes 7.6 (3.2-13.5) cm long, 4.5 (2.4-6.7) cm wide, and 10.2 (6.5-15.8) mm thick. Leaves dark green, ascending, parallel to the cladode surface, 5.2 (3.5-7) mm long. Glochids straw yellow (stramineous), areolar trichomes white. Spines mostly 2 per areole, but oftentimes 3 on terminal cladodes, but generally more on basal cladodes (up to 6), which continue to produce new spines, when 3 spines on terminal cladodes, 2 long and 1 short, the spines dark reddish-brown when young, turning white when mature and gray in age, strongly retrorsely barbed, twisted to cylindrical in cross section, most spines twisted at least at the base, 4.01 (2.5-5.6) cm long, 0.66 (0.33-0.99) mm in diameter. Flowers: outer tepals dark green, ovate, tepal tips erect to incurved in bud, apex of bud rounded to acute (Fig. 7-2E), inner tepals 8, dark yellow (Fig. 7-2G-H), 23.8 (21-26) mm long, stamens with yellow filaments turning orange-red as flower ages (Fig. 7-2G), stigma cream colored with 6 lobes. (The diploid population at Big Pine Key produces mutant flowers with inner tepals producing anthers at their tips and finally with normal stamens in the center of the flower surrounding the gynoecium (Fig. 7-2G; this aberrant flower type has also been seen in *O. austrina* and *O. drummondii*). The two tetraploid populations have completely normal flowers (Fig. 7-2H).) Berries barrel shaped, dark purple or yellow-green (Fig. 7-2I), 2.7 (2.1-3) cm long. (Tetraploids appear to only produce sterile fruit.) Seeds 3.3 (3.1-3.6) mm long, funicular girdle 0.80 (0.66-0.88) mm wide, funicular envelope smooth, i.e., with no impression of the embryo apparent on seed surface.

Phylogenetic placement. *Opuntia abjecta* is sister to *O. austrina* (Majure et al. 2012a; Fig. 7-1).

Ploidy. *Opuntia abjecta* is diploid, $2n=22$, and tetraploid, $2n=44$ (Majure et al. 2012b). The diploid population occurs at the type locality of the species (Majure et al. 2012b). There are cryptic morphological differences among the diploid and tetraploid populations (cladode shape, spine length), although, these minor differences are not suggestive of species boundaries. Based on phylogenetic studies (Chapter 6) and morphological similarity, it appears most likely that the tetraploid populations are autopolyploid in origin.

Phenology. *Opuntia abjecta* blooms in early spring (late March-mid April) in southern Florida, although, individuals transplanted further north bloom later (e.g., early May), demonstrating the plasticity in blooming time relative to climate.

Distribution. As far as is known, *Opuntia abjecta* is restricted to the Florida Keys, Monroe Co. (Fig. 7-3) and has only been recorded from three populations.

Habitat. *Opuntia abjecta* is restricted to Key Largo limestone of the lower Florida Keys where it can be found growing in depressions in the limestone containing enough humus to support root establishment.

Notes. Benson (1982) considered this species to be synonymous with the Caribbean taxa, *O. triacantha* (Willd.) Sweet and *O. militaris* Britton and Rose. However, it is clear from morphology and DNA sequence data that *O. triacantha* is more closely related to other Caribbean taxa, such as *O. caracassana*, *O. jamaicensis*, and *O. repens* Bello (Majure et al. 2012b; Chapter 4) rather than members of the *Humifusa* clade. Britton and Rose (1920) also considered *O. triacantha* to be more closely related to other Caribbean taxa, and even included the species in *Opuntia* Series *Tunae*, which includes *O. caracassana* and *O. jamaicensis*. *Opuntia militaris* is closely related to *O. caracassana*, *O. jamaicensis*, and *O. triacantha* but is likely not conspecific with *O. triacantha* (Chapter 4).

Opuntia triacantha is typically erect with a single, well-defined trunk, whereas *O. abjecta* has numerous ascending stems produced from the base of the plant but never produces an erect main trunk. *Opuntia triacantha* produces chalky yellow spines when immature, which mature chalky white. The spines of *O. abjecta* are darker reddish-brown when immature and mature bright white, not chalky white. Cladodes of *O. triacantha* are oblong to obovate or narrowly elliptic, while cladodes of *O. abjecta* are mostly rounded, obovate or broadly elliptic. *Opuntia triacantha* also has large tufts of yellow glochids associated with yellowish-clear trichomes, which are more pronounced than the stramineous glochids and white-clear trichomes of *O. abjecta*.

Additional specimens examined. United States. Florida. Monroe Co.: Big Pine Key, 12 May 1919, *P. Barrrtsch s.n.* (US); Long Key, rocky, open, low ground, 23 Apr 1966, *C. Byrd s.n.* (FLAS); Big Pine Key, 4 May 1951, *E.P. Killip 41332* (US); *ibid*, 10 Jan 1952, *E.P. Killip 41708* (US); SE end of Big Pine Key, Cactus Hammock, National Key Deer Refuge, 6 Mar 2010, *L. C. Majure 3908* (FLAS); Big Pine Key, 22 Feb 1935, *G.S. Miller, Jr. 1710* (US); Crawl Key, Jul 2008, *K. Sauby s.n.* (FLAS); Big Pine Key, 17 May 1922, *J.K. Small s.n.* (NY, US).

2. *Opuntia ochrocentra* Small in Britton and Rose, *The Cactaceae*, p. 262. 1923. —TYPE:

Florida, Monroe Co.: Big Pine Key, hammock, southern end of Big Pine Key, 11 Dec

1921, *J.K. Small s.n.*, with *G.K. Small*, *P. Matthews* (holotype: NY!; see Fig. 7-4A).

Large, scrambling to slightly erect shrub from 0.4-0.5 m tall, usually with one main trunk, although, branching heavily above; roots fibrous. Cladodes mostly elliptical or rarely obovate in outline, with slightly scalloped margins, terminal cladodes disarticulating with only slight force, cladodes light green, 15.6 (11.6-19) cm long, 7.5 (5.9-8.9) cm wide, 14.3 (13.2-16.2) mm thick, with 3-4 areoles per diagonal row. Leaves light to dark green, small, 3.6 (3.2-3.9) mm

long. Glochids bright yellow (as in *O. dillenii*), conspicuous. Spines developing from the areoles in a stellate pattern (as in *O. dillenii*), 1-5 spines per areole, central spines delicate, 5.3 (4.7-5.8) cm long, 1.04 (0.86-1.3) mm in diameter, cylindrical in cross section or basally twisted, radial spines flattened at the base and deflexed along the face of the cladode in age, immature spines yellow aging white or mottled cream and brown and then gray. Flowers: outer tepals broadly ovate or triangular-ovate, yellow-green or reddish with light green margins, inner tepals 8, entirely yellow or yellow green, obovate or emarginate, with a mucronate tip, the abaxial surface often reddish down the center, 3.5 (2.8-3.7) cm long, stamens with yellow filaments, stigma white or light yellow-green, 6-lobed. Berries clavate or barrel-shaped, although, mature fruit not been seen in cultivated material from Big Pine Key or Big Munson Island, immature fruit 3 (2.8-3.3) cm long, and mature fruit reported to be red and to 2 cm long (Small 1923). Seeds not seen (and not present on lectotype or any other material available for study), but described as 2.5-3 mm long, and numerous (Small 1933).

Phylogenetic Placement. This species is a pentaploid (Majure et al. 2012b) of interclade hybrid origin most likely between *O. abjecta* and *O. dillenii* (Ker-Gawl.) Haw. (Majure et al. 2012a, Chapter 4), with which it is largely sympatric on Big Pine Key.

Ploidy. *Opuntia ochrocentra* was reported as pentaploid, $2n=55$, from three individuals that have been analyzed (Majure et al. 2012b).

Phenology. *Opuntia ochrocentra* flowers in late spring to early summer (early April – May) growing in cultivation in north Florida.

Distribution. *Opuntia ochrocentra* is only known from the lower Florida Keys and co-occurs with *O. abjecta* on Big Pine Key, where it has nearly been extirpated through attack by *Cactoblastis cactorum* Berg. (Majure 2010; Majure pers. obs.) and anthropogenic disturbance

(Benson 1982, Majure pers. obs.). It is also known from Big Munson Island, just west of Big Pine Key (see collections *L.C. Majure 3968-69*), where it has also been seen under attack by *C. cactorum* (Majure pers. obs.). It has been recorded from Cape Romano as well (Small 1933), but no specimens have been seen from that locality (Benson 1982; Majure pers. obs.).

Habitat. *Opuntia ochrocentra* occurs essentially in the same habitat as *O. abjecta*, one of its putative parents (Majure et al. 2012b).

Notes. This species was placed in synonymy with *O. cubensis* Britton and Rose by Benson (1982). Molecular, morphological, and cytological data show that *O. ochrocentra* is not conspecific with *O. cubensis* and thus should not be considered synonymous with that Cuban species (Chapter 4). As noted by Britton and Rose (1923), *Opuntia ochrocentra* most closely resembles *O. dillenii*, one of its putative progenitors, although, its spines are more delicate and age gray as in *O. abjecta* (its other putative progenitor; Majure et al. 2012a; Chapter 4). *Opuntia ochrocentra* also forms a smaller, more delicate shrub compared to the more erect and robust growth form of *O. dillenii*.

Additional specimens examined. United States. Florida, Monroe Co.: Big Pine Key, 12-18 Feb 1935, *E.P. Killip 31423* (US); Big Pine Key, hammock, 2 Mar 1936, *E.P. Killip 31712* (US); Big Pine Key, SE hammock, 19 Mar 1952, *E.P. Killip 42026* (US); S end of Big Pine Key, 6 Mar 2010, *L.C. Majure 3907* (FLAS); Big Munson Island, 8 Mar 2010, *L.C. Majure 3968-69* (FLAS); hammock, S front of Big Pine Key, 17 May 1922, *J.K. Small s.n.* (US).

3. *Opuntia austrina* Small Fl. SE. US. p. 816. 1903. *Opuntia compressa* (Salisbury) J.F.

Macbride var. *austrina* (Small) L.D. Benson, Cact. Succ. J. 41: 125. 1969. *Opuntia*

humifusa (Raf.) Raf. var. *austrina* (Small) Dress, Baileyana 19 (4): 164. 1975.—TYPE:

United States. Florida, [Miami-Dade Co.:] Miami, in pinelands, 28 Oct-28 Nov 1903,

J.K. Small 1216, with J.J. Carter (lectotype designated by L.D. Benson (1982): US!; isolectotype: NY!; see Fig. 7-5A).

Opuntia ammophila Small, J. New York Bot. Gard. 20: 29. 1919. *Opuntia compressa* (Salisbury)

J.F. Macbride var. *ammophila* (Small) L.D. Benson, Cact. Succ. J. 41: 124. 1969.

Opuntia humifusa (Raf.) Raf. var. *ammophila* (Small) L. D. Benson, Cact. Succ. J. 48:

59. 1976. —TYPE: United States. Florida. [St. Lucie Co.:] hammock on sand dune, St.

Lucie Sound, 6 mi S of Ft. Pierce, 20 Dec. 1917, *J.K. Small 8456* (holotype: two sheets, NY!).

Opuntia pisciformis Small in Britton and Rose, Cactaceae 4: 258. 1923.—TYPE: United States.

Florida. [Duval Co.:] dunes, Pilot Island, 26 April 1921, *J.K. Small s.n.* (holotype: NY!).

Opuntia turgida Small in Britton and Rose, Cactaceae 4: 265. 1923.—TYPE: United States.

Florida. [Volusia Co.:] about 5 mi S of Daytona, 30 Nov 1919, *J.K. Small s.n.* (holotype: NY!, two sheets; isotype: US!).

Opuntia atrocapensis Small, Man. SE. Fl. 905. 1933.—TYPE: not found, and therefore a neotype

is designated here: United States. Florida. Monroe Co.: sand dunes; Middle Cape Sable, 28 Nov 1916, *J.K. Small s.n.* (US!).

Opuntia cumulicola Small, Man. S. E. Fl. 907. 1933.—TYPE: United States. Florida. [Miami-

Dade Co.:] beach. Bull Key, opposite Lemon City, 6 Nov. 1903, *J.K. Small 970 with J.J. Carter* (holotype: NY!; isotypes: NY!; US!).

Opuntia nitens Small, Man. S. E. Fl. 906. 1933.—TYPE: United States. Florida. [Volusia Co.:]

hammock, 5 mi S of Daytona, Florida, 23 Aug 1922, *J.K. Small s.n. with G.K. Small, J.B. DeWinkler* (holotype: NY!; isotype: US!).

Opuntia polycarpa Small, Man. S. E. Fl. 905. 1933.—Type: United States. Florida. [Collier Co.:]

sand-dunes, Caxambas Island, 11 May 1922, *J.K. Small s.n.* (holotype: NY!, isotypes: NY!; US!).

Small to large shrubs or small treelets, 0.2-1.2 (-2) m tall, usually erect but in some cases merely ascending, but with a central trunk, which may be cylindrical or flattened (Fig. 7-5B, D-E), but plants damaged at the base of the trunk (e.g., burned, cut off, scarred, damaged by insects) often producing numerous branches from the base, and in age basal-most cladodes often strongly fused and appearing as a single unit (Fig. 7-5D; instead of several stem segments), the plants typically heavily branched towards the apex and frequently semaphore-like; roots commonly tuberous (Fig. 7-5C) or fibrous, the tubers more commonly produced in very well drained, deep sands. Cladodes highly variable, generally elliptic, but commonly obovate or rarely completely round, dark or light green, sometimes slightly glaucous, never cross wrinkling unless under severe drought stress, 14.5 (6.5-29.5) cm long, 6.5 (3.7-9.5) cm wide, thin 8.2 (6.4-10.9) mm thick, mostly with slightly scalloped margins, but margins sometimes non-scalloped, from 2-6 (mostly 4) areoles per diagonal row, cladodes occasionally easily disarticulating during winter months (in the *polycarpa* entity, see below), but generally with cladodes not easily detaching (the *ammophila* entity, see also below). Leaves dark green or sometimes glaucous, 9.3 (6.7-13.8) mm long, ascending (parallel to the cladode surface; Fig. 7-5F) or commonly spreading with the tips recurved. Glochids conspicuous, exserted from the areole, stramineous, forming adaxial crescent in older cladodes from the compression of the areole, trichomes mostly clear or appearing clear-white. Spines mostly 1-2 per areole on terminal cladodes, although up to 3, or plants occasionally spineless, the trunks occasionally with up to 18 spines per areole, round in cross section or commonly twisted longitudinally, the spines highly variable in length, 6.1 (2-

10.3) cm long, 0.9 (0.6-1.2) mm in diameter, strongly retrorsely barbed or relatively smooth to the touch, developing spines dark reddish-brown or mottled (banded) brown-yellow and white, turning white after maturity and finally gray in age, often deflexed upon maturation. Flowers: outer tepals dark green, triangular or triangular-subulate, tips ascending, incurved or commonly recurved in bud (Fig. 7-5G), inner tepals 8, dark yellow to light sulfur yellow (Fig. 7-5H-I), obovate 3.8 (3.4-4.2) cm long, with a mucronate tip, staminal filaments yellow or greenish yellow, stigmas white with generally 6 lobes. Berries clavate or barrel shaped (Fig. 7-5J-K), dark purple, red, pink, or yellow-green when mature, 3.8 (2.8-5.0) cm long. Seeds 4.2 (3.9-4.7) mm long, funicular girdle 0.96 (0.67-1.26) mm wide, funicular envelope smooth with the cotyledon and hypocotyl region of the embryo only moderately raised.

Phylogenetic placement. *Opuntia austrina* is sister to *O. abjecta*, as shown in Majure et al. (2012b, Chapter 6) (Fig. 7-1).

Ploidy. *Opuntia austrina* is diploid, $2n=22$, throughout its range (Majure et al. 2012b).

Phenology. *Opuntia austrina* begins flowering in southern Florida during late March – early April. However, plants grown in more northern areas (e.g., central Mississippi) typically produce flowers around the beginning of May. Thus, flowering time appears to be strongly correlated with changes in climate.

Distribution. *Opuntia austrina* is mostly restricted to the Florida peninsula (Fig. 7-6). One specimen from Gadsden Co., Florida has been tentatively identified as *O. austrina*, and one specimen from Lowndes Co., Georgia (UNC), was described with essentially the same growth form as *O. austrina*, but the specimen is insufficient to confirm the its specific identity.

Habitat. *Opuntia austrina* is most common in peninsular Florida scrub habitat dominated by scrub oaks, *Quercus chapmannii*, *Q. geminata*, *Q. myrtifolia* and sand pine, *Pinus clausa*, as well as sandhills dominated by *Pinus palustris* or *Pinus elliottii*.

Notes. *Opuntia austrina* is the most common species in the Florida peninsula and is most often found in remnant scrub habitats. *Opuntia austrina* is a highly polymorphic species and has by some workers been divided into a number of other taxa that are here placed in synonymy: *O. ammophila*, *O. nitens*, *O. polycarpa*, and *O. turgida*. Of those four taxa, *O. ammophila* and *O. polycarpa* are quite distinctive and easily recognizable in parts of their ranges and are here informally referred to as “entities” of *O. austrina*. The *O. ammophila* entity is most common from the Ocala National Forest in Lake, Marion, and Putnam counties south to St. Lucie Co., where it was first described (Small 1903). *Opuntia ammophila* can form relatively large shrubs or treelets up to 1.2 m tall with a large diameter, cylindrical trunk (up to 40 cm in circumference). John K. Small recorded individuals up to nearly 2 m tall (Small 1919, 1933) but no such individuals have been found since. The *O. polycarpa* entity is primarily found in Highlands and Polk counties along the Lake Wales’ Ridge but individuals have also been seen from Lee County. The *O. polycarpa* entity is recognized by its extremely long spines, sometimes up to 10 cm long that are strongly retrorsely barbed, easily disarticulating cladodes, and generally strongly recurved tips of the tepals when in bud. The *O. polycarpa* entity may form relatively large shrubs or even small treelets to 1 m tall. Although, both the *O. ammophila* and *O. polycarpa* entities are strikingly distinct in certain populations, they form a gradation of morphological characters that overlap with other populations of *O. austrina* including growth form, spine production and color of spines, the degree of spine barbedness, cladode shape and size, and tepal shape. Hence, morphological variation within most populations of both of these

entities directly overlaps with those characters seen in typical *O. austrina* and for this reason as well as the lack of phylogenetic structure, these taxa are treated as part of *O. austrina*.

Another entity of *O. austrina*, which in contrast to the *O. polycarpa* and *O. ammophila* entities has not been formally named, is noteworthy because it forms erect shrubs, which are basically miniature forms of the *O. ammophila* entity, ranging in height from 20-30 cm tall. This entity produces copious spines and in certain specimens resembles an erect form of *O. drummondii* (see below). The spines are usually strongly barbed, tuberous roots are produced, and a cylindrical trunk is also a common feature of this entity. I have collected it in Osceola and Orange counties and have seen another specimen from Lee Co.

Typical *Opuntia austrina* forms erect shrubs from 40-60 cm tall, although, with a relatively flat trunk. Plants may or may not be heavily covered with spines, and the spines are slightly retrorsely barbed to the touch or oftentimes smooth. Cladodes do not disarticulate easily and plants are generally smaller and less robust than the *O. ammophila* and *O. polycarpa* entities.

Benson (1982) included *O. austrina*, at the infraspecific level, in his broad concept of *O. humifusa*, however, phylogenetic analyses have shown that *O. austrina* and *O. humifusa* are not synonymous (Majure et al. 2012a, Chapter 6). Benson (1982) cited *O. humifusa* var. *austrina* (here *O. austrina*) from Big Pine Key, although, the photo presented (p. 442; Fig. 443) is actually of *O. abjecta*, not *O. austrina*. Benson (1982) also concluded that *O. pisciformis* (included here under synonymy with *O. austrina*) was of hybrid origin between *O. humifusa* and *O. stricta*. However, characters possessed by the type specimen of *O. pisciformis* fall completely within the bounds of *O. austrina*, as circumscribed here. So a hybrid origin of *O. pisciformis* appears dubious.

Additional specimens examined. United States. Florida. Brevard Co.: off of A1A SW of Jetty Park, Cape Canaveral; 17 0539480N 3141126E, 16 Mar 2007, *L.C. Majure 2087* (MISSA). Charlotte Co.: Port Charlotte Beach State Recreation Area, N portion of park, bayside, Manasota Key, 7 Mar 1991, *S. Erickson PC0031* (USF). Citrus Co.: FL 491, ca. 1 mi E of Holder, 26 Mar 1965, *J. Beckner 662* (FLAS). Collier Co.: 1 mi E of North Naples on FL 846, 7 Aug 1967, *O. Lakela 30902B* (USF). Duval Co.: Florida Beach, 17 Nov 1929, *H.N. Moldenke 5233* (NY). Flagler Co.: Flagler Beach: off of Hwy. A1A S, just E of Silver Lake, 17 May 2008, *L.C. Majure 3222* (FLAS). Gadsden Co.: Bear Creek Educational Forest (E of Rt. 267 and ca. 10 air mi SSW of Quincy); 30.47650°N 84.62331°W, 28 Jun 2011, *L.C. Anderson 25542* (FSU). Glades Co.: 7.4 km E of Charlotte Co. line, 9.7 km S of Highlands Co. line, NE corner of C-731 and FL 74, 8 May 2010, *A.R. Franck 2131* (USF). Highlands Co.: off of Hwy. 27N, ca. 21 km S of the town of Lake Placid; lower portion of Lake Wales Ridge, 20 Jul 2008, *L.C. Majure 3450* (FLAS). Indian River Co.: off of Hwy. 1S, jct. of 65th St. and Old Dixie Hwy. at Winter Beach, 11 Feb 2011, *L.C. Majure 4182* (FLAS). Lake Co.: Ocala National Forest, off of Hwy. 40 W ca. 4 km W of Aster Park and 2 km E of jct. with Hwy. 19; along roadside; 24 May 2008, *L.C. Majure 3246* (FLAS). Lee Co.: Wulfert, western Sanibel, 28 Mar 1973, *W.C. Brumbach 8290* (FLAS). Manatee Co.: South Fork State Park, 4 Apr 1992, *R. Owens SF0050* (USF). Marion Co.: Ocala National Forest, Salt Springs off of Hwy. 19S at jct. with Hwy. 314, 24 May 2008, *L.C. Majure 3244* (FLAS). Miami-Dade Co.: Miami, 28 Oct - 28 Nov 1903, *J.K. Small s.n.* (NY); Miami, 1 Feb 1911, *J.K. Small s.n.* (NY). Okeechobee Co.: 0.25 km S of the Okeechobee County line, off of Hwy. 441W, 11 Feb 2011, *L.C. Majure 4185* (FLAS). Orange Co.: 3.5 mi SE of Hwy. 528 along Hwy. 520 under powerline; 17 0504805N 3142822E, 16 Mar 2007, *L.C. Majure 2086* (MISSA). Osceola Co.: off of Hwy. 441S (192), S of St. Cloud at Harmony, 28 Mar 2009 *L.C.*

Majure 3702 (FLAS). Palm Beach Co.: Near Boca Raton Airport, E of Interstate 95; NW 40th St. & 6th Way, 9 Mar 2010, *L.C. Majure 3970* (FLAS). Pinellas Co.: sand dunes, Long Key, 28 Nov 1921, *J.K. Small s.n.* (NY). Polk Co.: S of Frostproof along Rt. 27 ca. 3.6 mi N of Highlands Co. line, 1 Nov 1980, *W.S. Judd 2841* (NY). Putnam Co.: Ocala National Forest, Delancy Lake, along FR-75-2, just W of Hwy. 19, 24 May 2008, *L.C. Majure 3248* (FLAS). Seminole Co.: off of Hwy. 419S, W of Mills Lake Park; 17 0487398N 3166757E, 16 Mar 2007, *L.C. Majure 2085* (MISSA). St. Johns Co.: Crescent Beach, 1 Jan 1942, *H. Kurz 279* (MICH). St. Lucie Co.: Ancient Dunes, near Ft. Pierce, 6 Sep 1922, *J.K. Small s.n.* (NY). St. Lucie Co.: vicinity of Ft. Pierce, off of Hwy. A1A, E of Jack Island, 28 Mar 2009, *L.C. Majure 3705* (FLAS). Volusia Co.: off of SR 40 ca. 3 km W of jct. with Hwy. 11N, 18 May 2008, *L.C. Majure 3232* (FLAS).

4. *Opuntia cespitosa* Raf., Bull. Bot. Seringe. 216. 1830.—TYPE: United States. Kentucky.

Woodford County, Hwy. 60 N at jct. of Hwy. 62; just N of Versailles, *L.C. Majure 3275* with *B. Patenge*, 9 Jun 2008 (neotype, here designated: FLAS!; isoneotype, US!; see Fig. 7-7A).

Opuntia rafinesqueii Engelm. var. *microsperma* Engelm., Proc. Amer. Acad. 3: 295. 1856.

Opuntia mesacantha Raf. var. *microsperma* (Engelm.) J.M. Coult., Contr. U.S. Natl.

Herb. 3: 429. 1896. *Opuntia humifusa* (Raf.) Raf. var. *microsperma* (Engelm.) A. Heller.

Cat. N. Amer. Pl., ed. 2. 8. 1900. *Opuntia compressa* (Salisb.) MacBride var.

microsperma (Engelm.) L.D. Benson, Proc. Calif. Acad. Sci. 4th ser. 25: 250. 1944. —

TYPE: United States. Missouri. Cultivated in Missouri Botanical Garden 1854 (lectotype designated by Benson (1982): MO!).

Opuntia rafinesqueii Engelm. var. *minor* Engelm., Proc. Amer. Acad. 3: 295. 1856.

Opuntia mesacantha Raf. var. *parva* J.M. Coult., Contr. U.S. Natl. Herb. 3: 429. 1896.
nom. superfl. *Opuntia humifusa* (Raf.) Raf. var. *parva* (Coult.) A. Heller., Cat. N. Amer.
Pl., ed. 2. 8. 1900. nom. superfl. *Opuntia humifusa* (Raf.) Raf. subsp. *minor* (Engelm.) R.
Crook & Mottram., Bradleya 16: 135. 1998. TYPE— United States. Missouri. Sandstone
rock in southern Missouri. *Engelmann s.n.* (lectotype designated by Benson (1982):
MO!).

Sprawling shrub, to 0.3 m tall, with chains of up to 2-6 cladodes, the cladodes generally produced with the flat (broad) surface parallel to the ground surface; roots fibrous or tuberous, apparently depending on the substrate. Cladodes mostly obovate, rotund, or elliptical in outline, margins not scalloped, with 4-6 (generally 5) areoles per diagonal row, cladodes strongly glaucous-green (gray-green) when developing, aging dark green or light gray-green, cross-wrinkling during the winter months, 10.5 (3.8-18.7) cm long, 8.0 (3.2-11.3) cm wide, 10 (4-19.2) mm thick. Leaves glaucous, gray-green, ascending parallel to the cladode surface or slightly spreading, 6.0 (5.5-6.8) mm long. Glochids dark red, crimson red, or dark amber, aging light to dark brown. Spines robust or delicate, smooth to the touch, 1-2 (3) per areole (most commonly 1), 2.9 (1.5-4.3) cm long, these castaneous at the base during development but maturing bony-white, and finally dark gray in age, typically spreading in one plain from the areoles (i.e., in line with one another) with primarily 1 spine, or occasionally 2 of roughly the same length or 1 long and 1 short and slightly deflexed (these characters can be seen in individuals in the same population or even on the same plant), rarely 3 spines produced from the areoles, but in this case the central spine typically not porrect (as in *O. macrorhiza*); in age the mid-cladode and especially the basal cladode spines tend to deflex. Flowers: outer tepals triangular to ovate, inner tepals 9-10, 3.0 (2.5-5.5) cm long, basally tinged dark red, crimson, orange-red, or reddish-pink,

obovate with a mucronate tip, glaucous-green, staminal filaments yellow, reddish basally, stigmas white to cream, lobes 6-10. Berries dark red, or orange-red, 3.9 (2.7-4.5) cm long. Seeds 5.1 (4.9-5.4) mm long, funicular girdle 1.1 (0.95-1.3) mm wide, funicular envelope bumpy from the enlargement of the cotyledons and hypocotyl, the funicular girdle also tends to be slightly irregular or bumpy.

Phylogenetic placement. *Opuntia cespitosa* is an allopolyploid derivative of the southwestern *O. macrorhiza* species complex (SW clade) and the *O. humifusa* species complex (SE clade) of the southeastern United States. The southeastern progenitor of *O. cespitosa* was most likely the tetraploid, *O. humifusa* subsp. *pollardii*, or an ancestor thereof, which was derived solely from the SE clade (Chapter 6).

Phenology. Flowering time for *O. cespitosa* appears to be directly related to latitude, with more southerly populations blooming before more northerly ones. For instance, plants growing in central Mississippi generally begin flowering around the first or second week of May, while material from Michigan and Wisconsin flowers around late June or early July (see Introduction).

Distribution. *Opuntia cespitosa* is the most common species in the eastern United States occurring mostly west of the Appalachian Mountains west to Wisconsin, Iowa, Missouri, Arkansas, and eastern Texas, south to Mississippi and Alabama, and north to Michigan, in the United States, and also in southeastern Ontario, Canada. Populations are occasionally found in the eastern Appalachians as well (Fig. 7-8).

Habitat. *Opuntia cespitosa* is most commonly found in sandy or blackland prairies, juniper glades, or growing on rock outcrops (generally limestone or sandstone). It is commonly associated with *Juniperus virginiana*, *Ratibida pinnata*, *Rhus aromatica*, *Xanthoxylum clava-herculis* among many other species.

Ploidy. *Opuntia cespitosa* is tetraploid, $2n=44$, throughout its range (Majure et al. 2012b).

Notes. Engelmann (1856) was the first to truly recognize the difference between *O. cespitosa* and *O. humifusa*, although, he recognized *O. cespitosa* under the superfluous name, *O. rafinesquei*, apparently in an attempt to reconcile the taxonomic confusion surrounding the eastern United States' material. *Opuntia humifusa* at the time was recognized as *Opuntia vulgaris*.

Central United States populations (in Arkansas, Iowa, Illinois, Michigan, Missouri, and nearly all populations in Wisconsin) often show evidence of introgression with the eastern flank of *O. macrorhiza*, as they have spreading spines in more than one plain and occasionally one small, bristle-like radial spine produced at the base of the areole (e.g., MI, Musekegon Co.: *L.C. Majure 3259*; WI, Dane Co.: *D. Ugent 60-11J*). There also is apparent introgression with *O. humifusa* at the eastern boundary of the two species (e.g., eastern NY, Orange Co.: *H.M. Dunslow s.n.*, Nantucket Island, MA), and populations in Bibb County, Alabama (e.g., *L.C. Majure 2042*) are nearly identical to *O. humifusa* subsp. *pollardii*, except for the red-centered flowers, lack of strong barbs on the spines, and the typical rotund cladodes of *O. cespitosa*.

Additional specimens examined. Canada. Ontario. Essex Co.: Pelee Island, W side of Fish Point, near N end, 9 May 1981, *A.A. Reznicek 6230* (MICH). Kent Co.: Harwich Township, Bethel Cemetery, UTM 107042, map 40J/8, square 17MT10, 6 Sep 1986, *M.J. Oldham 6867* (note: most likely planted, originating from Pelee Island) (MICH). United States. Alabama. Bibb Co.: off of Hwy. 219N from Hwy. 5 N at jct. with Schultz Cr. Rd.; 16 0486461E 3653643N, 7 Mar 2007, *L.C. Majure 2042* (MISSA). Colbert Co.: off of Natchez Trace, just S of jct. with Hwy. 72, 34.7525N 88.0269W, 25 Jul 2007, *L.C. Majure 2610* (MISSA). Franklin Co.: along Spruce Pine Hwy., ca. 4 mi S of Russelville, 30 Aug 1966, *R.C. Clark 8004* (UNC). Jackson Co.:

Hwy. 79 0.2 mi N of jct. with US Hwy. 72, 8 Jul 1966 *R.C. Clark* 4582 (UNC). Lawrence Co.: Prairie Grove Glades, 3.6 km NE of Mt. Hope, 34.4859N 87.5005W, 25 Jul 2007, *L.C. Majure* 2609 (MISSA). Limestone Co.: Elkmont, 14 Jul 1913, *E.G. Holt* 13-64 (NY). Marshall Co.: 7 mi W of Guntersville on Georgia Mt., 9 Apr 1966, *D.H. Brown* 7 (UNA). Morgan Co.: off CR-55 in Massey east of Emmanuel Church, Moulton Valley district of the Highland Rim section; 34°22'12"N 87°01'12"W, 6 Jul 2003, *D.D. Spaulding* 11977 (UNA). Perry Co.: Uniontown, 1 Jan 1912, *A.H. Howell* 12-47 (NY). Arkansas. Bradley Co.: Warren, 21 May 1937, *D. Demaree* 15046 (NY). Garland Co.: Ouachita Nat'l Forest, N of FS rd. 130 and Cedar Fourche Landing of Lake Ouachita; 34.6660°N, 93.2834°W, 6 Apr 2007, *L.C. Majure* 2198 (MISSA). Grant Co.: 5 Jun 1940, *D. Demaree* 21180 (MO). Hempstead Co.: near Tokis, 22 Oct 1932, *D. Demaree* 10024 (US). Hot Springs Co.: Magnet Cove, 10 Oct 1937, *D. Demaree* 16493 (MO). Independence Co.: along Pine Hollow Rd. where it crosses Lafferty Creek, 3.5 mi W of Cushman, Sec. 14, T14N, R8W, 6 Jun 1968, *R.D. Thomas* 8023 (TENN). Izard Co.: Guion, 16 Aug 1913, *W.H. Emig* 187a (MO). Marion Co.: opposite Cotter (in Baxter Co.), 15 Sep 1960, *G.N. Jones* 31045 (ILL). Miller Co.: Stateline Rd., S of jct. with Hwy. 134 on W side of Miller County Sandhills Natural Area, 33° 11.137N, 94° 2.569W, 2 Oct 2008, *B. Snow* 2062 (FLAS). Pulaski Co.: Levy, 4 Nov 1931, *D. Demaree* 8849 (NY). Saline Co.: Just N of Detonti; E of Bauxite Cutoff Rd.; 34.5300°N, 92.5043°W, 6 Apr 2007, *L.C. Majure* 2194 (MISSA). Washington Co.: White River, Fayetteville, *G. Engelmann* 931, 1 Jun 1835 (MO). Yell Co.: 1.95km NNW of Dardanelle, just W of Arkansas River, 1 Oct 2011, *G.P. Johnson* s.n. (FLAS). Connecticut. New Haven Co.: Milford, exposed ledges, 8 July 1892, *E.H. Eames* s.n. (ILL). Illinois. Adams Co.: Mississippi Bottom SE of Quincy, 15 Jul 1943, *R. Brinker* 2824 (ILLS). Calhoun Co.: Cap au Gris Hill Prairie, 2.5 mi SE of Batchtown, T15N, R2W, Sec. 29, 4 Jun

1987, *K. Robertson 4514* (ILLS). Cass Co.: Chandlerville 7.5 min topo map, 6 Jul 1994, *L.R. Phillipe 24911* (ILLS). Crawford Co.: SE of Palestine, 26 Nov 1949, *R.A. Evers 22044* (ILLS). Fayette Co.: bluffs of Dismal Creek NE of Laclede, 23 Jul 1947, *R.A. Evers 5587* (ILLS). Gallatin Co.: sandstone outcrop NE of The Pounda, SW of Gibsonia, 24 Sep 1947, *R.A. Evers 8550* (ILLS). Hardin Co.: above Ohio River, 1 mi E of Rosiclare, 29 May 1949, *G.S. Winterringer 1963* (ILL). Henderson Co.: N of Oquawka, *R.A. Evers 14786*, 18 Sep 1948 (ILLS). Jackson Co.: cliff summits in Giant City State Park, SE Jackson Co.: 1 Oct 1931, *H.S. Pepoon s.n.* (ILLS). Jersey Co.: Riehl Station, *A.H. Horrell 09-168*, 30 May 1909 (US). Jo Daviess Co.: Savanna Army Depot, Green Island 7.5 min quad E of Building D-107, 18 Jul 1996, *L.R. Phillipe 27862* (ILLS). Johnson Co.: Marion quad, R2E T11S, 1.75 mi S of Goreville, E of Dunntown school, 27 Jul 1931, *J. Schopf 858* (ILLS). La Salle Co.: SW of Naplate, 30 Aug 1974, *R.A. Evers 113834* (ILLs). Lake Co.: N of Waukegan and E of the glacial Glenwood Ridge, 1 Jul 1908, *F.C. Gates 2802* (ILL). Lee Co.: near Amboy, 8 Jul 1956, *J.B. Long 293* (ILL). Mason Co.: 0.2mi S of Batts, on St. Rt. 78 on 600N heading W turn on 1400E, 7 Jun 2007, *R. Altig s.n.* (MISSA). Menard Co.: Athens, *E. Hall s.n.*, 1 Jan 1862 (NY). Mercer Co.: SE of Keithsburg, 2 Jul 1964, *R.A. Evers 80855* (ILLS). Monroe Co.: rock ledges, 3 mi S of Valmeyer, 24 May 1950, *R.A. Evers 23057* (ILLS). Morgan Co.: S of Meredosia, 31 May 1947, *R.A. Evers 3521* (ILLS). Perry Co.: N of Pinckneyville, 24 Sep 1953, *R.A. Evers 41629* (ILLS). Pike Co.: rock ledge of Kinderhook, 7 Sep 1949, *R.A. Evers 20829* (ILLS). Pope Co.: Brownfield Quad R5E, T12S, Pine Hollow 2.5 mi E of Dixon Springs, 18 Aug 1931, *J. Schopf 1360* (ILLS). Putnam Co.: 1 mi S of Hennepin, 15 Jul 1955, *R.A. Evers 7661* (ILLS). Randolph Co.: rock ledges, 1 mi N of Prairie du Rocher, 24 May 1950, *R.A. Evers 23137* (ILLS). Scott Co.: 3.5 mi W of Winchester, T14N R13W Sec. 27 NW 1/4, 23 Jun 1981, *K.R. Robertson 2607*

(ILLS). St. Clair Co.: on bluff top 2.5 mi S of Falling Springs near Dupo, 31 Aug 1947, *J. Neill 1427* (ILLS). Tazewell Co.: near Spring Lake, 10 mi SW of Pekin, 6 Jun 1948, *H.R. Hoehn s.n.*

(ILL). Union Co.: rocky slopes of Pine Hills, SE of Aldridge, 2 Oct 1948, *R.A. Evers 15475*

(ILLS). Whiteside Co.: along CB&Q & CMSP&P RRs, N of Fulton, 29 Jan 1968, *C.J. Sheviak 206* (ILL). Will Co.: Kankakee River Watershed, Sand Ridge Savanna Nature Preserve, 2.7 mi E of Braidwood (jct. of Rt. 53 and 113) along Rt. 113, in the Kankakee Sand Area Section of the Grand Prairie Natural Division, 41.26025°N 88.1662°W, 13 Aug 2007, *L.R. Phillipe 40000*

(ILLS). Indiana. Adams Co.: Sec. 20, T3S, R8W, 22 Jun 1943, *R.A. Evers 1205* (NY). Clark Co.: 1 mi E of Charlestown, 9 May 1953, *F.B. Buser 3047* (ILL). Fountain Co.: Covington, 1 Aug 1953, *F.B. Buser 3167* (ILL). Jasper Co.: Walker TP and SJ Wheatfield, 8 Jul 1924, *W. Welch 619* (ILL). Jefferson Co.: just N of Madison, 22 Jun 1913, *C.C. Deam 13412* (MO). Lake Co.: Long Lake, 20 Jul 1927, *W.B. Welch 5616* (NY). Porter Co.: just S of Lake Michigan off of Hwy. 12, 8 Jun 2008, *L.C. Majure 3274* (FLAS). Tippecanoe Co.: SW of Lafayette, 17 Jun 1941, *C.M. Ek* (NY). Iowa. Muscatine Co.: E of Cedar River R.R. bridge, W of Bayfield, 3 Jul 1915, *B. Shimek* (NY). Kentucky. Anderson Co.: off of Hwy. 127S, ca. 1 mi S of jct. with Bluegrass Parkway, 9 Jun 2008, *L.C. Majure 3276* (FLAS). Boyle Co.: just SW of Perryville, 27 Aug 1959, *C.F. Reed 45203* (MO). Breckinridge Co.: W of Cloverport, Rt. US 60, Breckinridge, 17 Aug 1961, *C.F. Reed s.n.* (MO). Caldwell Co.: Pennyriple St. Park, 22 Jun 1966, *G.E. Hunter 1676* (UNC). Clark Co.: 1.5 mi SE of Indian Fields, 31 Aug 1939, *H.A. Gleason, Jr. 115* (MICH). Crittendon Co.: Rt. 60, 1 mi N of Mattoon, 23 Jun 1974, *C.F. Reed 138388* (MO). Cumberland Co.: ca. 0.25 mi W of KY Hwy. 704; ca. 1 mi S of Adair-Cumberland county line, 15 Apr 1999, *R.C. Clark 24276* (EKY). Edmonson Co.: Mammoth Caves, 7 Jun 1949, *C.F. Reed 15304* (MO). Franklin Co.: Dad's farm, 10 Oct 1980, *S. Rice FR-109* (EKY). Garrard Co.: W bank Paint Lick

Creek, and directly W of Geo. Caldwell farm, this on KY 21, 1.7 mi E of jct. rd. and KY 52, Blue Grass Province, 5 Oct 1963, *E.M Browne 8150* (EKY). Hancock Co.: USGS Topo, Cloverport 3786-86, 450-650' Jeffry Cliff entered from US 60 on the gravel rd. 1.35 km SE of US 60 and KY 1406 jct., 18 Jun 1980, *R. Hannan 4259* (EKY). Henry Co.: 1.4 mi SW of Lockport, 6 Jun 1962, *J.L. Gentry, Jr. 301* (NY). Jefferson Co.: Goose Creek, 30 Apr 1947, *Davies s.n.* (UNC). Jessamine Co.: Jessamine Cr.; Blue Grass Province, 17 Jun 1961, *E.M. Browne 4221* (EKY). Johnson Co.: C. Ferguson farm near Flat Gap, Mud Lick, rd. to Paintsville, 22 Jun 1949, *O. McKenzie s.n.* (MO). Lyon Co.: western KY, Kuttawa, 2-18 Jun 1909, *W.W. Eggleston s.n.* (NY). Madison Co.: G. Caldwell farm, 1.7 mi E of Paint Lick on KY 21, 5 Oct 1963, *E.M Browne 8130* (EKY). Owen Co.: Gilbert Tract WMA; Brown Bottom., 3 Oct 2003, *R.L. Jones 9542* (EKY). Pike Co.: Old US 460, 4.6 mi E of jct. this rd. and KY 80 near Fishtrap Dam, 9 Jun 1964, *E.M. Browne 8641* (EKY). Pulaski Co.: Pumpkin Hollow, Burnside Q., E of Burnside, Rte N side of Lake Cumberland in Williams Bend in Pumpkin Hollow, 11 Apr 1979, *R. Hannan 1190* (EKY). Warren Co.: Bowling Green, 1 Jan 1899, *S.F. Price s.n.* (MO). Wayne Co.: Cooperville Rd., 15 Apr 1940, *E.L. Braun 2814* (US). Maryland. Baltimore Co.: Factory Rd., 0.5 mi N of Harford Rd., 10 Dec 1981, *C.F. Reed 121328* (MO). Washington Co.: Kemps Mills N of Williamsport, 14 Jun 1952, *C.F. Reed 29115* (MO). Massachusetts. Nantucket Co.: Nantucket Island, Coatue Point, 1 Sep 1964, *F.C. MacKeever* (NY). Michigan. Allegan Co.: 3 mi W of Allegan, summit of bluffs above Kalamazoo River, 15 May 1950, *R. McVaugh 11260* (MICH). Manistee Co.: S end of Maple Grove Township Cemetery immediately E of Kaleva on the N side of Nine Mile Rd; SE quarter of Sec. 21, T23N, R14W, 30 Jul 1978, *A.B. Johnsen 1596* (MICH). Monroe Co.: S side of Tunnicliffe Rd., ca. 5 mi SE of Petersburg, 7 May 1992, *A.A. Reznicek 8931* (MICH). Muskegon Co.: off of Hwy. 31N, 6.2 KM SE of Whitehall, 2 km

NW of Lakewood, 2 Jun 2008, *L.C. Majure 3259* (FLAS). Newaygo Co.: off of Hwy. 31N; ca. 8.3 km NE of Newaygo and 6.7 km SE of White Cloud, 2 Jun 2008, *L.C. Majure 3260* (FLAS). Oceana Co.: off of Hwy. 20, W of jct. with 132 Ave., 2 Jun 2008, *L.C. Majure 3262* (FLAS). Van Buren Co.: 0.03 mi N of 28th Ave. and 0.39 mi W of 77th St., 42°18'57.795N 86°17'57.904W, 14 Jul 2008, *T.L. Walters 11972* (MICH). Mississippi. Carroll Co.: Holly Property adj. to Hwy 82 W, 8 Mar 2005, *L.C. Majure 799* (MISSA). Clay Co.: off of Herman-Shirley Rd., ca. 0.25 mi S of Hwy. 50, 16 May 2006, *L.C. Majure 1442* (MISSA). Holmes Co.: off of Hebron Rd., Loess Hills, 33.07420 90.15976, 15 May 2007, *L.C. Majure 2365* (MISSA). Lee Co.: Tombigbee State Park, W side of lake shore, 30 Dec 2005, *L.C. Majure 1292* (MISSA). Lowndes Co.: Old West Point Rd., ca 0.5 mi E of Catalpa Cr., 18 Dec 2004, *L.C. Majure 736* (MISSA). Madison Co.: Natchez Trace Parkway, *W.B. McDougall 1651*, 20 May 1948 (US). Montgomery Co.: Sam Marter property, N of CR 404, 13 Jan 2005, *L.C. Majure 768* (MISSA). Noxubee Co.: off Hwy. 14 behind St. John's Church, 25 Jun 2005, *L.C. Majure 1543* (MISSA). Oktibbeha Co.: property of Anne Daniels, just W of Hwy. 389, near Trim Cane Creek, N of Starkville, 6 May 2006, *L.C. Majure 1380* (MISSA). Pontotoc Co.: vicinity of Troy, off of Shannon Rd., 7 Jun 2006, *L.C. Majure 1519* (MISSA). Scott Co.: S of town of Forest off of Hwy. 501S, ca. 1km NW of Norris, 32.3000°N, 89.4448°W, 25 Jun 2007, *L.C. Majure 2563* (MISSA). Tishomingo Co.: J.P. Coleman State Park, E of boat launch at end of Steel Br. Rd., 24 May 2007, *H. Sullivan s.n.* (MISSA). Missouri. Adair Co.: terrace on E side of Chariton River, ca. 2.5 mi N of State Hwy. 6 on W side of dirt CR, ca. 5 mi NW of Kirksville; T63N, R16W, S15/22 boundary, 29 Jun 1994, *D. Ford 719* (MO). Barry Co.: Eagle Rock, *B.F. Bush 614*, 25 Sep 1896 (MO). Cape Girardeau Co.: South of Delta, 19 May 1926, *R.E. Woodson s.n.* (MO). Carter Co.: along Hwy. 103, just W of Ozark National Scenic Riverways entrance gate, 3 May

1993, *B. Summers* 5607 (MO). Crawford Co.: Savanna Ridge Glade, T40N R2W sec. 23 NE1/4, 5 Jun 1997, *C.E. Darigo* 2888 (MO). Greene Co.: vicinity of Willard, 30 Aug 1912, *P.C. Standley* 9634 (US). Harrison Co.: T66N, R26W, S10, 7 May 1985, *P. Delozier* 1722 (MO). Henry Co.: bluffs of Grand River, 3 mi NE of Piney, near Benton Co. line, 8 Oct 1934, *J.A. Steyermark* 15977 (MO). Howell Co.: Tingler Lake Conservation Area, ca. 7 mi S of West Plains on S Fork of Spring River, T22N R08W S06 N1/4, 23 May 1997, *B. Summers* 8132 (MO). Jasper Co.: Joplin, barrens on Turkey Creek, 19 May 1909, *E.J. Palmer* 2278 (MO). Jefferson Co.: 5 mi SE of Catawissa, 22 Jun 29, *J.A. Steyermark* 1279 (MO). Laclede Co.: Sweet Hollow Creek, 3 mi W of Eldridge, off Hwy. NN, T36N R17W S28, 9 Oct 1991, *B. Summers* 4759 (MO). Lincoln Co.: west of bridge on Cuivre River, 23 Oct 1982, *M.R. Crosby* 14618 (MO). Monroe Co.: near Victor, 27 Jun 1933, *E.J. Palmer* 40740-A (MO). New Madrid Co.: off of I-55N at mi 61.2, S of Sikeston, 36.80077 89.53167, 25 May 2007, *L.C. Majure* 2435 (MISSA). Newton Co.: Reding's Mill, 12 Aug 1908, *B.F. Bush* 5072 (MO). Phelps Co.: Duke, 1 Aug 1913, *W.H. Emig* 187 (MO). Scott Co.: off of I-55N beside Best Western Hotel, Miner and N Sikeston; 36.8883°N, 89.5320°W, 26 May 2007, *L.C. Majure* 2441 (MISSA). Shannon Co.: Ozark National Scenic Riverways, Jerktail Mt., ca. 8 mi des NE of Eminence, 37°13'30"N, 91°18'00"W, 25 Oct 1996, *C. Dietrich* 461 (MO). Taney Co.: Mark Twain National Forest, Ozark, T23N R18W, along Blair Ridge Rd., 2 Jun 1978, *J.L. Hicks* 988 (MO). Washington Co.: 7 mi N of Potosi on state rd. F at Mineral Fork Creek, 38° 00'N, 90°4.9'W, 19 Apr 1990, *J.S. Miller* 4879 (MO). New York. Columbia Co.: Hudson, NY, 25 Aug 1904, *G.T. Hastings* (NY). Orange Co.: Coronham, 7 Aug 1912, *H.M. Dunslow* (NY). Ohio. Adams Co.: Hwy. 52E along the Ohio River (N of river and Hwy. 52) in graveyard of Sand Springs Church, 1 Jun 2008, *L.C. Majure* 3251 (FLAS). Brown Co.: Huntington Township, above Aberdeen, Ohio - station # 1., 1

Apr 1975, *J. Bryant s.n.* (MU). Erie Co.: Cedar Point, Sandusky, Ohio; in the sand near the new Lake Laboratory, 1 Jul 1903, *A. Wetzstein s.n.* (MU). Gallia Co.: off of Hwy. 141 E just (ca. 1 km) E of Raccoon Creek, 1 Jun 2008, *L.C. Majure 3252* (FLAS). Hamilton Co.: Ft. Dennison, E of Cincinnati, 10 Sep 1922, *E.T. Wherry s.n.* (NY). Jackson Co.: dry cliff 0.5 mi west of Jackson, 14 Jun 1936, *Bartley 59*, (NY). Lucas Co.: beside Swan Creek, along Oak Openings Preserve, just E of Wilkins Rd., S of Reed Rd., and W of Berkeley Southern Rd., 1 Jun 2008, *L.C. Majure 3254* (FLAS). Ottawa Co.: Cedar Point, 23 Jun 1894, *W. Worrallo s.n.* (MU). Woods Co.: off of Zepernick Rd. from Hwy. 6 E ca. 8 mi E of Bowling Green, 1 Jun 2008, *L.C. Majure 3253* (FLAS). Pennsylvania. Lancaster Co.: Peach Bottom, 26 Jun 1893, *Castez s.n.* (NY). Tennessee. Bledsoe Co.: Off of Lowes Gap Mt. Rd., vicinity of Litton, 21 Dec 2006, *L.C. Majure 1938* (MISSA). Blount Co.: Cave Springs near Townsend, 14 Aug 1942, *A.J. Sharp S-4247* (TENN). Cannon Co.: jct. of Poplar Bluff Rd. (TN hwy. 96) and Hurricane Cr. Rd.; 16 583120N 3980169E, 9 Mar 2007, *N. Sondermann s.n.* (MISSA). Cheatam Co.: just W of Nashville along Hwy. 24W, ca. 0.5km E of exit 40 (Old Hickory Blvd.), 10 Jun 2008, *L.C. Majure 3280* (FLAS). Davidson Co.: Knapp Farm about 500 yards E of Mills Creek, 13 Jun 1937, *N.H. Woodruff s.n.* (TENN). De Kalb Co.: 36.05907°N, 85.80175°W, Edgar Evans State Park, Center Hill Dam quad. 15 Sep 2002, *L.R. Phillippe 34887* (TENN). Decatur Co.: eastern shore of TN River, 13 mi above Perryville, 22 Aug 1907, *W. Clark 456* (US). Fayette Co.: Gordon Hill area, S of Lagrange, 4 Oct 1980, *V. Bates, Jr. 1925* (TENN). Fentress Co.: 19 Apr 1949, *N.C. Fassett 27936* (TENN). Franklin Co.: along Old Stage Rd., SW of Cowan about 4 mi, 2 Jun 2001, *M. Rhinehart s.n.* (TENN). Giles Co.: SW of Pulaski, on north side of Hwy. 11, 100 yards N of Circle Rd., 14 May 2001, *D. Estes 1626* (TENN). Hamilton Co.: off of Hwy. 321, 21 Dec 2006, *L.C. Majure 1937* (MISSA). Haywood Co.: SE corner of the Hillville Loop Rd., 20 Mar 1984, *P.*

Lewis 1769 (TENN). Jackson Co.: Flynn Creek Rd., about 9 mi W of Rt. 56, 11 Jun 1992 *V.E.*
McNeilus 92-545 (TENN). Jefferson Co.: Friends Station, 1 mi W of New Market, 17 Jun 1934,
M. Weaver 1586 (TENN). Knox Co.: 4 mi from Knoxville, on old Sevierville Rd., 5 Jun 1934,
S.A. Cain 555 (TENN). Lewis Co.: Natchez Trace Parkway, Jacks Branch pulloff; 35° 24' 50"N
87° 30' 59"W, 19 Jun 2009, *J.G. Hill s.n.* (FLAS). Marion Co.: High limestone bluffs N of Lee
Highway Bridge; Cedar Mtn., 1 Jan 2003, *J. Beck 4398* (TENN). Marshall Co.: just W of Henry
Horton State Park, South side of Caney Springs Rd., at a point ca. 0.7 mi W of Hwy. 31A;
35°36'10"N, 86° 42'43"W, 6 Jun 2005, *M. Rhinehart s.n.* (TENN). Maury Co.: off of I-65 N; mi
marker no. 32-51, 20 Jul 2007, *N. Sondermann s.n.* (MISSA). Roane Co.: Pisgah Ridge, facing
Watts Bar Lake at river mi 570.8 (Bacon Gap quad), 14 Jun 1984, *B.E. Wofford 84-40* (TENN).
Rutherford Co.: Rt. 99, between Murphreesboro and Rockvale, 11 Oct 1958, *A.J. Sharp 25493*
(TENN). Shelby Co.: S of Cordova, 20 Aug 1947, *A.J. Sharp 6605* (TENN). Smith Co.: Caney
Fork; Upchurch Rd., ca. 4.9 mi W of jct. with Horseshoe Bend Rd., due E of Carthage, TN, 8
May 1999, *T.J. Weckman 4888* (EKY). Sumner Co.: 5 mi N of Gallatin, 13 Oct 1968, *K.E.*
Rogers 42903 (TENN). Wayne Co.: 2.2 mi E of Clifton Junction, N side of Hwy. 64; 35°
18.416N, 87° 54.276W, 3 Aug 2008, *B. Snow 2061A* (FLAS). Wilson Co.: S end of Lebanon, 8
Aug 1968, *K.E. Blum 2863* (FLAS). Virginia. Fredrick Co.: off of Hwy. 50 W at Hayfield; jct. of
N Hayfield St. and Hwy. 50 W, 30 May 2009, *L.C. Majure 3806* (FLAS). Page Co.: Luray, 20
Sep 1926, *E.T. Wherry s.n.* (US). Wythe Co.: Barren Springs, Rt. 100, *C.F. Reed 97725*, 6 Mar
1975 (MO). West Virginia. Cabell Co.: sandy field, near Milton, 9 Oct 1935, *L. Williams 366*
(MO). Wisconsin. Columbia Co.: sandy roadside near Lake Wisconsin, 24 Sep 1955, *R.C.*
Koeppen s.n., Dane Co.: off of Hwy. 78S just N of Mt. Horeb, (T7N, R6E, Sec. 26), 21 Jul
1960, *D. Ugent 60-11J* (WISC). Grant Co.: sec. 5, Woodman Township, T7N, R4W; W side of

Hwy. 132 N of Mt. Hope, 4 Oct 1968, *L.J. Musselman 2291* (WISC). Green Co.: 1 mi off county Hwy. on Sawmill Rd. (T4N, R6E, Sec. 20), 4 Dec 1970, *J. Cram 27* (WISC). Green Lake Co.: Granite Knobs, Marquette, 18 Sep 1929, *N.C. Fassett 9203* (WISC). Jackson Co.: near Rezin Marsh, 23 mi SE of Black River; Falk (Sec. 25, T26N, R1W), 20 Aug 1947, *D.F. Grether 6618* (WISC). La Crosse Co.: Trunk quad, T18N, R6E, Sec. 9; Farmington Twp., 28 Jun 1956, *T.G. Hartley 923* (WISC). Marquette Co.: Budsins Corner (T17N, R10E, Sec. 28), 9 Aug 1960, *D. Ugent 60-16* (WISC). Monroe Co.: Dalton Ave. 0.75 mi E of hwy. 71; T16N, R3W, Sec. 3, 4 Sep 1988, *J.M. Graber 310* (WISC). Richland Co.: ca. 1 mi NW of Hub City, off of Hwy. 80S, 8 Jun 2008, *L.C. Majure 3273* (FLAS). Sauk Co.: vicinity of Troy (T9N, R5E, Sec. 35, SW1/4), 17 Jul 1960, *D. Ugent 60-8c-8a*, (WISC). Waushara Co.: T18N, R9E, Sec. 30, SE of Coloma and SW of Richford W of Hwy. 22, 1 Sep 1959, *R.F. Pochmann 15152* (WISC).

5. *Opuntia drummondii* Graham in Maund., *Botanist* 5: 246.1846. — TYPE: United States.

Florida. St. Johns Co.: FL, dunes 5 mi S of Ponte Verde, 2 Sep 1954, *L. & R.L. Benson 15388* (neotype designated by L.D. Benson (1982); POM!; see Fig. 7- 9A).

Opuntia pes-corvi LeConte ex Engelmann, *Proc. Amer. Acad.* 3: 346. 1856. —TYPE: United States. Florida. [Franklin Co.:] Apalachicola, FL, April, July, Nov., 1860, *Chapman s.n.* (neotype designated by L.D. Benson (1982); MO!).

Opuntia frustulenta Gibbes, *Proc. Elliott Soc. Nat. Hist.* 1: 273. 1859. —TYPE: United States.

South Carolina. Charleston Co.: Folly Island, near Charleston, 15 Feb 1916, *J.K. Small s.n.* (neotype designated by L.D. Benson (1982); US!).

Opuntia tracyi Britton, *Torreyia* 11: 152. 1911. —TYPE: United States. Mississippi. [Harrison Co.:] Coast, Biloxi, 11 May 1911, *S.M. Tracy s.n.* (holotype: NY!).

Small shrubs 0.2-0.3 m tall, often forming large mounds as a result of disarticulating stems coupled with a high degree of branching, typically consist of numerous (3-5 or more) radiating branches (Fig. 7-9B) from a thick but shallow rootstock; older stems with a thin scaly bark; roots are mostly fibrous but commonly expand in girth for a short distance proximally (Fig. 7-9E). Cladodes typically cylindrical, but flattened as well, especially in larger basal cladodes, dark green, or yellow-green, not glaucous, small relative to other species, 3.6 (0.8-11.1) cm long, 1.8 (0.6-3.4) cm wide, 10.4 (5.3-14.8) mm thick, elliptical, oblong, or rounded in shape, with 1-2 areoles per diagonal row at midstem, the terminal cladodes easily disarticulating at the nodes, leading to frequent vegetative dispersal. Leaves green, 2.8 (2.3-3.5) mm long, spreading or ascending parallel to cladode. Glochids stramineous, usually exserted and conspicuous. Spines 3.0 (1.5-4.9) cm long, 0.6 (0.2-0.9) mm in diameter, dark brown or mottled brown and white during development, aging white and finally gray, the basal cladodes usually producing spines throughout their lifetime, with up to 5 spines per areole, the terminal cladodes usually with 2-3 spines per areole; all spines are strongly retrorsely barbed, but spines on the terminal, easily disarticulating cladodes with more pronounced barbs, presumably aiding in vegetative dispersal. Flowers: outer tepals green or yellow-green, triangular or triangular ovate, erect and generally incurved in bud, generally small, inner tepals 8, dark yellow or occasionally light sulfur yellow, obovate with a mucronate tip, 2.6 (2.2-3.2) cm long, staminal filaments yellow or yellow toward the apex and greenish-yellow at the base, stigmas white, with 3-6 lobes. Berries small, barrel shaped or clavate (Fig. 7-9G), 2.6 (1.8-3.5) cm long, purple, pink, reddish-pink, or green at maturity. Seeds 4.7 (4-5.4) mm long, funicular girdle 0.7 (0.4-0.9) mm wide, funicular envelope smooth (with no prominent expansion from the embryo).

Phylogenetic placement. *Opuntia drummondii* is sister to the rest of the diploid species in the SE clade of the *Humifusa* clade (Chapter 6; see Fig. 7-1).

Phenology. *Opuntia drummondii* flowers from mid-April through mid-May with occasional flowers produced through June depending on environmental conditions.

Distribution. *Opuntia drummondii* is found in coastal areas from North Carolina to western coastal Mississippi and can be found substantially far inland in Alabama and Mississippi (Majure and Ervin 2008). This species is slightly disjunct from the Gulf of Mexico to the Atlantic Coast (i.e., contiguous populations have not been found stretching across the Florida peninsula to the Atlantic Coast; Fig. 7-10). Interestingly, disjunct mountain populations and introgressive forms produced from hybridization with *O. humifusa* have been found in Georgia and South Carolina, suggesting a distribution pattern coincident with changing sea levels during interglacial cycles with the subsequent extinction of populations of the species in parts of the outer coastal plain.

Habitat. *Opuntia drummondii* is most commonly found in coastal strand vegetation of the Gulf of Mexico and the Atlantic Coast commonly associated with *O. humifusa* subsp. *pollardii* or *O. humifusa* subsp. *lata*. It is most common in non-shifting sands behind primary dunes, although, the species is also very common in certain parts of its range along major river systems with open, sandy habitats (see Majure and Ervin 2008). *Opuntia drummondii* is occasionally found on rock outcrops, as well, almost always associated with *O. humifusa* subsp. *pollardii*.

Ploidy. *Opuntia drummondii* has been recorded as diploid ($2n=22$), triploid ($2n=33$), and tetraploid ($2n=44$) (Majure et al. 2012a). There are very minor morphological differences associated with cytotype, however, sufficient differences have not been observed that would suggest different ploidal levels should be recognized as separate species. Phylogenetic analyses reveal that these ploidal levels are also most closely related to one another, suggesting that the

polyploids may be autopolyploids (Chapter 6). Polyploids are mostly limited to coastal areas, under presumable harsher environmental conditions, whereas diploid members of the species are more widespread.

Notes. Benson (1982) placed this taxon in synonymy with *O. pusilla* with no clarification as to why he thought this southeastern United States species belonged within *O. pusilla*. Benson (1982) designated the neotype of *O. pusilla* as the line drawing by Pfeiffer & Otto of *O. foliosa* (see Britton and Rose 1920, p. 106), which although somewhat conforming to the morphology of *O. drummondii*, does not show a sufficient number of diagnostic characters to be identified to species. Thus, it is unreasonable to use this name for the southeastern United States material, since no type locality was ever given for *O. pusilla* (Haworth 1803, 1812), and the actual identity of *O. pusilla* is ambiguous. Britton and Rose (1920) mentioned that the species was typically assigned to South America and may even belong within *Tephrocactus*, another genus within subfamily Opuntioideae. Haworth (1812) also thought that the species may have been from South America and in his monograph described it alongside *O. curassavica*, a species from the Lesser Antilles (Venezuelan Caribbean). It is highly likely that *O. pusilla* could have been confused with the southeastern United States material, as many European cactus collectors often traded and sold *O. drummondii* under the mistaken identity of *O. pusilla* (Britton and Rose 1920). As far as is currently known, no material closely related to the southeastern US species has been found in the West Indies or South America, although, the closely related *O. abjecta* is found in the Florida Keys, which shares some Caribbean taxa with Antillean islands. However, *Opuntia drummondii* has never been recorded from the West Indies and was described from the southeastern United States (Appalachicola, Florida; Graham 1846), so this name should be used

for the southeastern United States material instead of the ambiguous and undeterminable taxon *O. pusilla*. Further study is needed to determine the correct identity and affinity of *O. pusilla*.

Although, *O. drummondii* was described from Appalachicola, Florida (along the Gulf coast of Florida), Benson (1982) designated a neotype for the species from the Atlantic coast in St. John's County. However, he designated the neotype for *O. pes-corvi*, a synonym of *O. drummondii* described from South Carolina, from Appalachicola, Florida. Anderson (2001) placed *O. drummondii*, *O. pes-corvi*, *O. pisciformis*, and *O. tracyi* (all taxa described from the southeastern US) under synonymy with *O. pusilla*, and further stated that the species is found in the West Indies. By his placement of these other taxa in synonymy with *O. pusilla*, it is clear that Anderson (2001) understood neither where these other taxa were actually native, nor from where they were originally described. *Opuntia drummondii* is listed for Louisiana (under *O. pusilla*; USDA, NRCS, 2012), but the collections actually represent *O. nemoralis* Griffiths. Benson (1982) placed *O. macateei* under synonymy with *O. pusilla*, which also conforms to *O. nemoralis*. Likewise, Weniger (1967) encountered what he identified as *O. drummondii* on Galveston Island, Texas. His collection also is *O. nemoralis* not of *O. drummondii*. Weniger described the glaucous color of the stems, as well as a slightly reddish hue of the inner tepals, both characters exhibited by some populations of *O. nemoralis*. *Opuntia drummondii* never exhibits reddish-coloring of the inner tepals, as *O. drummondii* is derived solely from the yellow-flowered southeastern subclade of the *Humifusa* clade (Chapter 6).

Additional specimens examined. United States. Alabama. Baldwin Co.: Bon Secour National Wildlife Refuge, off of Mobile St., 29 May 2006, *L.C. Majure* 1512 (MISSA). Butler Co.: Hwy. 7, 2.5 mi N of Butler, Co. Hwy. 54, 31°56'50.38"N 86°51'25.19"W, 6 Sep 2007, *A.R. Diamond* 18045 (TROY). Coffee Co.: CR 43, mi marker 3, *K.E. Childree* 7 (TROY). Conecuh

Co.: US Hwy. 31 at N side of the Sepulga River, W of the rd, 31°27'16.5"N 86°47'8.5"W, 17 May 2008, *A.R. Diamond 19255* (TROY). Mobile Co.: Dauphin Island, off of Hwy. 163 S; 30.2645°N, 88.1156°W, 8 Jul 2007, *L.C. Majure 2570* (MISSA). Florida. Baker Co.: along FL 2, at Breakfast Branch, 3.5 mi NW of Eddy Tower, 4.2 mi SE of Georgia St. line, 11 Jul 1984, *B. Hansen 9964* (USF). Bay Co.: off of Hwy. 98W just W of Bay County Canal & W of Pt. St. Joe at Highland View, 6 Nov 2011, *L.C. Majure 4223* (FLAS). Columbia Co.: off of Hwy. (CR) 246W, 7.7km E of White Springs, 15 Feb 2011, *L.C. Majure 4191* (FLAS). Duval Co.: at Jacksonville Beach, ca. 1.9 km S of Neptune Beach, 28 Mar 2009, *L.C. Majure 3700* (FLAS). Escambia Co.: Near Pensacola, S of Navy Blvd., W of Pace Blvd., just E of RR tracks and bridge, 3 Jun 1979, *J.R. Burkhalter 6405* (FLAS). Flagler Co.: off of Hwy. A1A S, just S of Sumner; roadside, 17 May 2008, *L.C. Majure 3221* (FLAS). Franklin Co.: jct. of NE 12th St. and Hwy. 98W at Carrabelle, 6 Nov 2011 *L.C. Majure 4222* (FLAS). Gulf Co.: KS 325 FLAS; Hamilton Co.: East side of Alapaha River, 6 mi W of Jasper off of Hwy. 41W, at jct. with Hwy. 6, 15 Feb 2011, *L.C. Majure 4192* (FLAS). Nassau Co.: Fernandina, 21 Aug 1922, *J.K. Small s.n.* (NY). Okaloosa Co.: just S of Botanical Facility, ca. 3 mi S of Valparaiso, 9 May 1968, *R.R. Smith 2395* (FLAS). St. Johns Co.: just S of St. Augustine, off of Hwy. A1A S, E side, vicinity of Anastacia Beach area; off of Magnolia Ave., 17 May 2008, *L.C. Majure 3218* (FLAS). Wakulla Co.: Panacea Springs, 28 Jul 1942, *H. Kurz 290* (MICH). Walton Co.: off of Hwy. 30A, Grayton Beach State Park, 25 Jun 2005, *L.C. Majure 1066* (MISSA). Georgia. Camden Co.: sand dunes, St. Marys, 20 Aug 1922, *J.K. Small s.n.* (NY). DeKalb Co.: off of Hwy. 124 N from exit 75 off of Interstate 285N, 27 May 2009, *L.C. Majure 3788* (FLAS). Glynn Co.: Jeckyll Island, Atlantic Coast side in center of the island, 1 Sep 2008, *Tom Mann s.n.* (FLAS). Mississippi. Clarke Co.: off of Hwy. 45, just N of jct. with Hwy. 512, 22 Oct 2005, *L.C. Majure 1270*

(MISSA). Forrest Co.: Hattiesburg off of Edwards Rd., 5 Jan 2005, *L.C. Majure 756* (MISSA). George Co.: Charles Deaton Nature Preserve, Nature Conservancy Property off of US Hwy 98; vicinity of Merrill; Hog Island, 22 Jan 2005, *L.C. Majure 771* (MISSA). Greene Co.: Charles Deaton Nature Preserve, Nature Conservancy Property off of US Hwy 98; vicinity of Merrill, 22 Jan 2005, *L.C. Majure 772* (MISSA). Hancock Co.: vicinity of Ansley, along Clairborne Rd., peninsula between tidal marshes, 9 Jun 2005, *L.C. Majure 1033* (MISSA). Harrison Co.: Biloxi, 1 Aug 1896, *C.L. Pollard s.n.* (NY). Jackson Co.: Belle Fontaine Beach, W end, 18 May 2005, *L.C. Majure 955* (MISSA). Jasper Co.: Hwy 503 S; S of Paulding, across from Willam Chapel, 9 Jan 2005, *L.C. Majure 766* (MISSA). Kemper Co.: off of Hwy. 39S between Daleville and Dekalb, 32.71215N 88.66920W, 16 Jun 2007, *L.C. Majure 2555* (MISSA). Lauderdale Co.: pipeline off Point Wanita Lake Rd., 9 Jan 2005, *L.C. Majure 763* (MISSA). Lowndes Co.: off of I-82, ca 0.5 mi W of Columbus, 12 Apr 2005, *L.C. Majure 843* (MISSA). Newton Co.: Chunky River; ca. 0.75 mi SE of Hwy 80, 5 Feb 2005, *L.C. Majure 776* (MISSA). Noxubee Co.: Gholson, off of Hwy. 21, 20 Aug 2005, *L.C. Majure 1155* (MISSA). Perry Co.: Mars Hill, Camp Shelby, DeSoto National Forest, 31 Dec 2004, *L.C. Majure 757* (MISSA). Smith Co.: Ainsworth property, S of Hwy 18, 29 Dec 2004, *L.C. Majure 753* (MISSA). North Carolina. Brunswick Co.: Smith Island, 3 Apr 1918, *McCorm s.n.* (NY). Carteret Co.: Ft. Macon, opposite Beaufort, 18 Aug 1922, *J.K. Small s.n.* (NY). Currituck Co.: 1.9 mi S of Mamie on US 158, 25 Jun 1958, *H.E. Ahles 44470* (UNC). Dare Co.: Town of Kitty Hawk off of Hwy. 158 W between Wachovia Bank and The Marketplace, in front of shopping center, 2 Jun 2009, *L.C. Majure 3826* (FLAS). Hyde Co.: Ocracoke, *T.H. Kearney 2275*, 13-17 Oct 1898 (US). New Hanover Co.: Wrightsville Beach at the end of Hwy. 76 E, 3 Jun 2009, *L.C. Majure 3830* (FLAS). Onslow Co.: at end of NC 210 near New River Inlet, 28 Apr 1969, *S.W. Leonard 2396* (UNA). South Carolina.

Charleston Co.: Porcher's Bluff, Christ Church Parish, 7 May 1911, *E.A. Mearees s.n.* (US).

Clarendon Co.: Rt. 15-301, E Santee, 26 Mar 1988, *C.F. Reed 126895* (MO). Horry Co.: at the S end of Folly Island County Park (Ashley Ave.), 3 Jun 2009, *L.C. Majure 3833* (FLAS). York Co.: ca. 3 mi NE of Clover off of Hwy. 321 N then off of Old Carriage Rd., 28 May 2009, *L.C. Majure 3792* (FLAS).

6. *Opuntia humifusa* (Raf.) Raf. Med. Fl. U.S. 2: 247. 1830. *Cactus humifusus* Raf. Annals Nat. 15. 1820.

Sprawling, decumbent shrubs, forming large patches, branching from the base forming chains of 1-4 cladodes; roots typically fibrous or proximally thickened. Cladodes light to dark green, 8.9 (3.1-17.7) cm long, 5.3 (2.0-9.0) cm wide, and 10.2 (3.6-19.9) mm thick, cross wrinkling during the winter, with 3-4 areoles per diagonal row at midstem. Leaves dark green, ascending parallel to developing cladode or slightly spreading, 7.4 (4.9-9.6) mm long. Glochids conspicuous, exserted or inconspicuous, included within the areole, stramineous when young, aging brown. Spines absent, or 1-2 per areole, dark brown, brown and white mottled, or brownish yellow and white mottled during development, aging white and then gray, relatively smooth to the touch or strongly retrorsely barbed, delicate or robust, 2.5 (0.9-4.9) cm long, 0.9 (0.7-1.3) mm in diameter. Flowers: outer tepals ovate, or triangular, dark green, or light green, erect or incurved in bud, inner tepals 8, entirely yellow, 3.4 (2.3-4.3) cm long, obovate with a mucronate tip. Berries clavate or barrel-shaped, red, pink, purple, or green at maturity, 3.4 (2.1-4.9) cm long. Seeds 5.2 (4-5.9) mm long, funicular girdle 0.9 (0.6-1.2) mm wide, funicular envelope smooth or bumpy, with or without protrusion from the cotyledons and hypocotyl region of the embryo.

Opuntia humifusa is most commonly found in the eastern United States, east of the Appalachian Mountains to the Atlantic Coast, south to Florida, and east to Louisiana (Fig. 7-11). The distribution for the species given here is much reduced from that of Benson (1982) or Pinkava (2003), as *O. cespitosa* is recognized as distinct from *O. humifusa*, as well as other less spiny forms of *O. macrorhiza* found along the eastern fringe of that species' distribution. *Opuntia austrina* also is considered specifically distinct from *O. humifusa*, and not at the varietal level of *O. humifusa*, as in Benson (1982).

Opuntia humifusa, as circumscribed here, consists of three subspecies, i.e., *Opuntia humifusa* subsp. *humifusa*, *O. humifusa* subsp. *pollardii*, and *O. humifusa* subsp. *lata*, which are considered genetically and morphologically distinct (Chapter 6). Additionally, *O. humifusa* subsp. *lata* is diploid, while the other two subspecies are tetraploid. Although, these three subspecies are generally morphologically recognizable, certain populations may exhibit morphological characters that make identification very problematic without other sources of data (i.e., molecular genetic or ploidy data). Thus, it is considered most appropriate to recognize these distinct taxa at the subspecific level within a broadly circumscribed *O. humifusa*. A listing of county records for *O. humifusa* that cannot be identified to the level of subspecies follows, but the specimens that can be completely identified are listed after each of the subspecies treatments.

Additional specimens examined. subspecies undertermined. United States. Alabama. Crenshaw Co.: off of hwy. 331S ca. 9 mi S of Brantly along powerline, 16 0572674N 3489826E, 7 Mar 2007, *L.C. Majure* 2044 (MISSA). Pickens Co.: Sipsey River at Al 14 crossing, 6.6 mi SSE of Aliceville, 2 Aug 1967, *R.C. Clark* 17281 (UNC). Georgia. Gwinnett Co.: Yellow River, near McGuire's Mill, 20 July 1893, *J.K. Small* s.n. (NY). Marion Co.: 2 mi SE of Juniper, Fall Line Sandhill, 25 Aug 2010, *J. Hill* s.n. (FLAS). Walton Co.: Hard Labor Creek State Park: 2 mi

N of Rutledge, 23 Sep 1979, *J.W. Hill 1206* (NY). Mississippi. Calhoun Co.: Calhoun Wildlife Management Area, in cemetery, 10 Oct 2010, *J.G. Hill s.n.* (FLAS). Marion Co.: E Marion County, just N of Hwy. 13 E of Lamar County, Lower Little River, 18 Jun 2009, *T. Mann s.n.* (FLAS). North Carolina. Alexander Co.: SW base of Rocky Face Mt. along CR 1426, 4 Jun 1973, *R.M. Downs 13671* (UNC). Bertie Co.: 8.2 mi SW of Woodard on rd. paralleling Roanoke River, 9 Jul 1958, *H.E. Ahles 46275* (UNC). Bladen Co.: Rt. 701, 4 mi N of White Lake, 25 Dec 1978, *C.F. Reed 103135* (MO). Buncombe Co.: outcrop above Parkway near Bull Gap, 2 mi N of Riceville, 3 May 1953, *A.E. Radford 6968* (UNC). Cabarrus Co.: N side of NC 49, 4.5 mi E of Harrisburg, 7 Oct 1970, *L.T. Musselmann 3963* (UNC). Cumberland Co.: near Cedar Creek, 28 Sep 1963, *R.C. Clark E216* (EKY). Dare Co.: Nag's Head, beside Blackman St. beach access parking area, off of S Virginia Dare Tr. (Rt. 12), 13 Jun 2001, *M.A. Vincent 9386* (MU). Lee Co.: 1 mi S of Juniper Spring Church, 17 Jun 1958, *S. Stewart 696* (UNC). Madison Co.: Hot Springs, on steep slopes of French Broad River on CR 1304, 3.6 mi W of Hot Springs, 5 Jun 1981, *D. Sather 1284* (UNC). Montgomery Co.: E shore of Yadkin River at Falls Dam, Uwharrie WMA, off NC 109 2 mi NW of Uwharrie, 15 Sep 1969, *E.F. Wells 2193* (UNC). Moore Co.: wildlife refuge E of Rosewood, 19 Oct 1957, *C.J. Burk 37-10* (MU). New Hanover Co.: S of Masonboro, cut-off on rd. to Myrtle Grove, 12 Jun 1958, *C. Ritchie Bell 12932* (UNC). Swain Co.: Ellen School, 1 Jul 1956, *Salloway s.n.* (UNC). Wake Co.: Rt. 55, 6.2 mi E of Mt. Olive; Rt. 117, 6 Oct 1982, *J. Doyle 319* (UNC). Wayne Co.: NC 55, 2.5 mi E of NC 403; ca. 10 mi E of Mt. Olive; S side of rd., 26 May 1986, *J. Doyle 802* (UNC). South Carolina. Abbeville Co.: near SC 81, 3 mi S of Calhoun Falls, 13 May 1957, *A.E. Radford 22826* (UNC). Chester Co.: Fishing Cr. pond dam, 13 May 1957, *C.R. Bell 7437* (UNC). Dorchester Co.: 0.8 mi SE of jct. CR 138 and 148 on CR 138 (SW of Reevesville), *H.E. Ahles 26267*, 27 May 1957 (UNC). Edgefield Co.:

near US 25, 9 mi SSW of Trenton, 13 May 1957, *A.E. Radford* 22825 (UNC). Fairfield Co.: 1.2 mi NE of Strother, 12 May 1957, *C.R. Bell* 7104 (UNC). Florence Co.: 2 mi N of Oak Grove school (E of Claussen), 24 May 1957, *C.R. Bell* 7521 (UNC). Hampton Co.: US Hwy. 601, ca. 2 mi NW of Miley, 11 May 1956, *C.R. Bell* 2565 (UNC). Kershaw Co.: Rt. 601 of Rt. 20, near Lugoff, 23 Mar 1986, *C.F. Reed* 125526 (MO). Lancaster Co.: Forty Acre Rock, 4 Apr 1977, *B. Jacobs* 14 (MU). Marion Co.: ca. 10 mi S of US Hwy. on CR 49 (S of Britton Neck), 26 May 1957, *C.R. Bell* 7859 (UNC). Marlboro Co.: 2 mi NW of Drake, 10 Jun 1956, *A.E. Radford* 12543 (UNC). Richland Co.: SC 12, 2 mi E of Ft. Jackson entrance, 13 May 1958, *Duke* 531 (UNC). Saluda Co.: SC 392, 2 mi S of Ridge Spring, 26 May 1957, *A.E. Radford* 23214 (UNC). Union Co.: near Farming Cr. of Robat, 5 Jun 1957, *C.R. Bell* 8547 (UNC). Tennessee. Fayette Co.: Ames Plantation, 19 Oct 1972, *H.R. Deselm s.n.* (TENN). Hardeman Co.: NE of Newcastle, 21 Aug 1947, *A.J. Sharp* 6629 (TENN).

Key to Subspecies of *O. humifusa*

1. Cladodes spineless, cladodes mostly elliptical, glochids mostly inconspicuous (included within the areole), except for older cladodes, areoles generally 4 per diagonal at midstem *O. humifusa* subsp. *humifusa*
1. Cladodes with spines, cladodes elliptical, obovate, or rotund, glochids usually conspicuous (exserted from the areole), areoles generally 3 per diagonal row at midstem 2
2. Seeds with funicular envelope smooth, only moderate, if any, protrusion of the cotyledons and hypocotyl, cladodes typically scalloped-margined, elliptical or rotund, spines delicate 0.8 (0.7-0.9) mm in diameter *O. humifusa* subsp. *lata*
2. Seeds with funicular envelope bumpy, cotyledons and hypocotyl noticeably protruding, cladodes typically smooth-margined, obovate or rotund, spines robust 1 (0.95-1.3) mm in diameter *O. humifusa* subsp. *pollardii*

6a. *Opuntia humifusa* (Raf.) Raf. subsp. *humifusa* Med. Fl. U.S. 2: 247. 1830. *Cactus humifusus*

Raf. Annals Nat. 15. 1820. *Opuntia rafinesquei* Engelm. Proc. Amer. Acad. 3: 295. 1856. nom. superfl.— TYPE: Berks Co.: PA, 0.75 mi southwest of New Jerusalem, 10 July 1927, *C.T. Wherry s.n.* (neotype designated by Leuenberger (1993); US!; see Fig. 7-12A).

Opuntia calcicola Wherry Jour. Wash. Acad. Sci. 16: 12. 1926. TYPE—United States. West Virginia. Jefferson Co.: growing on limestone edges, exact locality along B. & O. RR track about 2 mi N of Harper's Ferry RR station, 10 Jun 1925, *E.T. Wherry s.n.* (holotype: US!, isotype: NY!). Although, Wherry (1926) cited his specimen as collected, 9 June 1925, only collections from the 10 June, 1925, exist at the two repositories listed in his description (i.e., US and NY) and are here designated as the holotype and isotype based on the interpretation that Wherry merely mistakenly altered the date of collection in the protologue. These type specimens replace the lectotype designation by Benson (1982) of a specimen collected by Wherry in 1935.

Sprawling or slightly ascending shrub, during warmer months, forming large, often dense colonies, or cespitose clumps, the cladodes produced in chains of 1-4, often branching towards the tips of the plant and from the base; roots fibrous. Cladodes elliptical or rotund, dark green, not glaucous, cross-wrinkling during the winter, 12.6 (9-15) cm long, 7.2 (5.7-8.3) cm wide, 11.5 (9.6-15.7) mm thick, 3-4 (mostly 4) areoles per diagonal row at midstem. Leaves dark green, 8.2 (6.2-9.6) mm long, triangular-ovate, to lanceolate, ascending (parallel to the cladode surface). Glochids inconspicuous, generally only exerted in older, basal stems, stramineous, but turning light brown or amber in age. Spines absent. Flowers: outer tepals dark green to slightly gray-green, ovate or long triangular, erect or incurved, inner tepals 8, entirely yellow, 8-9, 3.9 (3.7-

4.0) cm long, obovate, stamina filaments yellow or yellow-green, stigma white, 6-7 lobed.

Berries green, red, or orange-red at maturity, 4.4 (4.2-4.8) cm long. Seeds 4.4 (4.0-4.6) mm long, funicular girdle 0.8 (0.6-0.9) mm wide, often bumpy or irregular, funicular envelope raised along the margin from the increase in size of the cotyledons and hypocotyl, bumpy, portion of the funicular envelope surrounding the radical not evidently raised.

Phylogenetic placement. *Opuntia humifusa* subsp. *humifusa* is an allotetraploid derivative of the southeastern and southwestern diploid subclades of the *Humifusa* clade (Chapter 6).

Phenology. *Opuntia humifusa* subsp. *humifusa* flowers from early May through June and July depending on latitude. Plants already in flower in northern Virginia in late May may just be developing flower buds on Cape Cod, Massachusetts.

Distribution. *Opuntia humifusa* subsp. *humifusa* is most common along the eastern edge of the Appalachian Mountains to the Atlantic seaboard. It also occurs sporadically in the southeastern United States (see Additional specimens examined). Although, not recorded from Alabama here, this subspecies certainly should occur there.

Habitat. *Opuntia humifusa* subsp. *humifusa* is most commonly found on rock outcrops (commonly slate) on the eastern slopes of the Appalachian Mountains or sandy soils of the Atlantic Coast. In other parts of its range, it is often found in sandy or clayey soils on xeric hilltops. This subspecies appears to be more tolerant of mesic conditions than the other two subspecies of *O. humifusa*.

Ploidy. *Opuntia humifusa* subsp. *humifusa* is tetraploid throughout its range (Majure et al. 2012b).

Notes. *Opuntia humifusa* subsp. *humifusa*, geographically, is most often found between regions dominated by *O. humifusa* subsp. *pollardii* and *O. cespitosa*, suggesting those two taxa,

or their ancestors could have been involved in the origin(s) of *O. humifusa* subsp. *humifusa*, which is derived from the SW (paternal lineage) and SE clade (maternal lineage), as is *O. cespitosa*. Although, *O. cespitosa* is suggested to have been derived from *O. humifusa* subsp. *pollardii* (maternal lineage), backcrosses of *O. cespitosa* to *O. humifusa* subsp. *pollardii* could have resulted in the formation of *O. humifusa* subsp. *humifusa*. Crossing studies need to be performed to further test this hypothesis.

Additional specimens examined. United States. Connecticut. Fairfield Co.: near Stratford, 25 Aug 1894, *C.L. Pollard* 256 (US). New Haven Co.: Leete's Island, 20 Jul 1958, *J.J. Neale* s.n. (FLAS). Middlesex Co.: Madison, 12 Sep 1970, *F.C. Seymour* s.n. (MO). Delaware. Sussex Co.: at Frankford off of Hwy. 113 S at jct. with Catmans Rd., 2 Jun 2009, *L.C. Majure* 3824 (FLAS). District of Columbia. Washington Co.: Bare rocks, island in Potomac, 22 Jun 1897, *E.S. Steele* s.n. (MU). Maryland. Alleghany Co.: between Interstate 68 and Hwy. 144 E just E (ca. 1 km) of the town of Flintstone, 30 May 2009, *L.C. Majure* 3810 (FLAS). Anne Arundel Co.: along Chesapeake Bay, Sandy Pt. St. Park, 17 Jul 1959, *C.F. Reed* 43690 (MO). Baltimore Co.: edge of field at Loch Raven, *C.F. Reed* 5362, 10 Jul 1946 (MO). Calvert Co.: S of Upper Marlboro, 4 Jul 1946, *C.F. Reed* 6075 (MO). Calvert Co.: S of Upper Marlboro, 4 Jul 1946, *C.F. Reed* 6075 (MO). Harford Co.: Cedar Church Rd. off Rt. US #1, near Dublin, 27 Feb 1982, *C.F. Reed* 121327 (MO). Montgomery Co.: Plummer's Island, 12 Sep 1910, *W.L. MacAtee* s.n. (NY). Worcester Co.: along Rt. 388, 8-12 mi W of Snow Hill, 3 Nov 1990, *C.F. Reed* 132951 (MO). Dorchester Co.: Wet Swag, 1 mi N of Eldorado, Rt. 313, 17 Jun 1970, *C.F. Reed* 93538 (MO). Prince George Co.: Rt. US 50 and 301, Priests Bridge, 3 May 1960, *C.F. Reed* 46305 (MO). Massachusetts. Barnstable Co.: off of Pilgrims Springs Rd., ca. 1.3 km S of WellFleet Harbor, 31 May 2009, *L.C. Majure* 3814 (FLAS). Hampden Co.: Southwick, 26 Apr 1910, *Gillette* 15057

(US). Mississippi. Attala Co.: off of rd. 5217, SE of Ethel from Kings Rd., 33.08230 89.42710, 15 May 2007, *L.C. Majure 2364* (MISSA). Choctaw Co.: 6.5 km E of Stewart, 1 Jun 2009, *K. Philley 499* (FLAS). Grenada Co.: Gore Springs, off of Betterton Dr. from Gore Springs Rd., 17 Sep 2006, *L.C. Majure 1833* (MISSA). Webster Co.: 1.6 km SW of Tomnolen, 1 Jun 2009, *K. Philley 498* (FLAS). New Hampshire. Hampshire Rockingham Co.: at Seabrook, 1 Aug 2011, *B. Nichols s.n.* (FLAS). New Jersey. Atlantic Co.: ca. 0.25 km SE of Nesco Rd. (Hwy. 542), E of Nescohague Lake, P.E. Marucci Center for Cranberry and blueberry Research, Hammonton, 1 Sep 2008, *P. Oudemans s.n.* (FLAS). Burlington Co.: ca. 1.5 km SE of Pemberton, along Hwy. 530 N, 1 Jun 2009, *L.C. Majure 3821* (FLAS). Camden Co.: without definitely locality, 25 Jun 1874, *J.H. Redfield 2459* (MO). Gloucester Co.: Wenonah, 1 Oct 1904, *G.T. Hastings* (NY). Ocean Co.: Tom's River, 14 Jul 1919, *F.W. Hunnewell 6465* (NY). Passaic Co.: 5 mi W of Boardville, 14 Jul 1907, *K.K. Mackenzie 2738* (NY). Somerset Co.: Rock Cliffs, near Biltmore, 1 Jun 1896, *Anonymous s.n.* (US). New York. New York Co.: NY, NY, Van Cortlandt Park and vicinity, 1 Jan 1925, *E.P. Bicknell s.n.* (NY). Orange Co.: West Point, 14 Mar 1905, *E.A. Means s.n.* (NY). Queens Co.: Hunters Point, 28 Jun 1865, *W.H. Leggett s.n.* (NY). Richmond Co.: Cooke's Point, Staten Island, 16 Jul 1914, *Photo* (NY). Rockland Co.: Rocky Knoll, S end of Palisades Interstate Park, sec., Iona Island, 30 Jun 1953, *J.H. Lehr 283* (NY). Suffolk Co.: at Ashrokan, Huntington, Long Island, 16 Oct 1926, *H.J. Banker 3844* (NY). Westchester Co.: White Plains, *O.R. Willis s.n.*, (NY). Worcester Co.: Pelham, 9 July 1882, *C.H. Day s.n.* (NY). North Carolina. Hertford Co.: 0.8 mi S of Barretts Crossroads, 8 Jul 1958, *H.E. Ahles 46028* (UNC). Jones Co.: Island Creek, E of the jct. of Trent River and Island Cr., 5 mi NE of Pollocksville, 3 Oct 1965, *M.N. Sears 6841* (UNC). Pennsylvania. Susquehanna Co.: Round Top Island, East of Harrisburg, 1 Jan 1912, *E. Gillett s.n.* (NY). Virginia. Accomac Co.: just S of

Wachopreague on CR 605, 5 Mar 1966, *F.C. James* 3843 (UNC). Amherst Co.: Rt. 130, 1.3 mi W of Rt. 635, NW of Elon, 25 Apr 1983, *J.Doyle* 444 (UNC). Augusta Co.: jct. of CR 1212 and CR 608, ca. 2 mi N of Vesuvius, 31 May 1986, *J.Doyle* 827 (UNC). Craig Co.: Rt. 311 ca. 0.1 mi W of Rt. 611 near Johns Creek, 5 Sep 1982, *J. Doyle* 310 (UNC). Culpeper Co.: near Cedar Hill, Rt. 522, 3 May 1969, *C.F. Reed* 87781 (MO). Franklin Co.: Bald Knob, Rocky Mount, 26 Jun 1976, *C.E. Stevens* 13064 (MO). Fredrick Co.: off of Hwy. 50 W at Hayfield, jct. of N Hayfield St. and Hwy. 50 W, 30 May 2009, *L.C. Majure* 3807 (FLAS). Gloucester Co.: G.P. Coleman Memorial Building on US 17 (S point of county), 5 Jul 1966, *F.C. James* 4926 (UNC). Goochland-Fluvanna Co.: cliffs, Rt. 6, at Columbia, 30 Jun 1970, *C.F. Reed* 97330 (MO). Isle of Wight Co.: near Franklin, 7-28 Jun 1893, *A.A. Heller* 916 (NY). King & Queen Co.: Rt. 360, just S of Stephen's Church, 25 Nov 1974, *C.F. Reed* 96621 (MO). Page Co.: off of Hwy. 34 N, 5.9 km N of town of Shenandoah and 0.10 km N of Shenandoah River, 29 May 2009, *L.C. Majure* 3798 (FLAS). Pittsylvania Co.: jct. of CR 734 and 730 (1 mi S of Ringgold), 2 Oct 1965, *F.C. James* 3227a (UNC). Powhatan Co.: 0.2 mi NW of jct. CR 711 & CR 641 on 641 (NE of Powhatan), 8 Jun 1967, *F.C. James* 6417 (UNC). Warren Co.: off of Hwy. 11 W just E of Middletown ca. 0.1 km at Cedar Creek Battleground, 29 May 2009, *L.C. Majure* 3800 (FLAS). West Virginia. Hampshire Co.: At the town of jct. just W (ca. 0.25 km) along shaly slopes of Hwy. 50 W, 30 May 2009, *L.C. Majure* 3808 (FLAS). Mineral Co.: ca. 3 km W of the town of jct. along Hwy. 50 W, 30 May 2009, *L.C. Majure* 3809 (FLAS). Pendleton Co.: ca. 1.2 km S of Brandywine, 1 Jun 2011, *E. Ribbens s.n.* (FLAS).

6b. *Opuntia humifusa* (Raf.) Raf. subsp. *pollardii* (Britton and Rose) Majure comb. nov.

Opuntia pollardii Britton and Rose, Smithsonian Misc. Coll. 50: 523.1908. —TYPE:

United States. Mississippi. Harrison Co.: Biloxi, 1 Aug 1896, *C.L. Pollard 1138*

(holotype: NY!; isotypes: MO! US! see Fig. 7-13A).

Opuntia mesacantha Raf. Bull. Bot. Seringe. 216. 1830.—TYPE: no type designated by author,

United States. Virginia. Hampton, 31 May 1878, *J.W. Chickering, Jr. s.n.* (neotype designated here, US!).

Opuntia macrantha Gibbes, Proc. Elliott Soc. Nat. Hist. 1: 273. 1859. —TYPE: within a few miles of Charleston (presumably destroyed in the US Civil War), United States. South Carolina. Charleston Co.: Isle of Palms, near Charleston, 14 Feb 1916, *J.K. Small s.n.* (neotype designated here, US!).

Sprawling shrubs, often slightly ascending, forming large colonies, sometimes several meters in diameter; roots typically fibrous, although generally thickening proximally. Cladodes mostly frequently obovate, but also elliptical, or rotund, dark green to light yellow-green, not glaucous, cross-wrinkling during the winter, 8.5 (3.1-17.7) cm long, 5.2 (2-9) cm wide, 10 (3.6-18.6) mm thick, occasionally cladodes disarticulating with ease in summer months, although, generally not disarticulating without force, areoles 3-4 (generally 3) per diagonal row at midstem. Leaves light or dark green, ascending parallel to the cladode or slightly spreading, triangular or ovate, 4.9 (4.8-5) mm long. Glochids conspicuous, exserted or inconspicuous, included within the areole, stramineous, aging light brown, or light amber. Spines relatively long or short, conspicuous in many specimens, robust, 2.1 (0.9-3.2) cm long, 1.0 (0.95-1.3) mm in diameter, strongly retrorsely barbed when young to several years old, this often being lost in older spines, dark-brown-white mottled, yellow-brown, or brown-yellow-white mottled during development, white when mature, aging gray. Flowers: outer tepals green, broadly triangular, erect or commonly incurved in bud, inner tepals 8, entirely yellow, 2.7 (2.3-3.0) cm long,

obovate to obtriangular, apex margins often moderately lacerate, stamen filaments yellow or yellow green, stigma white, 6-lobed. Berries green, red, orange-red, clavate to barrel-shaped. Seeds 5.5 (5.0-5.9) mm long, funicular girdle 1.0 (0.7-1.3) mm wide, often bumpy or irregular, funicular envelope raised along the margin from the increase in size of the cotyledons and hypocotyl, bumpy, portion of the funicular envelope surrounding the radical not evidently raised.

Phylogenetic placement. *Opuntia humifusa* subsp. *pollardii* is a tetraploid, apparently derived solely from the SE clade, and it is very closely related to *O. cespitosa* and *O. nemoralis* according to plastid DNA sequence data, and likely is was one of the progenitors of both species.

Phenology. This subspecies begins to flower at the end of April or beginning of May.

Distribution. *Opuntia humifusa* subsp. *pollardii* is mostly confined to the coastal plain of the eastern United States (see specimens examined), however this subspecies covers the broadest distribution of the three recognized within *O. humifusa*. *Opuntia humifusa* subsp. *pollardii* is one of the most common taxa of *Opuntia* along the Gulf Coast of Alabama, Mississippi, and along the panhandle of Florida.

Habitat. *Opuntia humifusa* subsp. *pollardii* is most common in the eastern United States pine belt in sandy soils in *Pinus palustris* sandhills or mixed *Pinus-Quercus* sandhills, although, it is frequently encountered on granitic outcrops in Georgia, South Carolina, and North Carolina. In Alabama, Mississippi, and the Florida panhandle it is common in non-shifting dunes behind primary dunes, similar to *O. drummondii*, with which it is commonly sympatric.

Ploidy. *Opuntia humifusa* subsp. *pollardii* is tetraploid, $2n=44$, throughout its range (Majure et al. 2012b).

Notes. The polyploid *Opuntia humifusa* subsp. *pollardii* apparently originated solely from the SE clade, although, the nature of its formation has not been determined. Considering

morphological characters as compared to its diploid relative, *O. humifusa* subsp. *lata*, it seems likely that *Opuntia humifusa* subsp. *pollardii* could have arisen via autopolyploidy, although this needs further study. *Opuntia pollardii* was elevated to subspecific rank over the two earlier names, *O. mesacantha* and *O. macrantha*, as *O. pollardii* was described with great detail by Britton and Rose (1908). There also are photos of live material of the type specimen and, and the type specimen itself was available for study. Although, the other two taxa, *O. mesacantha* and *O. macrantha* apparently belong here, there were no type specimens for them, and the original descriptions of the taxa were greatly lacking in detail.

Additional specimens examined. United States. Alabama. Baldwin Co.: Bon Secour National Wildlife Refuge, off of Hwy. 180, E of Ft. Morgan, 29 Jun 2005, *L.C. Majure 1082* (MISSA). Marion Co.: 6 mi SW of Hackelburg, 22 Aug 2007, *J. Hill s.n.* (FLAS). Mobile Co.: Mobile, Springhill, 12 May 1888, *C. Mohr s.n.* (UNA). Randolph Co.: granite outcrop (Bald Rock), Almond Community, 3.5 mi W of Wadley, 2 Aug 1966, *R.C. Clark 6526* (UNC). St. Clair Co.: Pottsville sandstone outcrops, E end of mt., 4 Jun 1963, *P.E. Bostick 284-1* (UNC). Winston Co.: 4.8 mi N of Haleyville, 10 May 1967, *R.C. Clark 13102* (UNC). Florida. Bay Co.: W of Panama City, 7 Jun 1938, *F.N. Young s.n.* (FLAS). Escambia Co.: W end of Santa Rosa Island at Gulf Islands National Seashore, S of Ft. Pickens Rd., ca. 0.7 mi W of Battery Langdon, 5 May 1979, *J.R. Burkhalter 6397* (FLAS). Levy Co.: T14S, R13E, Sec. 9, NW of SE, 28 Aug 1982, *K.A. Kron 1036* (FLAS). Okaloosa Co.: vicinity of Ft. Walton Beach, off of Hwy. 98 W, 25 Jun 2005, *L.C. Majure 1075* (MISSA). Santa Rosa Co.: jct. of US 98 and Hwy. 399, 25 Jun 2005, *L.C. Majure 1081* (MISSA). Walton Co.: off of Hwy. 30A, Grayton Beach State Park, 25 Jun 2005, *L.C. Majure 1067* (MISSA). Georgia. DeKalb Co.: off of Hwy. 124 N from exit 75 off of Interstate 285N, 27 May 2009, *L.C. Majure 3787* (FLAS). Emanuel Co.: Ochopee Dunes SNA,

32° 32' 15" 82° 27' 40", 16 May 2007, *J. Hill s.n.* (MISSA). Jackson Co.: off of Interstate 85 S; ca. 6.7 km NW of Commerce, 0.3 km NE of Oconee River, 27 May 2009, *L.C. Majure 3789* (FLAS). Taylor Co.: along side of GA-96, 3 mi W of Butler; 7 mi W of Howard, 20 May 1972, *H. Register s.n.* (UNC). Louisiana. Allen Co.: N side of RR tracks; S of LA 190; ca. 5 mi W of Kinder, 16 Apr 1983, *J. Doyle 358* (UNC). Washington Parish Co.: N of LA 437 via logging roads, ca. 1.5 mi W of Bogue Chitto River; ca. 1.5 air mi WSW of Enon, 1 Sep 2008, *Chris Reid s.n.* (FLAS). Natchitoches Co.: 3.25 mi W of Natchitoches on Hwy. 6, 8 Aug 1980, *W.C. Holmes 3951* (NY). Maryland. Anne Arundel Co.: Rt. 4 near Waysons Corner, 4 Jun 1969, *C.F. Reed 112805* (MO). St. Marys Co.: Piney Point, 26 Apr 1958, *C.F. Reed 40649* (MO). Mississippi. Forrest Co.: jct. of Hwy 49 S with Hwy 13 E; Vic of Maxie, 17 Mar 2005, *L.C. Majure 806* (MISSA). Hancock Co.: St. Joseph's Cemetery, vicinity of Diamondhead, 9 Dec 2006, *L.C. Majure 1924* (MISSA). Harrison Co.: Little Florida, DeSoto National Forest, 9 Jul 2006, *L.C. Majure 1603* (MISSA). Jackson Co.: Greenwood Island at Bayou Casotte, 14 Jan 2006, *L.C. Majure 1297* (MISSA). Lafayette Co.: off of Hwy. 7, S of Oxford, 34.35054°N 89.51003°W, 27 May 2007, *L.C. Majure 2448* (MISSA). Marshall Co.: off of Hwy. 78E ca. 1 mi NE of Wall Doxey State Park, 30 Dec 2005, *L.C. Majure 1293* (MISSA). Neshoba Co.: off of Hwy. 15 N, adjacent to Pearl River, ca. 0.25 mi S of bridge, 18 Sep 2005, *L.C. Majure 1201* (MISSA). Noxubee Co.: Gholson, off of Hwy. 21, 20 Aug 2005, *L.C. Majure 1156* (MISSA). Winston Co.: Tombigbee National Forest, off of Sturgis Rd., 16 Jan 2005, *L.C. Majure 769* (MISSA). Yalobusha Co.: trailside off of Co. rd 221, 13 Jan 2005, *L.C. Majure 767* (MISSA). New Jersey. Burlington Co.: Atsion along NJCRR, 29 Aug 1951, *W.A. Stern s.n.* (ILL). North Carolina. Brunswick Co.: C.C.C. Camp, Southport, 10 May 1935, *A.C. Matthews s.n.* (UNC). Chowan Co.: 4 mi W of Small's Crossroads, 24 Jun 1958, *H.E. Ahles 44231* (UNC). Cleveland Co.: along

the Broad River, ca. 4.5 mi S of Boiling Springs on NC Rt. 150, 22 Jun 1956, *H.E. Ahles 15375* (UNC). Currituck Co.: off of Hwy. 158 E just N of Kitty Hawk, 2 Jun 2009, *L.C. Majure 3825* (FLAS). Dare Co.: along Hwy. 12 S, S of the town of Kitty Hawk at jct. with Palmetto St., 2 Jun 2009, *L.C. Majure 3827* (FLAS). Davidson Co.: 1 mi E of Linwood-Southmont Rd., 0.25 mi N of Rockcrusher Rd., 19 Jun 1966, *S.W. Leonard 192* (UNC). Franklin Co.: Rt. 98; 0.2 mi E of Jct. 1001; 1.8 mi W of Bunn, S side, 7 Jun 1983, *J. Doyle 472* (UNC). Granville Co.: 0.7 mi W of Franklin Co. line on NC 56 and 1.5 mi NW of dirt rd., 28 Sep 1956, *H.E. Ahles 20204* (UNC). Halifax Co.: 3.3 mi S and E of Halifax on NC 561, 19 Jul 1956, *H.E. Ahles 17155* (UNC). Hoke Co.: 0.1 mi S of CR 1101 on 401; ca. 8 mi S of Raeford, 28 May 1986, *J. Doyle 814* (UNC). Onslow Co.: off of Hwy. 17 N ca. 1.8 mi N of jct. with Hwy. 172 E, 2 Jun 2009, *L.C. Majure 3829* (FLAS). Pender Co.: sandhill, 1 mi E of jct. of US 421 and NC 53 on NC 53 (W of Burgaw), 13 Jun 1957, *H.E. Ahles 28102* (UNC). Robeson Co.: 4.3 mi SE of Red Springs near ACLRR, 2 Jun 1958, *R.F. Britt 1972* (UNC). Rowan Co.: town of Granite Quarry off of Dunn Mt. Rd. at Dunn Mt. (summit), 28 May 2009, *L.C. Majure 3793*, (FLAS). Scotland Co.: Rt. 401, 2 mi S of Lumber River, 19 Apr 1967, *C.F. Reed 79087* (MO). Wake Co.: Mitchell's Mill, 3.6 mi E of 401 on 96; 0.2 mi SE on 2224, N 0.2 mi on 2300, 17 May 1983, *J. Doyle 447* (UNC). South Carolina. Anderson Co.: dry field 3 mi ESE of Fair Play, 31 May 1956, *H.E. Ahles 13415* (UNC). Bamberg Co.: 0.7 mi S of jct. CR 27 and 22 on CR 27 (SW of Govan), 26 May 1957, *H.E. Ahles 26037* (UNC). Beaufort Co.: 1 mi E of Co. Rt. 76 on US Rt. 21, 10 May 1956, *H.E. Ahles 12357* (UNC). Charleston Co.: Isle of Palms, Charleston, 1 Feb 1916, *J.K. Small s.n.* (NY). Chesterfield Co.: 0.5 mi S on CR 20 from jct. with US 1, S of Cheraw; 34°34'N 79°53'W, 16 May 1976, *J.C. Solomon 1945* (MO). Greenville Co.: slopes of Cesar's Head, North Cove, 3 Sep 1876, *G. Engelmann s.n.* (MO). Orangeburg Co.: Santee Club, 1 Jun 1910, *L.A. Beekauree s.n.*

(NY). Pickens Co.: Glassy Mt., ca. 3 mi NE of Pickens, 28 May 2009, *L.C. Majure 3790* (FLAS). York Co.: ca. 3 mi NE of Clover off of Hwy. 321 N then off of Old Carriage Rd., 28 May 2009, *L.C. Majure 3791* (FLAS). Virginia. Amelia Co.: Rock Sable, SW of Deatonville; 0.7 mi S on 1st dirt rd. to left, ca. 0.4 mi W of jct. 618 and 617; near Saylers Cr. St. Battlefield, 31 May 1986, *J. Doyle 815* (UNC). Brunswick Co.: off Rt. 626 and Rt. 705, near Gasburg, 19 Aug 1978, *C.F. Reed 103249* (MO). Gloucester Co.: sandy fields, Gloucester Point, 17 Apr 1983, *C.F. Reed 117312* (MO). Hampton Co.: Ft. Monroe, Hampton, 7 May 1977, *C.F. Reed 102057* (MO). Madison Co.: Rt. 29 at Robinson Run, N of Madison, 27 Apr 1981, *C.F. Reed 114429* (MO). Richmond Co.: Richmond, *D. Chalmot s.n.*(US). Suffolk Co.: Nansemond, ca. 1 mi E of Blackwater River and 6 mi N of VA-NC state line, 22 Jun 1963, *H.E. Ahles 58238* (UNC). Virginia Beach, Cape Henry, Rt. 6, 3 Sep 1940, *F.E. Egler 40-370* (NY).

6c. *Opuntia humifusa* subsp. *lata* (Small) Majure comb. nov., *Opuntia lata* Small, Jour. N. Y.

Bot. Gard. 1919. — TYPE United States. Florida. [Alachua Co.:] pine-woods, 12 mi west of Gainesville, 13 Dec 1917, J.K. Small s.n. (holotype: NY! on two sheets; see Fig. 7-14A).

Opuntia eburnispina Small in Britton and Rose, The Cactaceae 1: 24. 260. 1923. —TYPE: United States. Florida. [Collier Co.:] sand-dunes, Cape Romano, 10 May 1922, *J.K. Small s.n.* (holotype: NY!).

Opuntia impedita Small in Britton and Rose, The Cactaceae 4: 257. 1923. —TYPE United States. Florida. Duval Co.: Atlantic Beach, east of Jacksonville, 26 April 1921, *J.K. Small s.n.* (holotype: NY!; isotype: US!).

Small shrubs, procumbent or slightly ascending during warmer months, often with 1- numerous cladodes arising from the base; roots typically fibrous, although oftentimes thickened

proximally. Cladodes dark green, not glaucous, cross-wrinkling during the winter, generally not easily disarticulating, often heteromorphic (i.e., a single plant or population may have widely different cladode shapes), mostly elliptical, but also rotund or oblong (Fig. 7-14B-E), margins typically scalloped, 8.4 (4.1-13.0) cm long, 4.7 (3.6-5.9) cm wide, 11.6 (6.2-19.9) mm thick. Leaves green, 7.5 (7.2-7.8) mm long, ascending parallel to the cladode or slightly spreading, ovate. Glochids conspicuous, exserted, stramineous aging light brown. Spines 1-5 per areole (generally 1), moderately or occasionally strongly retrorsely barbed, this being lost with age of the spine, brown and white mottled during development, aging white after the first year, and then gray, 3.7 (2.4-4.9) cm long, 0.8 (0.7-0.9) mm diameter. Flowers: outer tepals green, triangular to ovate, erect or incurved in bud, inner tepals 8, entirely yellow, obovate with a mucronate tip, 3.9 (3.4-4.3) cm long, stamen filaments yellow or yellow-green, stigmas white to cream, 6 lobed. Berry clavate, red, pink, or green at maturity, 3.4 (2.1-4.9) cm long. Seeds 5.0 (4.7-5.3) mm long, funicular girdle 0.8 (0.6-1.1) mm wide, regular, generally not bumpy, funicular envelope smooth, usually not raised from the expansion of the cotyledons or hypocotyl, if slightly raised then generally not bumpy.

Phylogenetic placement. *Opuntia humifusa* subsp. *lata* is sister to the clade containing *O. abjecta* and *O. austrina* (Fig. 7-1).

Phenology. *Opuntia humifusa* subsp. *lata* begins flowering in southern Florida during mid-March. More northern populations soon follow and are in full flower typically in early to mid-April in northern Florida.

Distribution. *Opuntia humifusa* subsp. *lata* is distributed through the outer coastal plain of the southeastern United States from North Carolina south to Florida and west to Mississippi.

Habitat. *Opuntia humifusa* subsp. *lata* is most common in the southeastern United States in *Pinus palustris* or *P. elliotii* sandhills, or mixed *Quercus geminata*, *Q. incana*, *Q. laevis*, *P. palustris* xeric sandhills.

Ploidy. *Opuntia humifusa* subsp. *lata* is diploid, $2n=22$, throughout its range (Majure et al. 2012b).

Notes. Morphologically, *Opuntia humifusa* subsp. *lata* is the diploid version of *O. humifusa* subsp. *pollardii*. Both taxa have the same growth form and *O. humifusa* subsp. *lata* can be easily confused with *O. humifusa* subsp. *pollardii*. *Opuntia humifusa* subsp. *lata* tends to have non-uniform cladodes that are often scallop-margined, unlike *O. humifusa* subsp. *pollardii* that mostly has smooth-margined cladodes. *Opuntia lata* also tends to have seeds with a smooth funicular envelope, which contrasts with the bumpy funicular envelope of *O. humifusa* subsp. *pollardii*.

Benson (1982) considered *O. eburnispina* to be an interspecific hybrid between *O. humifusa* and *O. stricta*, based on the numerous spines produced from the areole. Here *O. eburnispina* is considered synonymous with *Opuntia humifusa* subsp. *lata*. The numerous spines per areole produced by *O. eburnispina* material are seen in all members of the SE diploid clade (i.e., *O. abjecta*, *O. austrina*, *O. drummondii*, *O. humifusa* subsp. *lata*), and therefore do not signify hybridization with *O. stricta*. Also, the spines are not produced in a stellate pattern as in *O. stricta* and related taxa or hybrids (see *O. ochrocentra* above).

Additional specimens examined. United States. Alabama. Autauga Co.: off of Hwy. 82E just N of Vida jct., 16 0529179N 3604707E, 7 Mar 2007, *L.C. Majure* 2043 (MISSA). Butler Co.: Hwy. 77, 0.93 mi N of Butler Co. Hwy. 62, 31°53'40.5"N 86°32'30.8"W, *A.R. Diamond* 19258, 17 May 2008 (TROY). Crenshaw Co.: off of Hwy. 331S, ca. 9 mi S of Brantly, 16

0572674N 3489826E, 7 Mar 2007, *L.C. Majure 2044* (MISSA). Dale Co.: Dale County Lake, 6 Jun 2000, *M. Woods 8106* (TROY). Dallas Co.: bluff on Mulberry Cr., vicinity of USGS stream gauge station, 2.9 mi ENE of Valley Creek jct., 12 Aug 1967, *R.C. Clark 18088*, (UNC). Elmore Co.: near Good Hope Church, 5 mi SE of Wetumpka, 27 Jul 1967, *R.C. Clark 17129* (UNC). Henry Co.: 7.5 air mi SE of Abbeville, Co. Rt. 65 off of State Rt. 95, 0.25 WSW of Hardwicksburg, 31°29'56"N 85°9'17"W, 23 Jul 2003, *R.R. Hayes 10413* (TROY). Marengo Co.: Myrtlewood, *E. Holt*, 1-Jan-1912 (NY). Mobile Co.: Mile marker 27, off of Hwy. 45N, 6 Mar 2011, *L.C. Majure 4194* (FLAS). Pike Co.: N of Ozark off of Hwy. 231N; near Bama Nut Shop, ca. 3 mi S of Brundidge, 6 Jul 2007, *L.C. Majure 2569* (MISSA). Sumter Co.: ca. 1 mi NE of Woodford on CR 23, 5 Aug 1966, *R.C. Clark 6743* (UNC). Wilcox Co.: along AL 41, ca. 5 mi SW of Camden, just N of Pebble Hill Community, 6 Jun 1967, *R.C. Clark 13941* (UNC).

Florida. Alachua Co.: Micanopy, off of Hwy. 234, just E of 441N, 11 Apr 2010, *L.C. Majure 3991* (FLAS). Calhoun Co.: open oak woods, cultivated at Missouri Botanical Garden, 31 May 1978, *J.C. Solomon 3836* (MO). Citrus Co.: along S41 and the railroad, 9 mi N of Inverness, 15 Apr 1976, *L.M. Baltzell 8272* (FLAS). Clay Co.: off of Hwy. 301 E (heading N), ca. 1.6 km S of CR 218, 28 Mar 2009, *L.C. Majure 3699* (FLAS). Collier Co.: TP Scrubs (Coll28), Secs. 2, 3 and 10, T48S, R25E (Bonita Springs quad.), 30 Mar 1986, *R.B. Huck 3954* (FLAS). Columbia Co.: off of US Hwy. 27 W, ca. 2 mi NW of Ft. White; 2.2 mi E of Itchatucknee State Park entrance, 24 Apr 2008, *L.C. Majure 3089* (FLAS). DeSoto Co.: Deep Creek, 800 ha on W side of Peace River, ca. 4.5km NE of jct. I-75 & Kings Hwy. (CR-769), 31 Jul 2008, *A. Franck 751* (USF). Dixie Co.: W of Suwanee River and Manatee Springs off of Hwy. 349S, 17 0306724E 32644465N, 8 Mar 2007, *L.C. Majure 2050* (MISSA). Duval Co.: St. John's Bluff, 1 Jan 1942, *H. Kurz 274* (MICH). Franklin Co.: St. Teresa, *H. Kurz 288*, 28 Jul 1942 (MICH). Gilchrist Co.: 5

mi W of Ft. White, 28 Apr 1961, *G.R. Cooley 8190* (UNC). Hamilton Co.: just S of Crossroads, off of Hwy. 141S, 24 Sep 2011, *L.C. Majure 4217* (FLAS). Hardee Co.: ca. 1 mi (by air) S of Ft. Green Springs, 9 Nov 1993, *B. Hansen 12444* (USF). Hernando Co.: off of Hwy. 93S, ca. 0.5 mi S of Withlacoochee River, 14 Feb 2010, *L.C. Majure 3948* (FLAS). Highlands Co.: Off of Hwy. 64W, just E of Avalon Park, corner of Dodd's Rd., empty lot, 11 Mar 2010, *L.C. Majure 3977* (FLAS). Hillsborough Co.: Little Manatee River State Park, E side of main park drive, 250 ft, S of Ranger Station, 27 Apr 1999, *J. Myers 360* (USF). Lafayette Co.: off of Hwy. 27 W; ca. 4 km NW of jct. with Hwy. 349, 28 Oct 2007, *L.C. Majure 2795* (FLAS). Leon Co.: along Tram Rd., 20 mi east of Tallahassee, 12 May 1976, *M. Blaker 40* (FSU). Levy Co.: off of Hwy. 24W, ca. 1 mi W of Alachua Co. line, 6 Dec 2008, *L.C. Majure 3645* (FLAS). Lake Co.: Palatakaha Park, off of Hull Rd., just S of Clermont, 11 Jun 2010, *L.C. Majure 4092* (FLAS). Liberty Co.: along SR 12, NE of Bristol, 9 May 1977, *L. Rosen 10* (FSU). Manatee Co.: Jct. of CR 675 and Jennings Rd., 0.6 km S of the east arm of Lake Manatee, 23 May 2010, *L.C. Majure 4065* (FLAS). Marion Co.: off of Interstate 75N, ca. 0.5 mi S of reststop, ca. 6.4 km S of Ocala Exit, 28 Mar 2009, *L.C. Majure 3709* (FLAS). Marion Co.: off of Interstate 75N, ca. 0.5 mi S of reststop, ca. 6.4 km S of Ocala Exit, 28 Mar 2009, *L.C. Majure 3709* (FLAS). Okeechobee Co.: off of Hwy. 441N, 0.5km N of Ft. Dunn, 11 Feb 2011, *L.C. Majure 4188* (FLAS). Orange Co.: ca. 2.5 km W of Oakland along Hwy. 438, 14 Nov 2010, *L.C. Majure 4174* (FLAS). Osceola Co.: off of Hwy. 441S, ca. 5 mi S of jct. with Hwy. 192, 28 Mar 2009, *L.C. Majure 3703* (FLAS). Pasco Co.: along C-587, 6.2 mi SW of jct. with FL 52; ca. 4.5 mi ENE of New Port Richey, 31 May 1984, *B. Hansen 9907* (USF). Putnam Co.: off of Hwy. 310 just W of Hwy. 19, just N of Rodman Reservoir, 24 May 2008, *L.C. Majure 3249* (FLAS). Santa Rosa Co.: along Choctaw OLF (Dillon Field) Rd., ca. 0.5 mi W of FL 87 and ca. 5 mi N of Holley, 4 May 1977,

K.D. Perkins 223 (FLAS). Sarasota Co.: Longboat Key, along Gulf of Mexico Dr. ca. 0.1 mi NW of golf course entrance, 2 Apr 1981, *R.P. Wunderlin* 8915 (USF). St. Johns Co.: Rt. 204, just W of US Rt. 1, 15 Apr 1982, *D.S. Correll* 53660 (NY). Sumter Co.: off of Hwy. 301N along railroad tracks ca. 2.5km S of Bushnell, 18 May 2008, *L.C. Majure* 3238 (FLAS). Suwanee Co.: ca. 3 mi N of Beachville off of Hwy. 247 W, 17 0323021E 3322521N, 8 Mar 2007, *L.C. Majure* 2049 (MISSA). Wakulla Co.: at the jct. of Hwy. 98W and Hwy. 365, 6 Nov 2011, *L.C. Majure* 4221 (FLAS). Walton Co.: Nekuse Preserve off of Hwy. 81, 30.53163N 85.94189W, 14 Jul 2007, *L.C. Majure* 2589 (MISSA). Georgia. Bullock Co.: ca. 8.5 mi S of G.S.C. off of US 301 at Lower Lotts Creek Church, 16 Jun 1965, *C.B. Oneal* 10 (UNC). Candler Co.: SE of Stillmore, 3.7 km SE of Emanuel Co. line on Stillmore Rd., 30 May 1988, *D.E. Boufford* 23886 (NY). Charlton Co.: off of Hwy. 121N, just S of St. George, 15 Feb 2011, *L.C. Majure* 4190 (FLAS). Chatham Co.: Savannah, 1 Apr 1927, *F.D. Heyward* s.n. (MICH). Crawford Co.: 2.6 mi SE of Knoxville, 10 Aug 2009, *J.G. Hill* s.n. (FLAS). Emanuel Co.: 5 mi S of Swainsboro on Hwy. 1, 4 May 1974, *C.L. Rodgers* 74081 (FLAS). Houston Co.: off of Interstate 75, ca. 1 mi SW of Perry at jct. with I-75 N, 27 May 2009, *L.C. Majure* 3786 (FLAS). Irwin Co.: off of Hwy. 32/125, ca. 0.15 km W of Alapaha River and Big Creek, ca. 2 km W of Irwinville, 27 May 2009, *L.C. Majure* 3785 (FLAS). Johnson Co.: E of Kite on Georgia Rte 57, just W of the Emanuel Co. line, elev. 80 m, 30 May 1988, *D.E. Boufford* 23890 (MO). Macon Co.: 6.4 mi E of Montezuma on Hwy. GA-224, 8 May 1976, *J.E. Taylor, Jr.* s.n. (UNC). Randolph Co.: off of Hwy. 82W, just W of Springvale, 16 0698524N 3522499E, 10 Mar 2007, *L.C. Majure* 2053 (MISSA). Sumter Co.: 9 mi WSW of Americus, 21 Mar 1966, *E. Parker* 140 (LSU). Tatnall Co.: Big Hammock, 2.7 km SE of Birdsville, 19 Jul 2007, *J.J. Hill* s.n. (FLAS). Mississippi. Greene Co.: Palestinian Gardens off of US Hwy 98, property of James Kirkpatrick,

22-Jan-2005, *L.C. Majure* 773 (MISSA). Jasper Co.: off of Hwy. 503 just N of Paulding, 5 Mar 2005, *L.C. Majure* 795 (MISSA). Lauderdale Co.: E of Lost Gap, N of Hwy. 80, 32.3465°N, 88.7887°W, 3 Mar 2007, *L.C. Majure* 2035 (MISSA). Newton Co.: off of Goodhope-Decatur Rd, ca 3.5 mi NE of Decatur, 26 Mar 2005, *L.C. Majure* 828 (MISSA). Wayne Co.: Gopher Farm, off of Brewerton Rd., off of Hwy. 63, 25 Nov 2005, *L.C. Majure* 1290 (MISSA). North Carolina. Cumberland Co.: NC 87, 12 mi S of Fayetteville, 21 Jun 1958, *J.A. Duke* 1204 (UNC). South Carolina. Aiken Co.: along Interstate 10 W, 10 mi NW of Aiken, 23 Jul 2008, *Jovonn Hill* s.n. (FLAS). Bamberg Co.: ca. 3 mi S of Branchville on Hwy 21, 29 Oct 1997, *B. Summers* 8475 (MO). Calhoun Co.: ca. 2 mi NNW of Lone Star on SC 267 and 0.9 mi ENE on paved rd., 19 May 1957, *H.E. Ahles* 25588 (UNC). Colleton Co.: Waterboro on US 15, ca. 5.5 mi W of 15-34, 14 May 1982, *J. Doyle* 148 (UNC). Darlington Co.: Lake at Hartsville, 20 May 1932, *B.E. Smith* s.n. (UNC). Georgetown Co.: 9 mi N of Georgetown, 7 Jul 1939, *R.K. Godfrey* 305 (MO). Horry Co.: Myrtle Beach off of Hwy. 17 S (Business) at jct. with 82 Ave., 3 Jun 2009, *L.C. Majure* 3832 (FLAS). Jasper Co.: edge of Ridgeland on US Hwy. 17, 12 May 1956, *C.R. Bell* 2579, (UNC). Lexington Co.: just S of I-20 at exit 44, side of SC 34 to Gilbert (ca. 30 mi W of Columbia), 3 May 1996, *C.M. Christy* 2745 (US). Williamsburg Co.: 5 mi S of Kingstree, 10 Jul 1939, *R.K. Godfrey* 375 (NY).

7. *Opuntia nemoralis* Griffiths Monastch. Kakteenk pp-133-134. 1913.—TYPE: United States.

Texas. Gregg County, Longview, Oct 1911, *D. Griffiths* 10480 (holotype: US!; see Fig. 7-15A).

Opuntia macatei Britton and Rose The Cactaceae 1: 113.1923. —TYPE: United States.

Texas.Aransas Co.: Rockport, 28 Dec 1910, *W.L. McAtee* 1992 (holotype: US!).

Plants forming small, spreading shrubs (Fig. 7-15B), oftentimes these forming masses (piles) of cladodes resulting in large patches, mounds, or clones with cladodes ascending to 30 cm tall in the summer; roots typically forming tubers (Fig. 7-15E), but this depends on substrate, and sometimes roots fibrous. Cladodes small, gray-green, glaucous, 6.3 (4.5-8.4) cm long, 3.9 (2.8-5.8) cm wide, 11.2 (8.1-14.2) mm wide, oblong, elliptical, or obovate, the terminal cladodes easily detaching, becoming strongly cross-wrinkled during the winter. Leaves glaucous, gray-green, ascending parallel to the cladode or slightly spreading, 5.4 (3.7-7.7) mm, ovate. Spines 1-6 produced per areole (typically 2), white or yellowish during development, aging bright white when mature and then gray in age, strongly retrorsely barbed when developing and into maturity, 2.3 (1.4-3.0) cm long, 0.6 (0.5-0.8) mm in diameter. Glochids bright yellow when young turning a dull brown in age. Flowers: outer tepals triangular to ovate, glaucous, gray-green, incurved in bud, inner tepals 7-8, yellow (or rarely tinged pink basally), obovate with a mucronate tip, 3.0 (2.7-3.5) cm long, staminal filaments yellow or greenish-yellow, stigmas creamy-white or more commonly light green, lobes 4-9. Berries clavate, 3.0 (2.3-4.2) cm long, dark red to pink, or occasionally light green at maturity. Seeds 4.8 (4.2-5.1) mm long, funicular girdle 1.0 (0.9-1.4) mm wide, funicular envelope only moderately raised by the hypocotyl and cotyledons, not smooth, bumpy (or rough), funicular girdle irregular, bumpy.

Phylogenetic placement. *Opuntia nemoralis* is an allopolyploid derivative of the southeastern and southwestern subclades of the *Humifusa* clade (Fig. 7-1). *Opuntia nemoralis* appears to be derived partially from an ancestor of *O. humifusa* subsp. *pollardii* (Chapter 6)

Phenology. Flowering mid to late April and into May.

Distribution. *Opuntia nemoralis* is found from the Oachita Mountains of Arkansas south to southwestern Louisiana in Cameron Parish and through parts of eastern Texas. In Louisiana *O.*

nemoralis is found in saline barrens, and in the Ouachitas, the species is found mostly on shale barrens. I have not seen live material from Texas. One specimen from Missouri has been tentatively identified as *O. nemoralis*. More fieldwork is needed and likely the distribution of the species is much greater than that shown here.

Habitat. *Opuntia nemoralis* commonly occurs on saline or sodic prairies in Louisiana and on rock outcrops in the Ouachita Mountains of Arkansas. In the Ouachitas, it is commonly associated with *O. cespitosa*. In southern Louisiana populations are in sandy prairies or sandhill communities.

Ploidy. This species is tetraploid, $2n=44$, throughout its range in Arkansas and Louisiana (Majure et al. 2012b), although material from Texas and Missouri has not been counted.

Notes. This species has long been placed in synonymy with either *O. macrorhiza* or *O. humifusa* (Benson 1982), but the small size of the cladodes, retrorsely barbed spines, easily disarticulating cladodes, and green stigmas set this species apart from the two aforementioned species. As a result of the disarticulating cladodes, Britton and Rose (1920) placed this species in *Opuntia* series *Curassavicae* along with *O. pes-corvi* and *O. drummondii*, a mostly synthetic series whose members are from various evolutionarily divergent clades (Majure et al. 2012a). Weniger (1967) described plants of *O. drummondii* from Galveston, Texas, but that material is referable to *O. nemoralis* with its sometimes faintly orange-centered flowers, and greenish stigma lobes, as well as glaucous-gray cladodes.

Additional specimens examined. United States. Arkansas. Garland Co.: Ouachita National Forest, shale barrens off of FR 11, 9.3 km N of Possum Kingdom and Ouachita Lake, 9 March 2011, *L.C. Majure 4204* (FLAS). Hempstead Co.: Fulton, sandy soil, 23 May 1909, *B.F. Bush 5718* (MO). Pulaski Co.: Hwy 65/I-540 about 0.9 miles north of Dixon Road exit, 34° 40'

57.96", 92° 16' 09.09", 5 Sep 2010, *B.L. Snow 2130* (FLAS). Yell Co.: S side of the Arkansas River, 0.75 mi S of the Hwy. 7 bridge at Dardanelle; 35.21494°N 93.14752°W, 4 Sept 2007, *T. Witsell* s.n. (FLAS). Louisiana. Beauregard Parish: off of Mouth of the Creek Rd., 24.5 km W of Deridder, 8 March 2011, *L.C. Majure 4197* (FLAS). Caddo Parish: Barron Road/Boggy Bayou Saline Prairie, T16N R14W, S27; N of Barron Rd. south of Boggy Bayou, 20 April 2006, *B.R. and M.H. MacRoberts 7396* (LSU). Cameron Parish: off of Hwy. 27N, at Johnson Bayou, 25 km E of Sabine Pass, 7 March 2011, *L.C. Majure 4196* (FLAS); DeSoto Parish, off of Hwy. 152S, ca. 4 km NNW of Kingston, 8 March 2011, *L.C. Majure 4198* (FLAS). Vernon Parish: Rt. 111, 9 mi N of Merriville, just past Bayou River on right, 16 April 1983, *J. Doyle 358* (UNC). Winn Parish: between Coldwater and Goldonna, near jct. of Hwy. 156 and Parish Rd. 882, 0.6 mi W of jct. of Hwy. 1233 and Hwy. 153, 27 Apr 2008, *B.L. Snow 2053* (FLAS). Missouri. Wayne Co.: Greenville quad, 37.20678°N 90.49950°W, Corps access rd., 23 Sep 2003, *K. Pocklington 445* (MO). Texas. Galveston Co.: Galveston Bay, *Weniger 687-688* (UNM) (Weniger 1967).

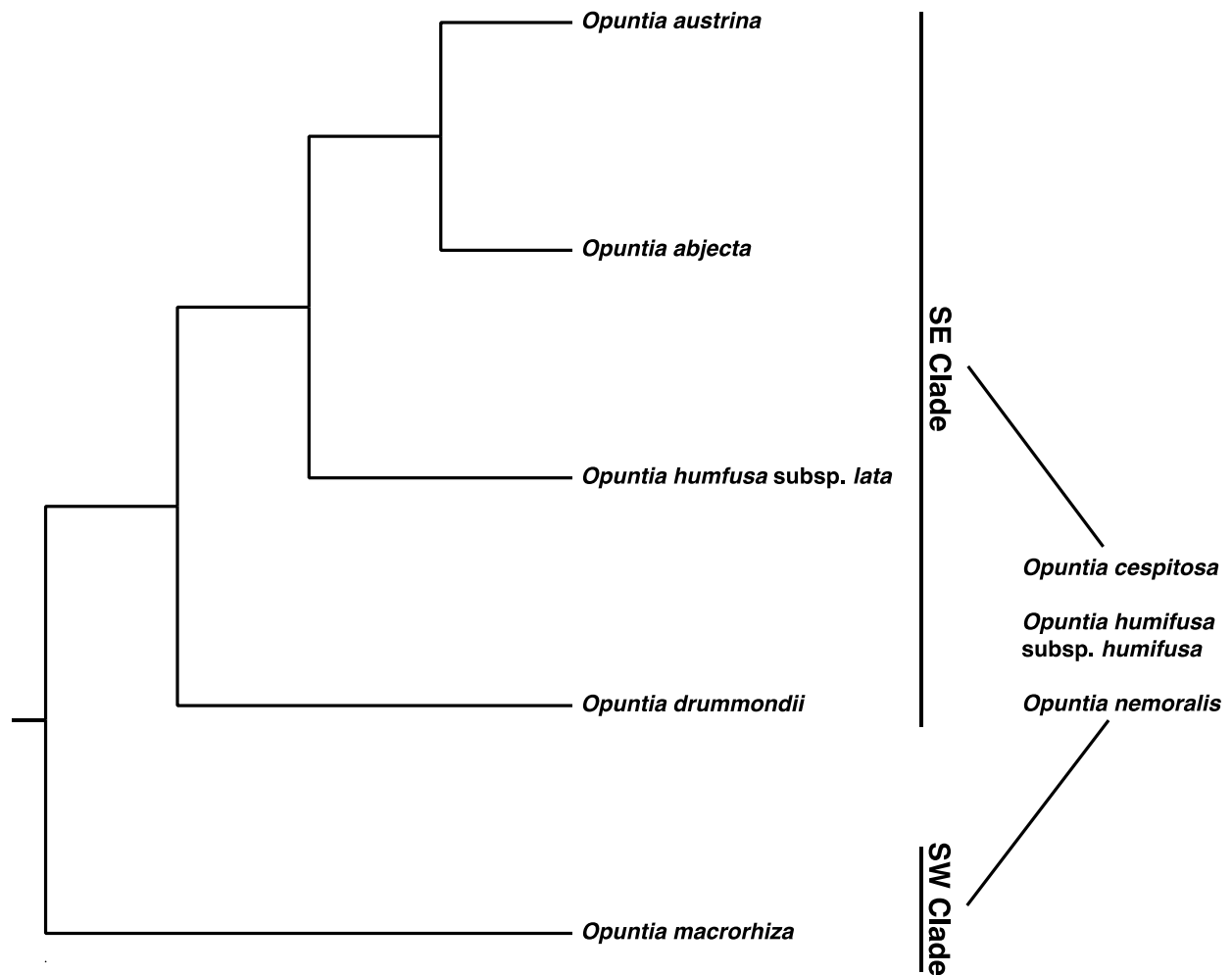


Figure 7-1. Phylogeny of the *O. humifusa* complex. This is a diploid phylogeny of the *Humifusa* clade, which consists of a SE and SW subclade. The *O. humifusa* complex is represented by the SE clade, as well as the reticulate taxa shown here (*O. cespitosa*, *O. humifusa* subsp. *humifusa*, and *O. nemoralis*), and the putative autotetraploid *Opuntia humifusa* subsp. *pollardii* (not shown here).

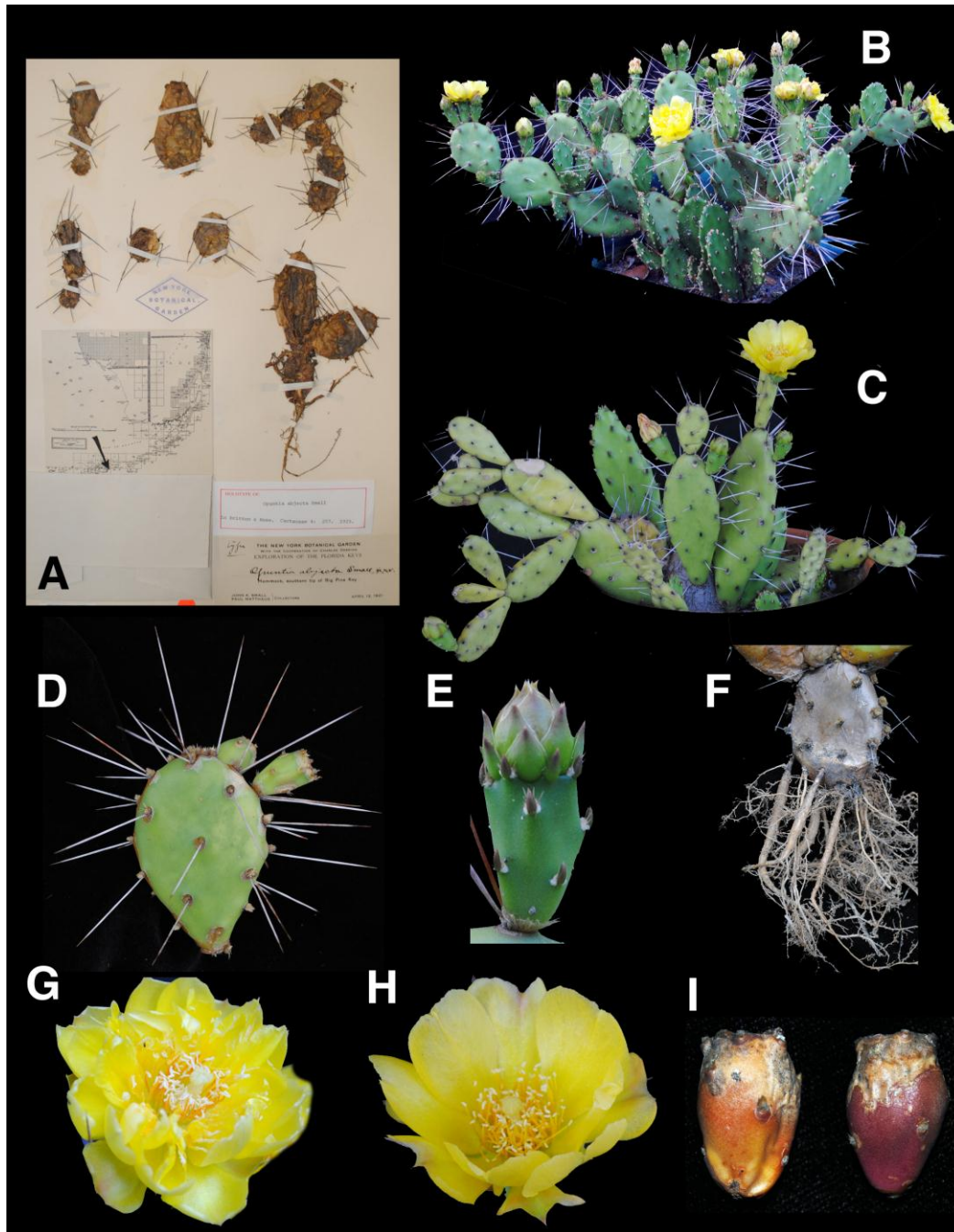


Figure 7-2. Morphological features of *O. abjecta*. A) type specimen, *J.K. Small s.n.*, Monroe Co., FL (NY), growth form of 2x, *L.C. Majure 3908* (B) and 4x *L.C. Majure 3318* (C) *O. abjecta*, D) spine development of a terminal cladode of 2x *O. abjecta* showing 1-3 spines produced per areole, E) flower bud of *O. abjecta*, F) slightly tuberous roots developing on a specimen planted for almost 2 years, flowers of 2x (G) and 4x (H) *O. abjecta*, and (I) barrel-shaped fruit of 2x *O. abjecta*.

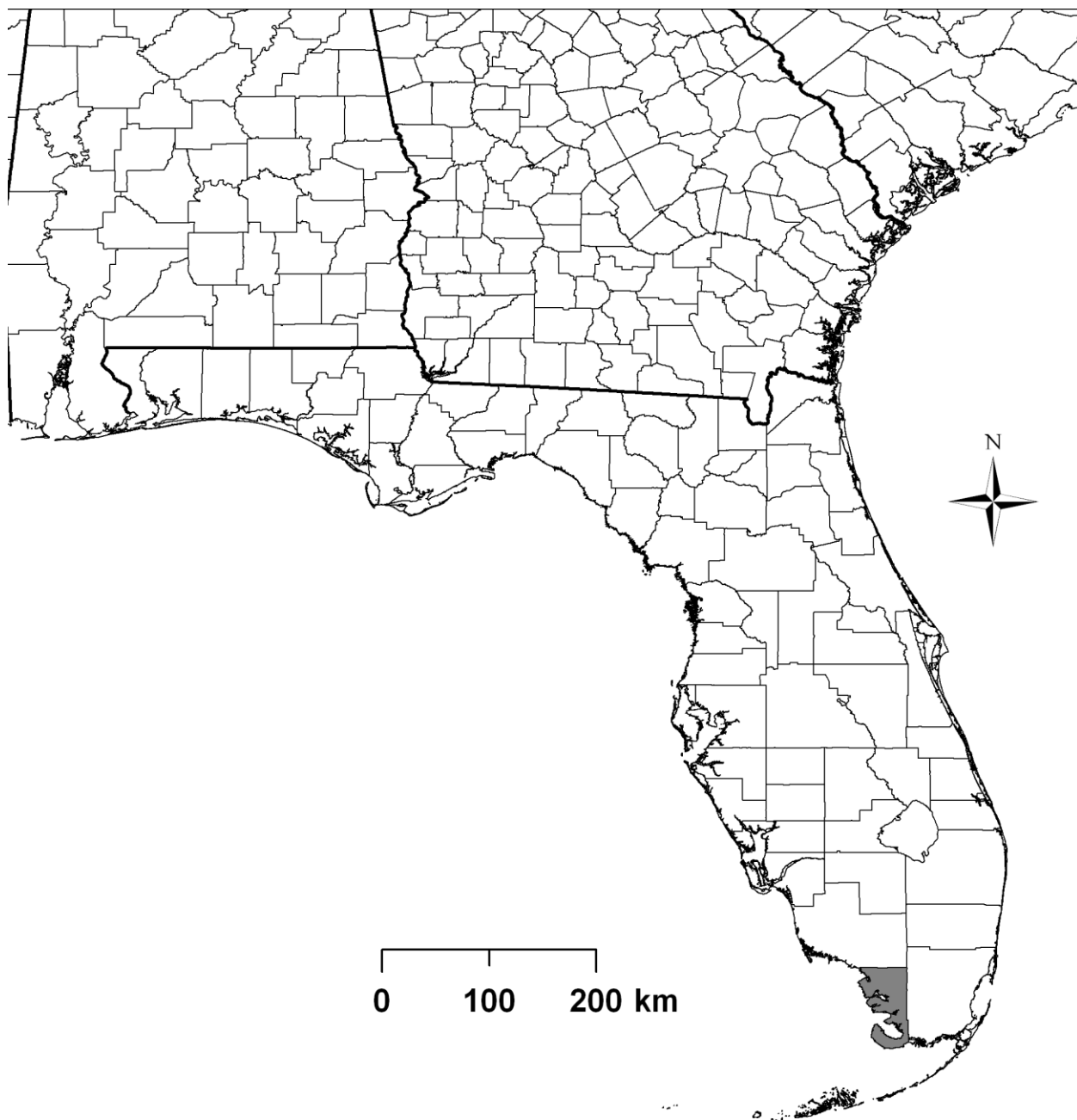


Figure 7-3. Geographic distribution of *O. abjecta*.

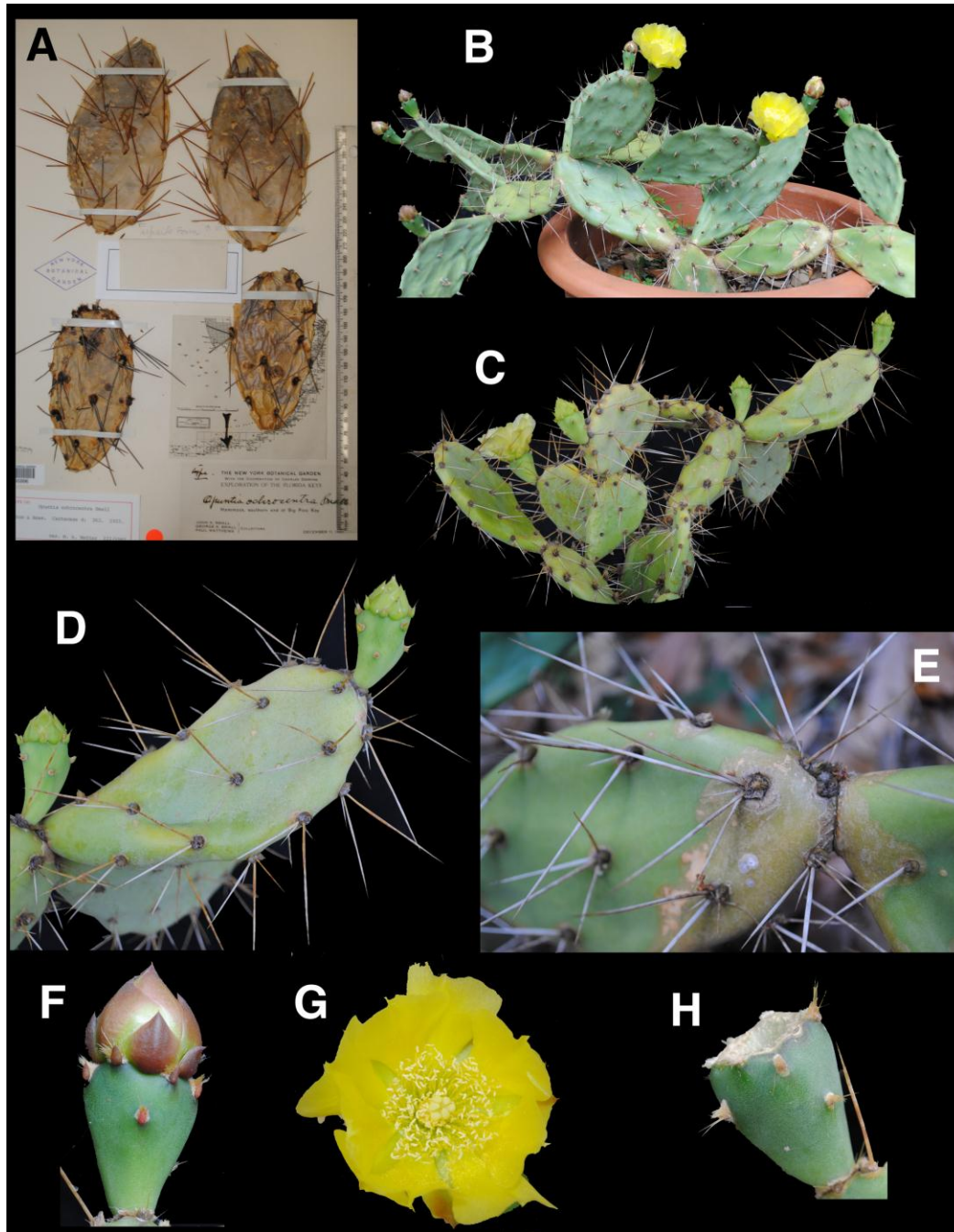


Figure 7-4. Morphological features of *O. ochrocentra*. A) type specimen of *O. ochrocentra*, J.K. Small s.n., Monroe Co., FL (NY), B-C) growth forms of *O. ochrocentra* from B) Big Pine Key, L.C. Majure 3907, and C) Big Munson Island, L.C. Majure 3968, D) spine characters and flower buds of L.C. Majure 3968, E) spine characters of L.C. Majure 3907 showing numerous radial spines flattened at the base and several central spines twisted or cylindrical at the base, F) flower bud, G) flower, and (H) immature fruit of L.C. Majure 3907.

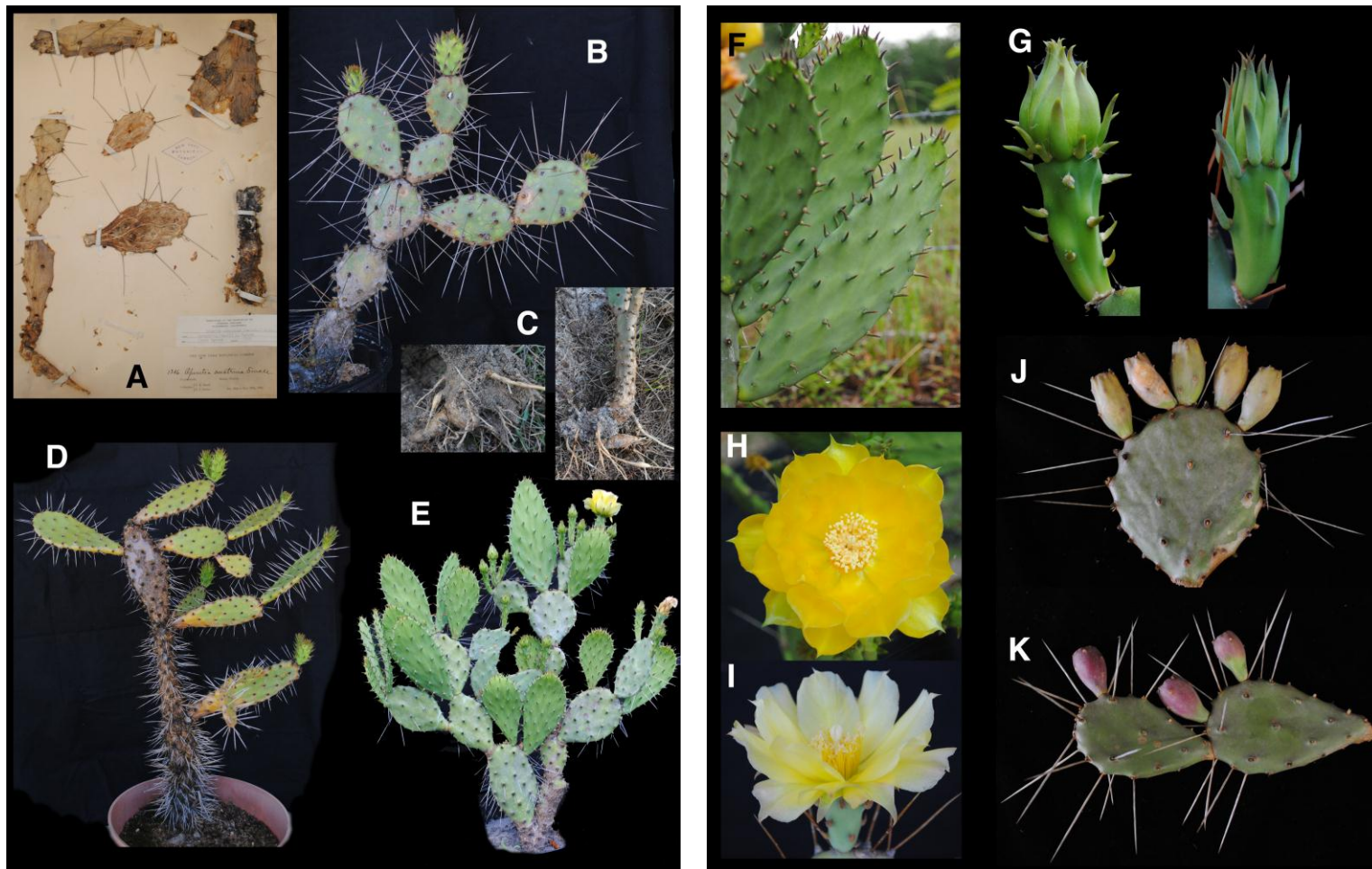


Figure 7-5. Morphological features of *O. austrina*. A) isotype specimen of *O. austrina*, J.K. Small s.n., Miami-Dade Co., FL (US), B) example of *O. austrina*, entity polycarpa, L.C. Majure 3975, with spines up to 10 cm long, C) tuberous roots of *O. austrina* (L.C. Majure 4184, left and L.C. Majure 4189, right), growth forms of *O. austrina*, ammophila entity D) L.C. Majure 2754, Marion Co., FL, E) L.C. Majure 4184, Indian River Co., FL., F) long shoot leaves of *O. austrina*, G) flower buds of *O. austrina*, H-I) color variation in flowers of *O. austrina*, and J-K) fruit color and shape variation of *O. austrina*.

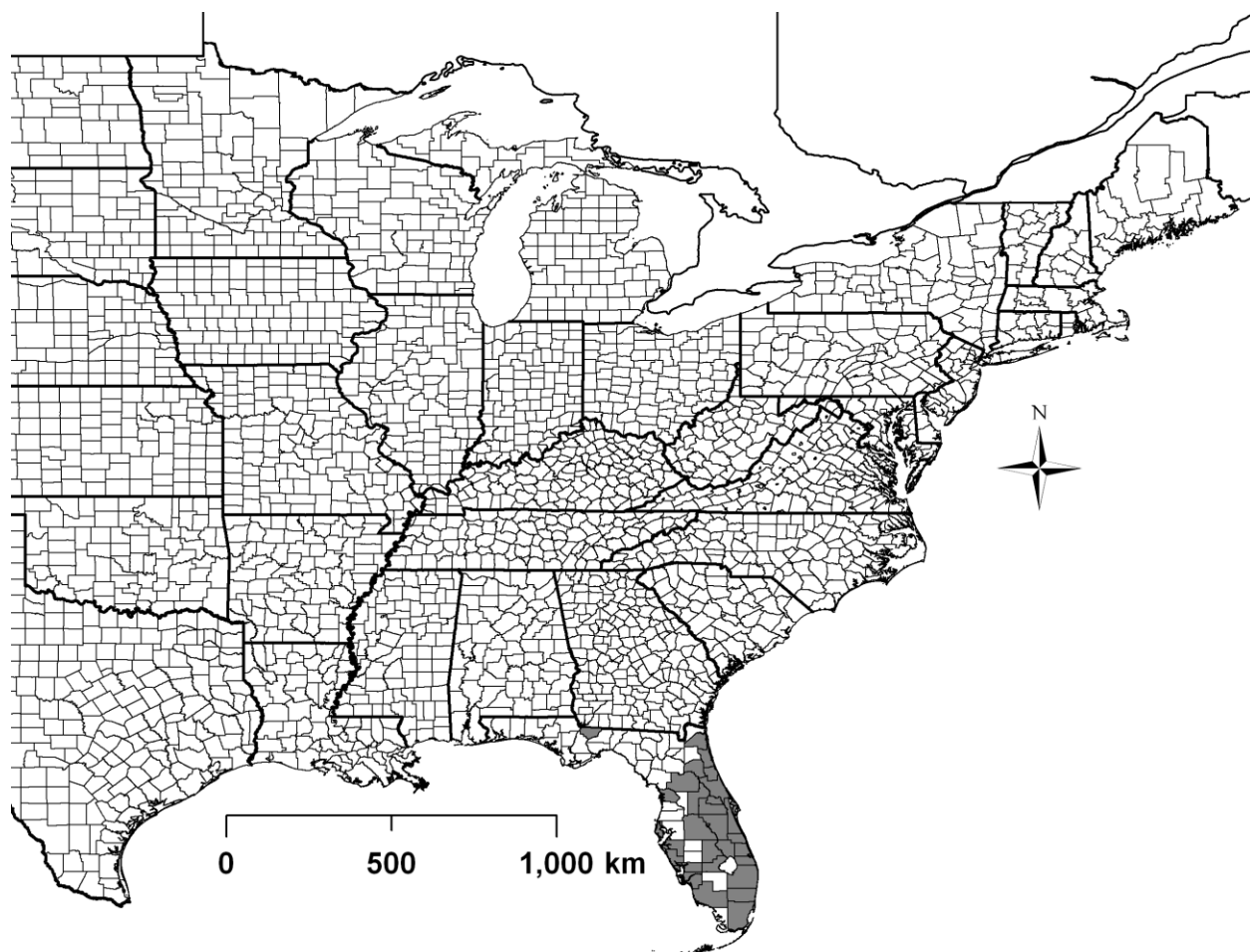


Figure 7-6. Distribution of *O. australis*.

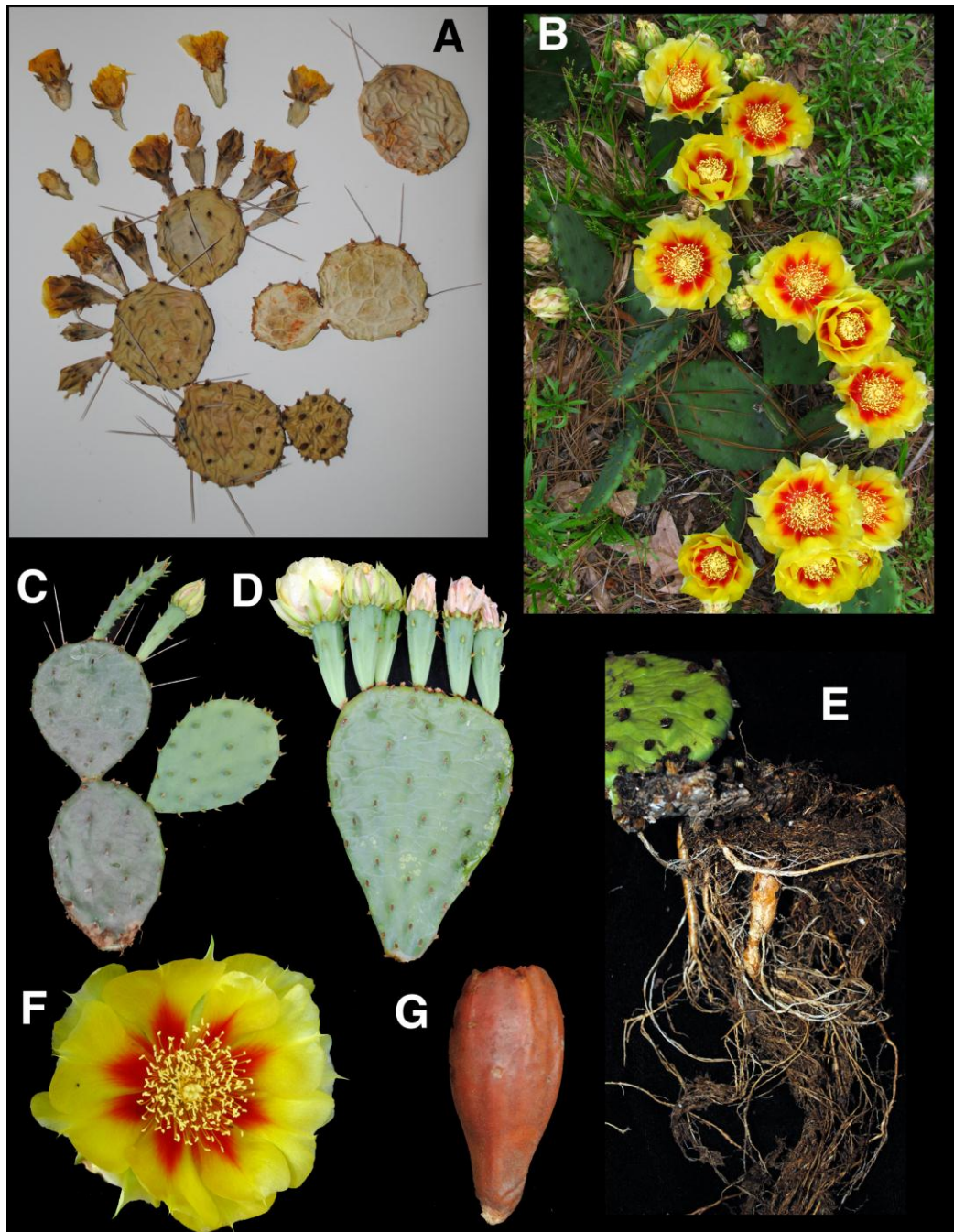


Figure 7-7. Morphological features of *O. cespitosa*. A) neotype specimen of *O. cespitosa*, L.C. Majure 3275, Woodford Co., KY (FLAS), B) spreading growth form of *O. cespitosa*, C-D) pad shape variation showing glaucous color and cladodes either spiny or spineless, E) occasional tuberous roots of *O. cespitosa*, F) flower and G) fruit of *O. cespitosa*.

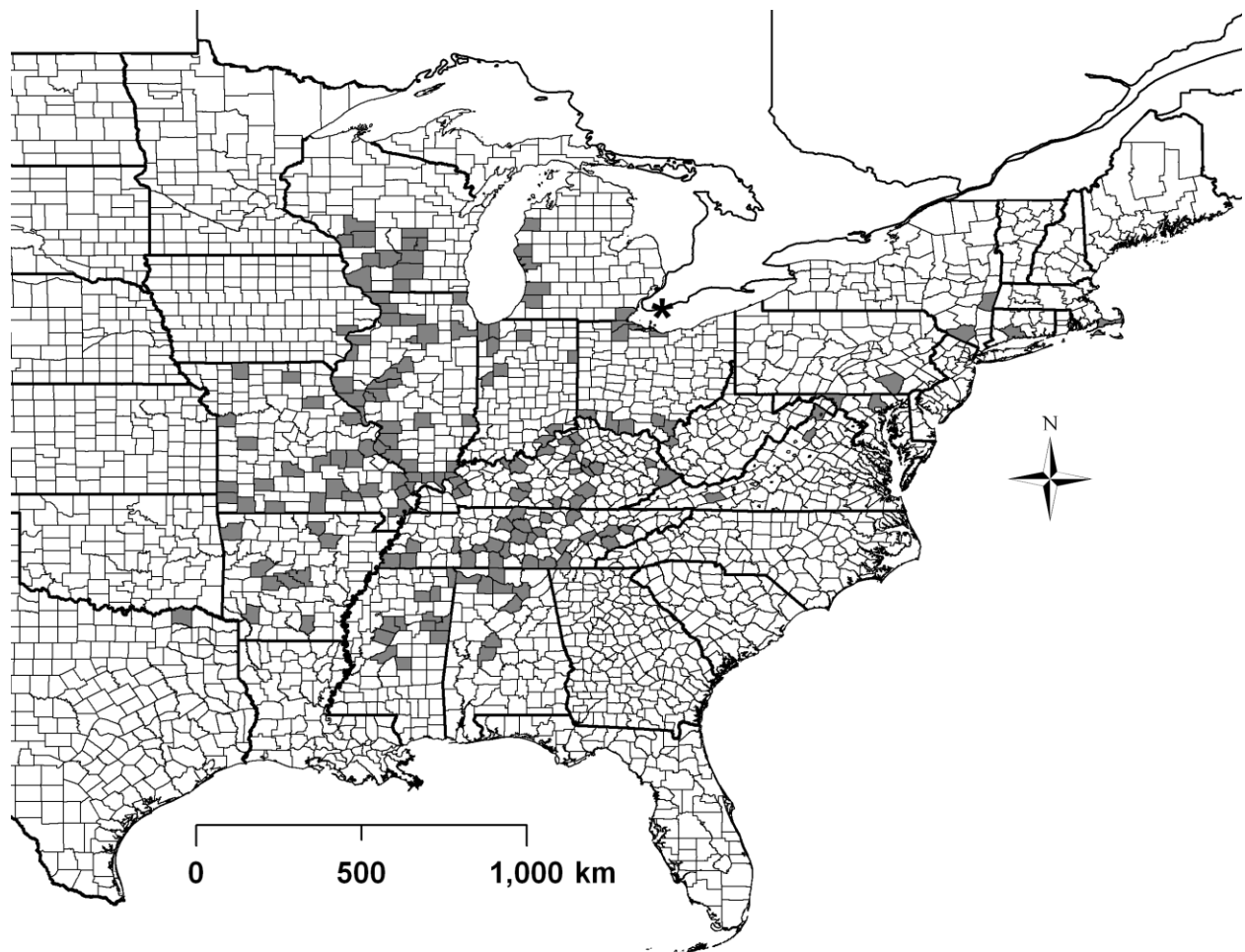


Figure 7-8. Distribution of *O. cespitosa*. Note: Essex County, Ontario is represented by *.

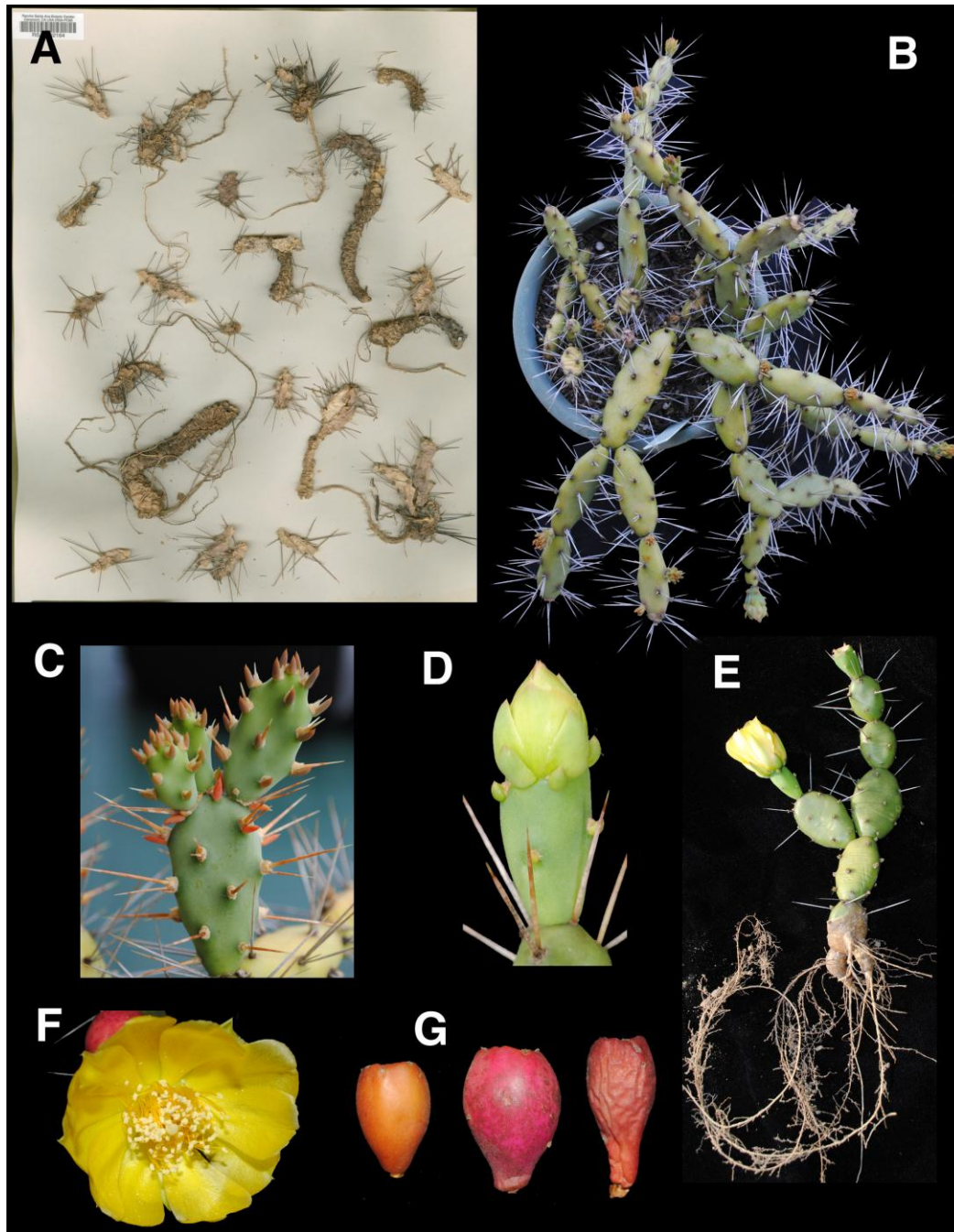


Figure 7-9. Morphological features of *O. drummondii*. A) type specimen of *O. drummondii*, L.D. Benson 15388, St. Johns Co., FL (POM), B) spreading/trailing growth form of *O. drummondii*, C) young cladodes, showing long shoot leaves and reddish-brown developing spines, D) flower bud of *O. drummondii*, E) fibrous roots of *O. drummondii* showing proximal thickenings, flower F) and fruit G) of *O. drummondii*.

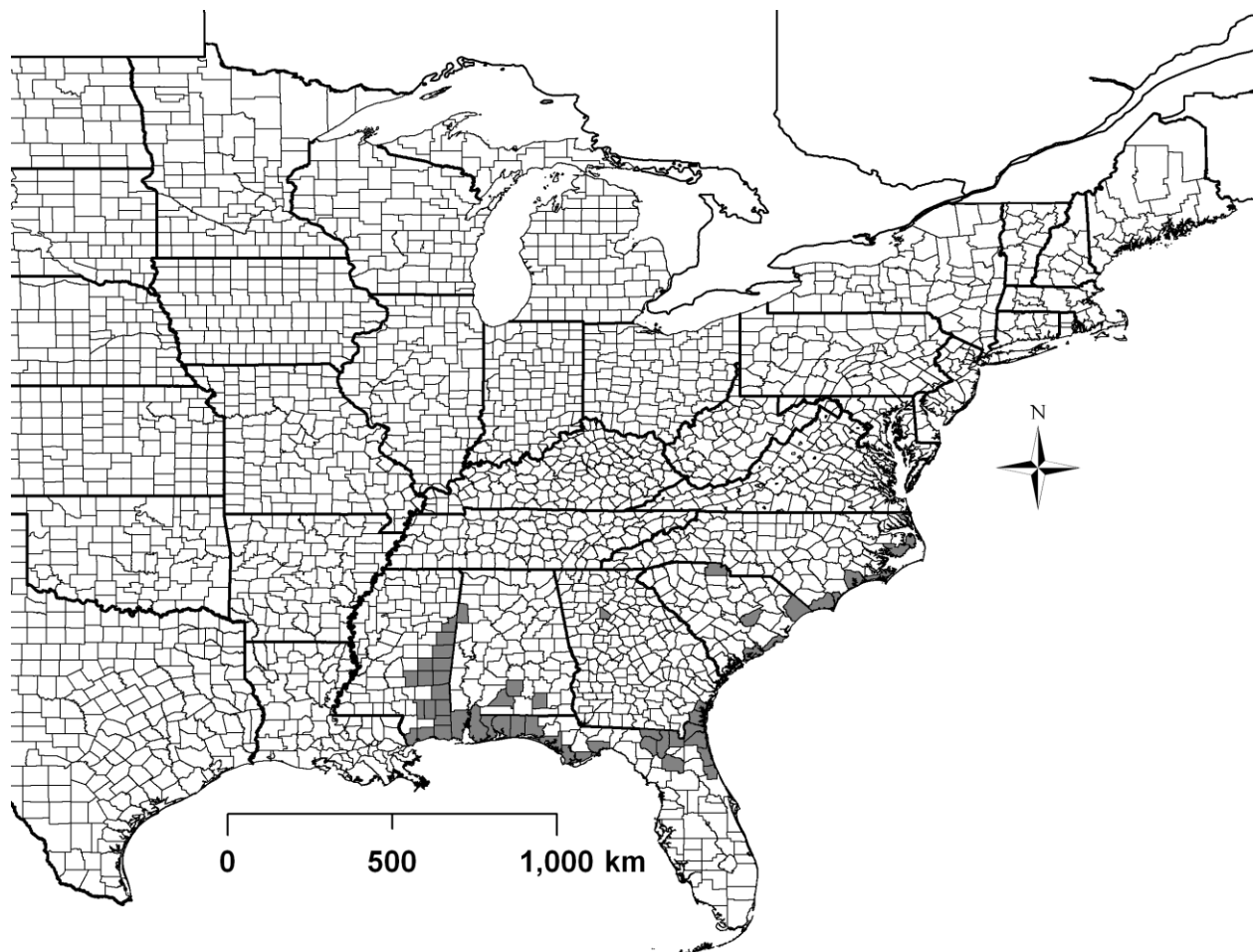


Figure 7-10. Distribution of *O. drummondii*.

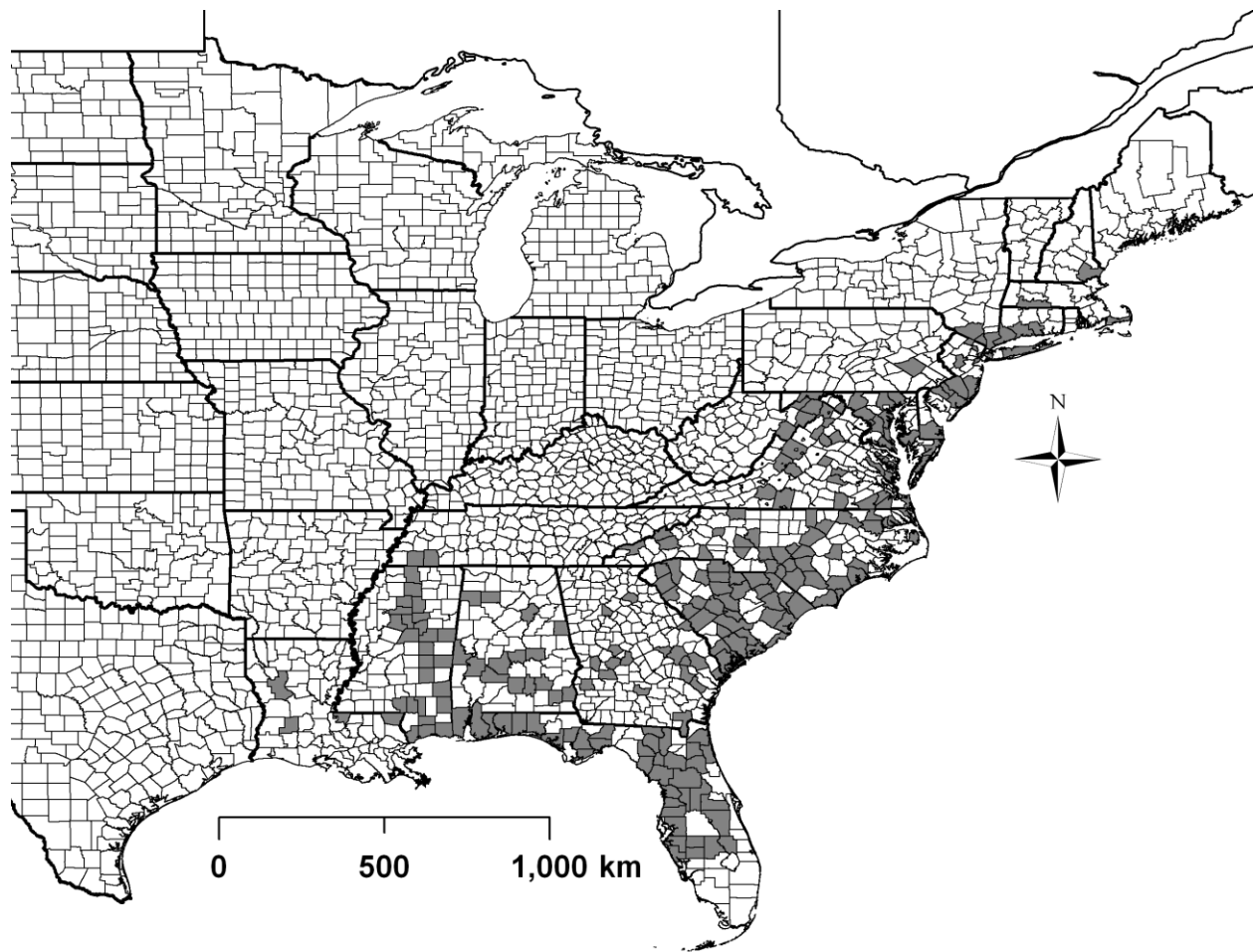


Figure 7-11. Distribution of *O. humifusa*.

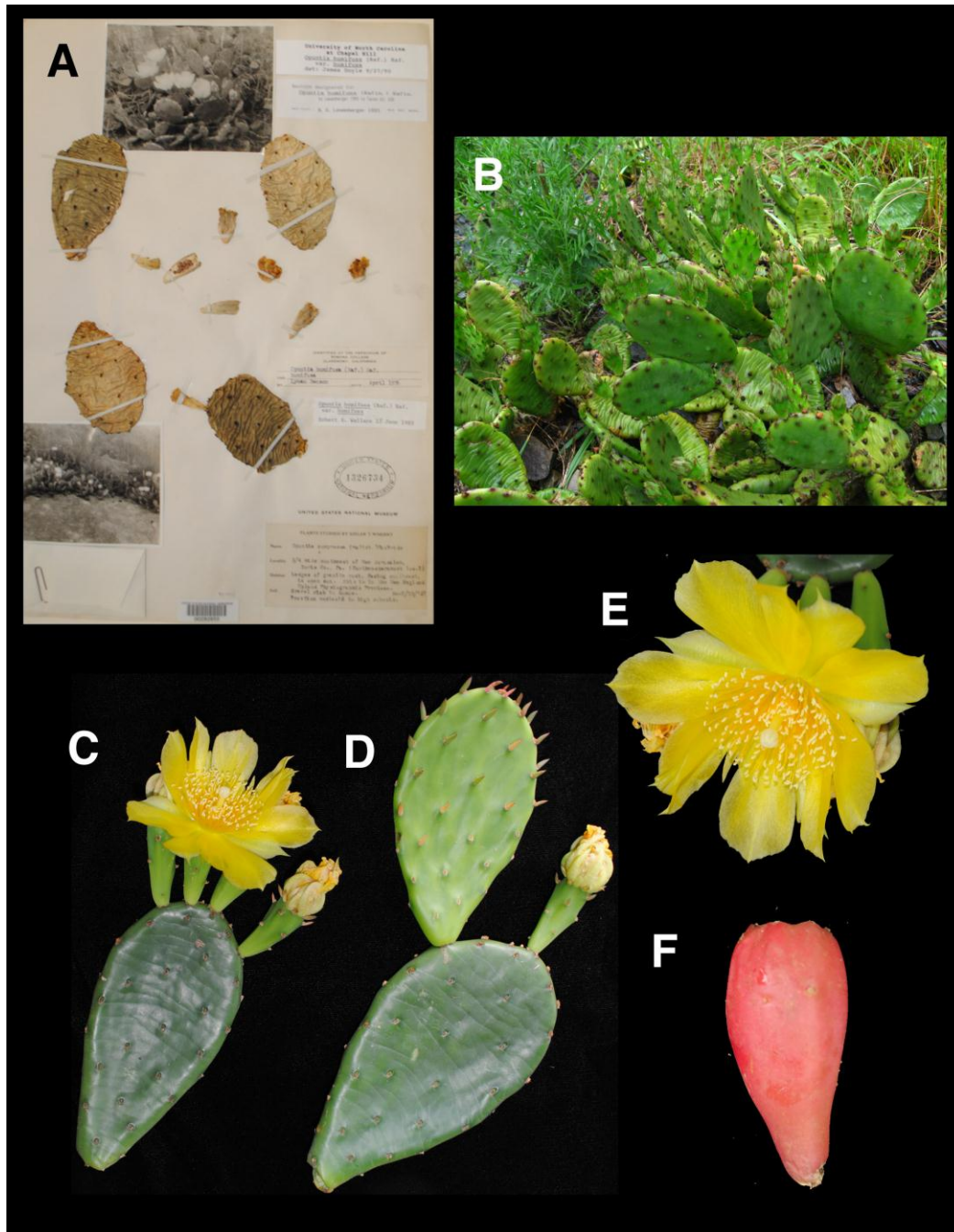


Figure 7-12. Morphological features of *O. humifusa* subsp. *humifusa*. A) type specimen of *O. humifusa*, C.T. Wherry s.n., Berks Co., PA (US), B) clumping/spreading growth form of *O. humifusa* subsp. *humifusa*, C-D) flower, mature cladodes showing inconspicuous glochids, and immature cladode with leaves, flower E) and fruit F) of *O. humifusa* subsp. *humifusa*.

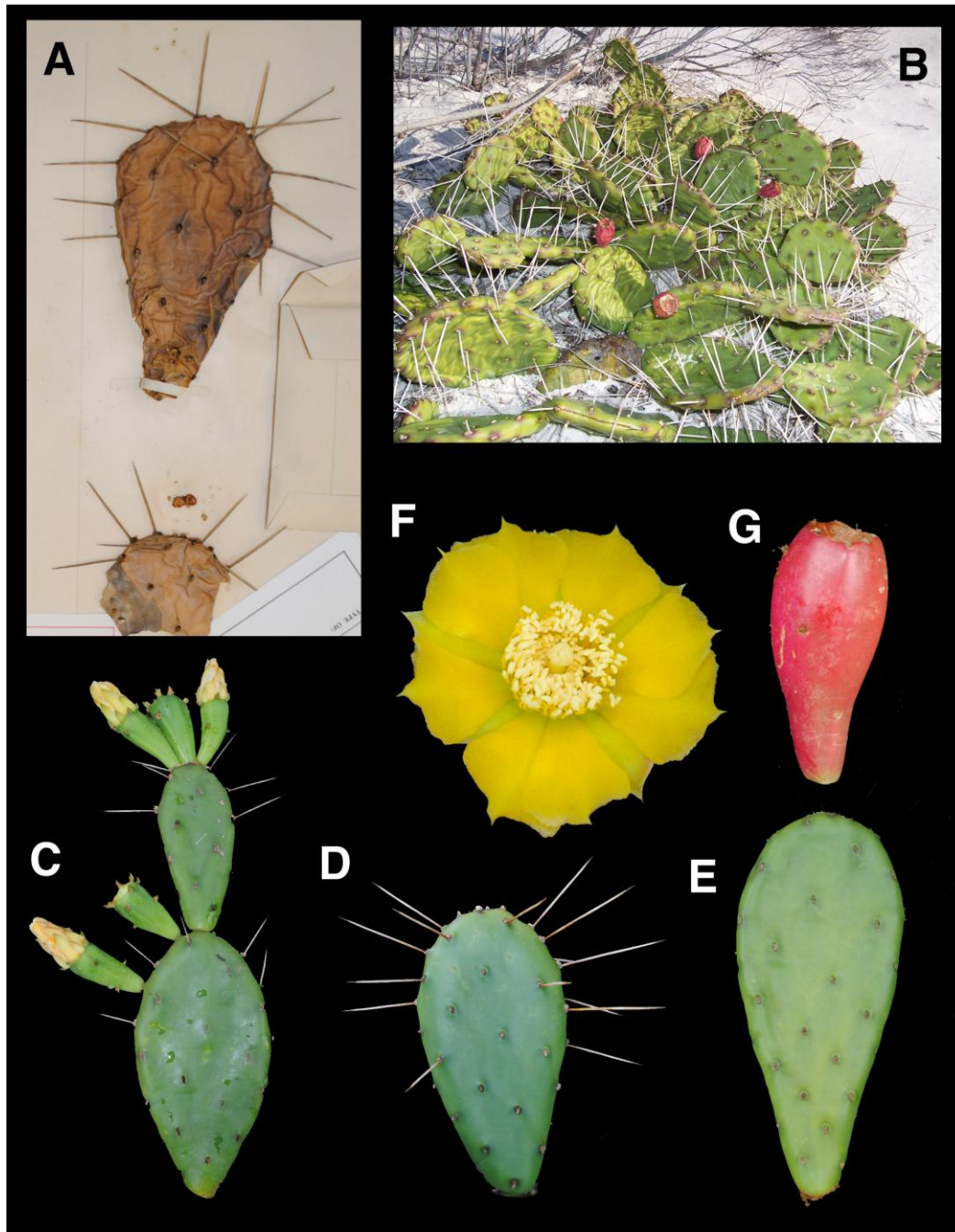


Figure 7-13. Morphological features of *O. humifusa* subsp. *pollardii*. A) type specimen of *O. pollardii*, C.L. Pollard 1138, Harrison Co., MS (NY), B) growth form of *O. humifusa* subsp. *pollardii*, C-E) cladode and spine production variation in *O. humifusa* subsp. *pollardii*, F) flower and G) fruit of *O. humifusa* subsp. *pollardii*.

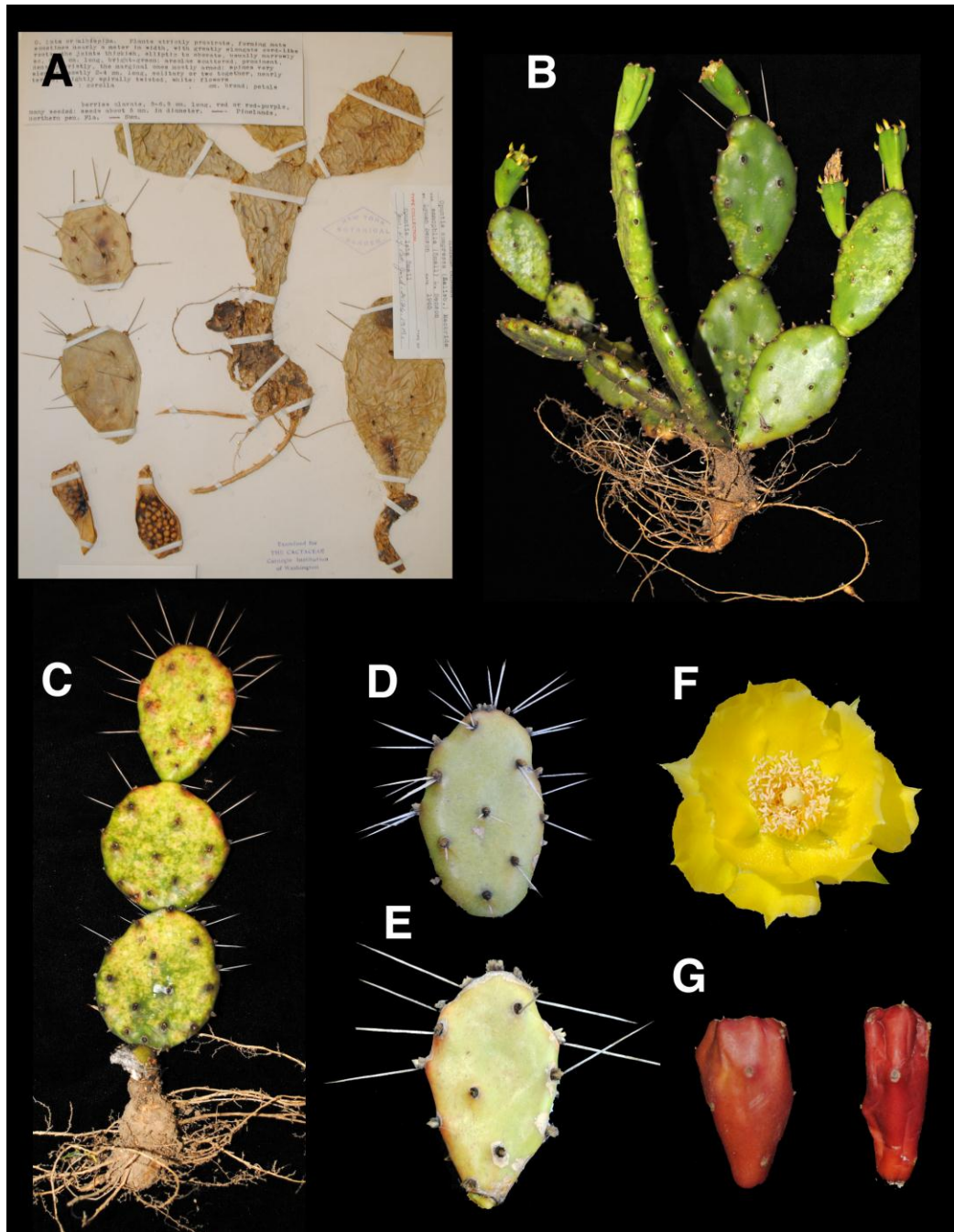


Figure 7-14. Morphological features of *O. humifusa* subsp. *lata*. A) type specimen of *O. lata*, J.K. Small s.n., Alachua Co., FL (NY), B-E) growth form, cladode shape, and spine production variation in *O. humifusa* subsp. *lata*, F) flowers and G) fruits of *O. humifusa* subsp. *lata*.

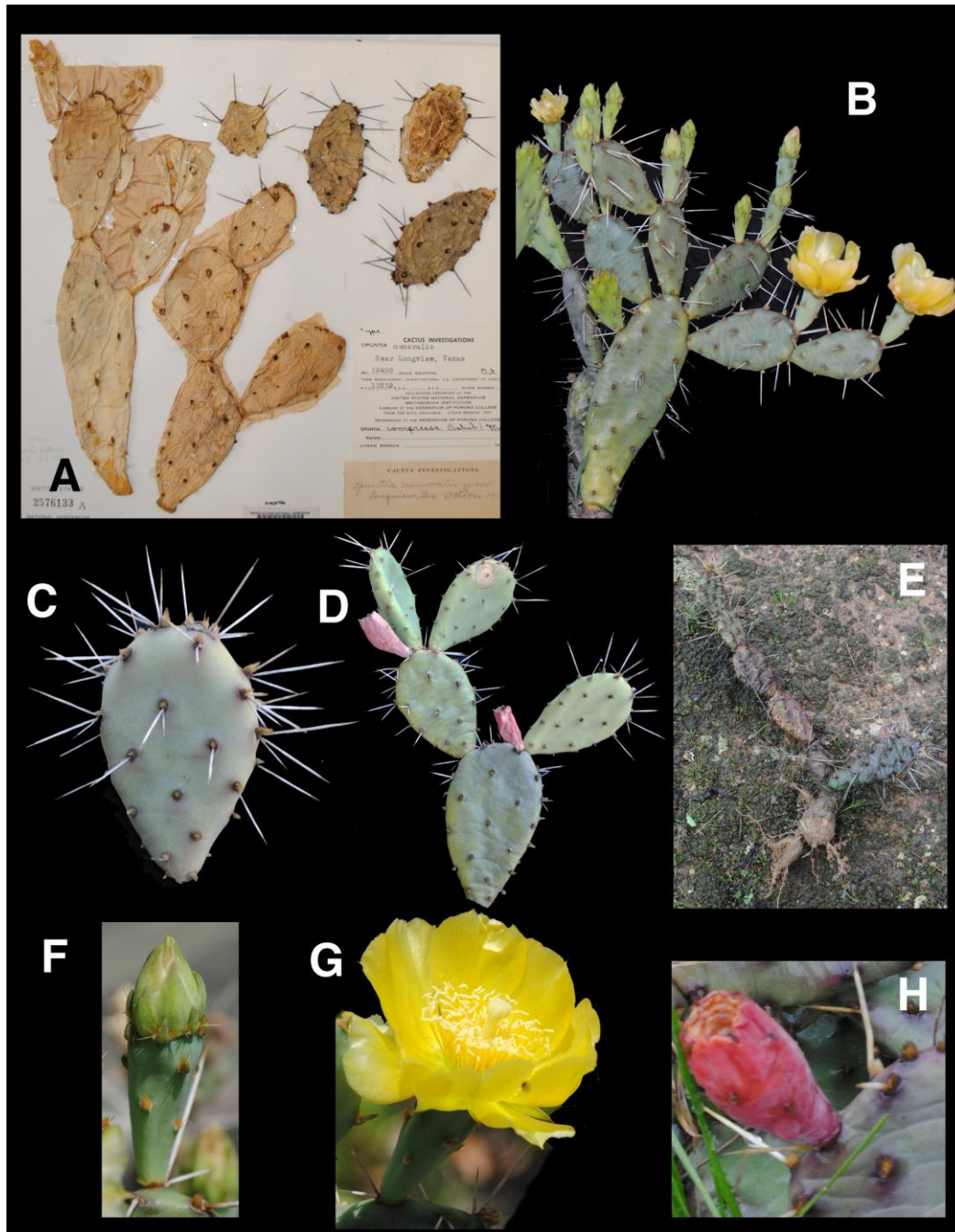


Figure 7-15. Morphological features of *O. nemoralis*. A) type specimen of *O. nemoralis*, D. Griffiths 10480, Gregg Co., TX (NY), B) growth form of *O. nemoralis*, C-D) cladode shape variation, glaucous, gray-green color, and spine variation in *O. nemoralis*, E) tuberous roots of *O. nemoralis*, F) flower bud, G) flower, and H) red fruit of *O. nemoralis*.

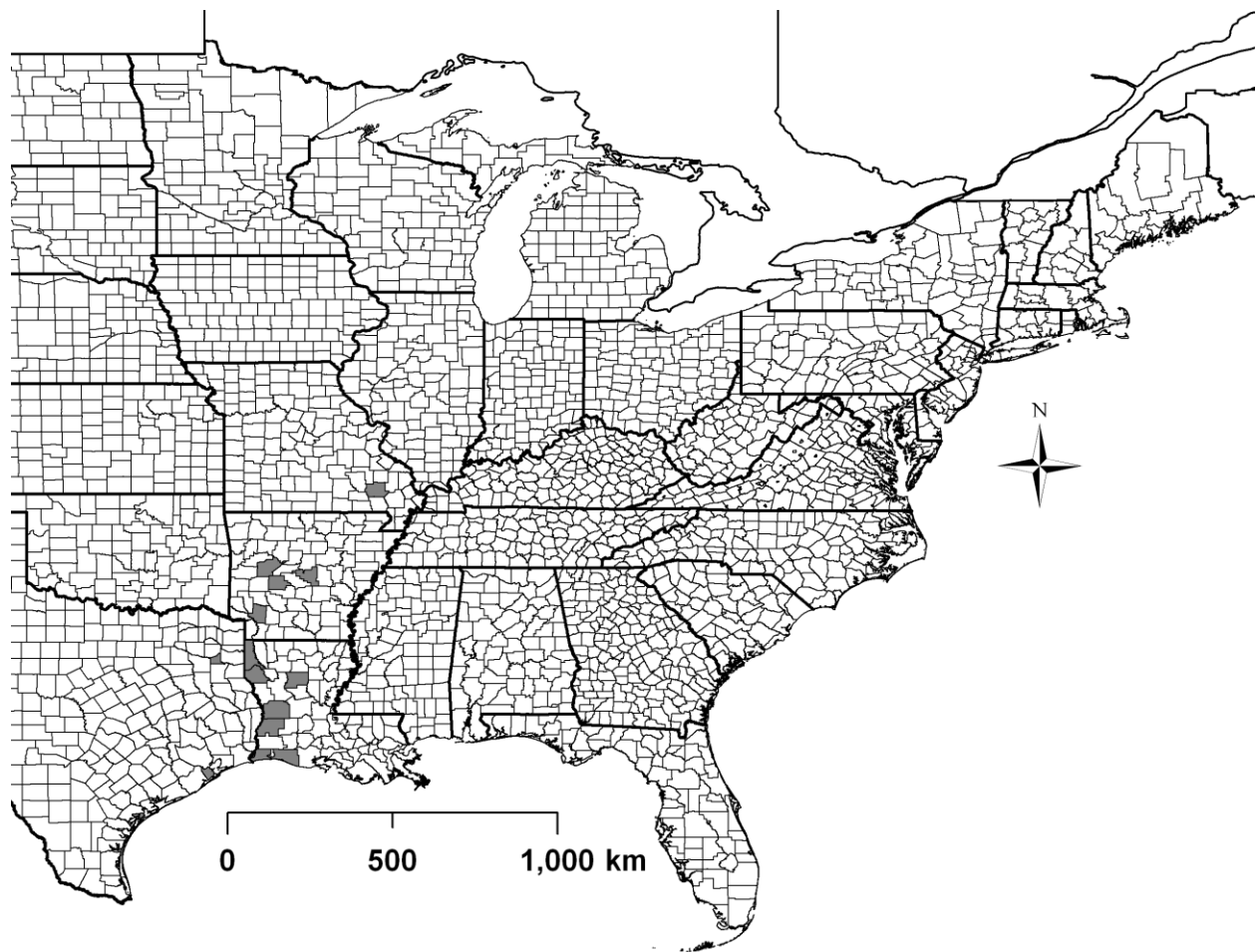


Figure 7-16. Distribution of *O. nemoralis*.

CHAPTER 8

GENERAL CONCLUSIONS

The overall goal of this work was to clarify the phylogenetic limits of *Opuntia* s.s. and the *Humifusa* clade (including the *O. humifusa* complex), as well as to provide an understanding of the distribution of ploidy in the group and the influence of reticulate evolution in species formation with the ultimate purpose of providing a taxonomic revision of the *O. humifusa* complex. The first objective was to determine the phylogenetic relationships among genera of Opuntieae, the circumscription of *Opuntia* s.s. and the *Humifusa* clade, as well as the biogeographic history and divergence date of *Opuntia* s.s. *Tacinga* and *Brasiliopuntia* along with *Opuntia lilae* and *O. schickendantzii* form a well-supported clade sister to *Opuntia* s.s. *Nopalea* is deeply nested within *Opuntia* s.s., and *Consolea* is resolved within *Opuntia* s.s. using ITS data and as sister to the *Tacinga* + *Brasiliopuntia* + *Opuntia* clade using plastid data. Thus, *Nopalea* is nothing more than a group of hummingbird-pollinated species of *Opuntia*. *Consolea* either evolved from a hybrid-derived ancestor that originated from *Opuntia* and a member of some other clade of Opuntieae (regarding plastid and ITS data placements), or ITS data could be confounded by homoplasy or incomplete lineage sorting. Regardless, although the position of *Consolea* within Opuntieae needs to be tested further, *Consolea* is monophyletic and should be recognized as a genus. *Opuntia* s.s. originated in southern South America in the late Miocene and then subsequently spread to the dry Central Andean Valleys of northwestern South America and the North American Desert region, where it further diversified and formed numerous species via reticulate evolution. Most of those species also are polyploid. The *Humifusa* clade is well supported. It apparently evolved in the western North American desert region at the end of the Pliocene and then migrated to eastern North America, leading to the evolution of the *O. humifusa* complex.

Opuntia lilae was resolved outside of *Opuntia* s.s. as sister to *Tacinga palmadora*. To resolve the position of *O. lilae*, I reconstructed a phylogeny of the genera of Opuntieae, including *O. lilae*, and mapped morphological characters on the phylogeny to determine the synapomorphies of *Tacinga* and to discover those characters shared with *O. lilae* and *Tacinga*. *Tacinga* exhibits deep umbilici, reduced perisperm relative to embryo size, hook-shaped embryos, and raised stomata, all characters shared with *O. lilae*. I subsequently transferred *O. lilae* to the genus *Tacinga*.

Next, I reconstructed the phylogeny of various clades of *Opuntia* s.s. to determine the position of *O. abjecta*, *O. militaris*, and *O. triacantha*. The polyploid taxa and putative hybrids *O. cubensis* and *O. chrocentra* were included to determine their origins. Although *O. militaris* and *O. triacantha* are closely related, they are not sister taxa, suggesting that they may represent different species. *Opuntia abjecta* is placed in the *Humifusa* clade, completely unrelated to *O. triacantha*. *Opuntia cubensis* and *O. ochrocentra* are resolved in different progenitor clades as well, suggesting that they are derived from separate crosses. Thus, *O. abjecta* and *O. militaris* are not synonymous with *O. triacantha*; *O. cubensis* and *O. ochrocentra* are not the same and should be recognized as distinct species.

Chromosome counts of the *Humifusa* clade were carried out to determine the geographic distribution of ploidal levels of members in the group. Diploids were confined to the southwestern (SW) and southeastern (SE) United States, while polyploids were distributed much more broadly: from the southern United States north to Canada. Many of the polyploids display morphological features from both the SW and SE diploids and are suggested to have originated via hybridization from members of both groups. An ITS phylogeny resolves the diploids in either a SW or a SE subclade, with the polyploids found in either subclade, supporting the hypothesis

that hybridization between members of these subclades led to the formation of certain polyploid taxa. This hybridization and polyploidy most likely occurred after the last glacial maximum, and polyploid taxa subsequently occupied open niches northward following glacial retreat.

Next, I reconstructed the phylogeny of the *Humifusa* clade to aid in the determination of species limits and to explore further the origin of polyploid taxa. Diploids again were resolved in two clades (i.e., SW and SE), and numerous polyploids were found to be of allopolyploid origin between the two clades, supporting the hypothesis of polyploid formation and subsequent increase in distribution after the last glacial maximum of the Pleistocene. *Opuntia humifusa* s.l. was found to be highly polyphyletic and inferred to represent several separate species.

I then presented a taxonomic revision of the *O. humifusa* complex. In the taxonomic treatment, I recognized seven species: *O. abjecta*, *O. austrina*, *O. cespitosa*, *O. drummondii*, *O. humifusa* (including three subspecies), *O. nemoralis*, and *O. ochrocentra*, provided a key for their identification, nomenclatural information, detailed species descriptions, and distribution maps (based on detailed assessment of living populations and herbarium material).

Evolution and species delimitation in *Opuntia* are very complex. Frequent hybridization and polyploidy, morphological variation and phenotypic plasticity, and the high distortion of dried material, coupled with the lack of basic biological data (e.g., lack of knowledge regarding variation in ploidy, sparse collections due to difficulty in preparing high-quality herbarium material), present obstacles in undertaking systematic studies. However, the occurrence of reticulate evolution, often with subsequent polyploidy, offers a very interesting system in which to study the consequences of their important evolutionary events. In addition, the ease of propagation of these plants allows a distinctive advantage over many other groups in studying

developmental patterns in a given species and for readily providing material (e.g., dividing roots tips) for chromosome counts.

Future studies will consist of resolving specific relationships among all major clades in *Opuntia* s.s. and providing systematic treatments (at the species level) of those taxa. The vast array of morphological diversity also is of interest, and more work will be carried out to determine the phylogenetic pattern of morphological character states in *Opuntia* s.s. and other clades of Opuntieae. Specifically, shifts in pollination syndrome are of interest as several clades in Opuntieae, including certain members of *Opuntia* s.s., have switched to hummingbird pollination. Resolving the placement of the problematic genus *Consolea*, is also of special interest.

APPENDIX A
VOUCHERS USED WITH GENBANK NUMBERS

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 and 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, (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. Bigelo, *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*
pachyrrhiza 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 taponia* 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.

APPENDIX B
ACCESSIONS USED WITH GENBANK NUMBERS

Specimens used in our phylogenetic analysis given with their GenBank accession numbers (*ndhF-rpl32*, *psbJ-petA*, *atpB-rbcL*, *trnL-F*, *matK*, *ycf1*, *ppc*, nrITS). Material obtained from botanical gardens is given with the garden acronym as follows: DBG (Desert Botanical Garden, Phoenix, AZ), HBG (Huntington Botanical Garden, San Marino, CA). Voucher information is provided following the garden acronym where applicable.

***Brasiliopuntia brasiliensis* (Willd.) A. Berger:** DBG 1990 0559 02, *Zimmerman 2606*, Cult. (DES), JF787309, JF787469, JF787155, JF712685, JF786712, JN387143, JN387207, JF786876; ***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; ***Opuntia arechavalatae* Spegazzini:** DBG, JF787338, JF787496, JF787181, JF712714, JF786742, JN387155, JN387220, JF786906; ***Opuntia lilae* Trujillo & Ponce:** DBG 1997 0369 01, *Trujillo & Ponce 18643*, Venezuela, Sucre (MY), JF787390, JF787612, JF787233, JF712769, JF786797, JN387171, JN387237, JF786955; ***Opuntia macbridei* Britton & Rose:** *L.C. Majure 3848*, United States, FL (cult., FLAS), JF787423, JF787616, JF787269, JF712806, JF786833, JN387184, JN387249, JF786990; ***Opuntia quimilo* K. Schumann:** DBG 2003 0111 0101, Argentina, cult., JF787421, JF787569, JF787267, JF712804, JF786831, JN387183, JN387248, JF786988; ***Opuntia schickendantzii* F.A.C. Weber,** DBG 2010 0049 01, Puente s.n., Cult., (DES), JF787437, JF787582, JF787282, JF712820, JF786848, JN387192, JN387257, JF787003; ***Salmiopuntia salmiana* (Parmentier ex Pfeiffer) Guiggi:** HBG 18366, JF787433, JF787579, JF787279, JF712816, JF786843, JN387189, JN387254, JF786999; ***Tacinga funalis* Britton & Rose:** —, —, —, —, AY042660, —; ***Tacinga inamoena* (K.Schumann) Taylor & Stuppy:** *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**) **Taylor & Stuppy**: DBG 1997 0392 01, Brazil, (DES), JF787460, JF787603, JF787307, JF712845, JF786872, JN387203, JN387267, JF787028; *Tacinga saxatilis* (**F.Ritter**) **Taylor & Stuppy**: DBG, *C. Hamann s.n.*, cult, (DES), JF787468, JF787620, JF787308, JF712846, JF786873, JN387204, JN387268, JF787029; *Tunilla corrugata* (**Salm-Dyck**) **Hunt & Illiff**: DBG 2001 0005, JF787461, JF787604, —, JF712847, JF786874, JN387205, JN387269, JF787030.

APPENDIX C
SPECIMENS EXAMINED FOR CHAPTER 4

Opuntia abjecta Small: **Florida**, Monroe Co., Big Pine Key, *P. Barrtsch s.n.*, 12 May 1919 (US); *E.P. Killip 41332*, 4 May 1951 (US), *E.P. Killip 41708*, 10 Jan 1952 (US); *L.C. Majure 3908* with I. Marino, M. Pajuelo, 6 Mar 2010 (FLAS); *G.S. Miller, Jr. 1710*, 22 Feb 1935 (US); *J.K. Small s.n.* w/ P. Matthaus, 12 April 1921 (type: NY); *J.K. Small s.n.*, 17 May 1922 (NY-US); Crawl Key, *K. Sauby s.n.*, July 2008 (FLAS); Long Key, small area of rocky, open, low ground, *C. Byrd s.n.*, 23 April 1966 (FLAS).

Opuntia cubensis Britton and Rose: **Cuba**, Guantánamo Bay, Oriente, dry sand, valley near coast, *N.L. Britton 2064*, 17-30 March 1909 (type: NY); *Areces-Mallea s.n.* (FLAS).

Opuntia militaris Britton and Rose: **Cuba**, Guantánamo Bay, Oriente, coastal hills, *N.L. Britton 1957*, 17-30 March 1909 (Type: NY); Guantánamo Bay, *R.N. Jervis 246*, 4 Sept 1950 (MICH); Schist hills and fringing fossil coral terrace from Escondido Bay to US Naval Operating Base, *R.N. Jervis 1033*, 7 Jan 1951 (MICH).

Opuntia ochrocentra Small: **Florida**, Monroe Co., Big Pine Key, Hammock, southern end of Big Pine Key, *J.K. Small s.n.* with G.K. Small, P. Matthews, 11 Dec. 1921 (type: NY); Hammock, southern front of Big Pine Key, *J.K. Small s.n.*, 17 May 1922 (US); Big Pine Key, *E.P. Killip 31423*, 12-18 Feb, 1935 (US); Big Pine Key, hammock, *E.P. Killip 31712*, 2 Mar 1936 (US); Big Pine Key, southeast hammock, *E.P. Killip 42026*, 19 Mar 1952 (US); southern end of Big Pine Key, *L.C. Majure 3907* with I. Marino, M. Pajuelo, 6 Mar 2010 (FLAS); Big Munson Island, *L.C. Majure 3968-69*, with I. Marino, 8 Mar 2010 (FLAS).

Opuntia repens Bello: **Puerto Rico**, Punta Melones, coastal rocks, *N.L. Britton s.n.*, 26 Feb 1915 (NY); Lajas, ca. 5km NW of La Parguera, off of Hwy. 116, *L.C. Majure 3838* with T. Majure, F. Axelrod, 15 June 2009 (FLAS); Cabo Rojo, Refugio de vida Silvestre, Salinas de

Cabo Rojo, *L.C. Majure* 3839 with T. Majure, F. Axelrod, 15 June 2009 (FLAS); **St. Thomas**, off of Hwy. 32E at Red Hook, ca. 1 km NE of interisland ferry, *L.C. Majure* 3837 with T. Majure, 13 June 2009 (FLAS).

Opuntia triacantha (Willd.) Sweet: **Antigua**, *H.E. Box* 1455, Galley Bay, xerophytic coastal areas, 21 May 1938 (US); *J.N. Rose* 3304 w/ W.R. Fitch, P.G. Russell, 4-16 Feb 1913 (NY-US); English Harbour, *A.C. Smith* 10442, 4 Apr 1956 (NY-US); **Guadeloupe**, Mornes Basaltiques secs., Isle les Saintes; Terre de Haut, Morne Charreau, *H. Stehlé* 1726, 15 May 1917 (NY); Raquette volante, Isle les Saintes, Désirade (Leprosérie), *Pére Duss* 3071, 1904 (NY-US); **Montserrat**, rocky cliffs, *J.A. Schafer* 543, 13 Feb 1907 (NY-US); **Puerto Rico**, Desecheo Island, coastal rocks, *N.L. Britton* 1565 w/ J.F. Cowell, W.E. Hess, 18-19 Feb 1914 (NY); **Saba**, SW corner of island; Giles Quarter Trail; 17° 36' 55"N 63° 14' 45"W, *S.A. Mori* 22693 w/ C.A. Gracie, W.R. Buck, P.C. Hoetjes, M. Hoetjes, H. Sipman, J. den Dulk, 4 Mar 2007 (NY); **St. Barthelemy**, *A. Anested* 924, 1939 (US); **St. Kitts**, Basseterre, *J.N. Rose* 3241 w/ W.R. Fitch, P.G. Russell, 2 Feb 1913 (NY); **St. Martins**, *N.L. Britton* , 1901 (US); **St. Thomas**, Buck Island, coastal rocks, *N.L. Britton* 1388 w/ J.A. Schafer, 25 Feb 1913 (NY).

APPENDIX D
ACCESSIONS USED FOR CHROMOSOME COUNTS

Currently recognized *Opuntia* species investigated are listed (1-6). Synonyms of recognized species (sensu Benson 1982, Pinkava 2003, and Powell et al. 2008 in part; see Table 1) and their respective ploidy are given below the recognized species name. Recognized species are split by ploidy, where species have more than one cytotype. Their somatic chromosome number is given along with locality, collector, and repository according to Index Herbariorum (Thiers 2011). Taxa counted for the first time or cytotypes not previously recorded for a species are delimited with an asterisk (*). All counts were made by L.C. Majure.

1) *Opuntia abjecta* Small

*** *Opuntia abjecta* Small; $2n = 22$ Florida, Monroe Co., LCM 3908 (FLAS). * *Opuntia abjecta* Small; $2n = 44$, Florida, Monroe Co., LCM 3318 (FLAS), Monroe Co., KS s.n. (FLAS).**

2) *Opuntia humifusa* (Raf.) Raf.

***Opuntia humifusa* (2x) taxa: *Opuntia ammophila* Small; $2n = 22$, Florida, Brevard Co., LCM 2087 (MISSA), Broward Co., KS 62 (FLAS), Flagler Co., LCM 3222 (FLAS), Indian River Co., LCM 4182 (FLAS), Indian River Co., LCM 4183 (FLAS), Indian River Co., LCM 4184 (FLAS), Lake Co., LCM 3246 (FLAS), Lake Co., LCM 4093 (FLAS), Marion Co., LCM 2753 (FLAS), Marion Co., LCM 2754 (FLAS), Marion Co., LCM 2826 (FLAS), Marion Co., LCM 3247 (FLAS), Okeechobee Co., LCM 4185 (FLAS), Okeechobee Co., LCM 4186 (FLAS), Orange Co., LCM 2086 (MISSA), Orange Co., LCM 3962 (FLAS), Osceola Co., LCM 3702 (FLAS), Osceola Co., LCM 4181 (FLAS), Osceola Co., LCM 4189 (FLAS), Putnam Co., LCM 3248 (FLAS), St. Johns Co., K.S. s.n. (FLAS), St. Lucie Co., LCM 3704 (FLAS), St. Lucie Co., LCM 3705 (FLAS), St. Lucie Co., LCM 3708 (FLAS), Seminole Co., LCM 2085 (MISSA), Volusia Co., LCM 3224 (FLAS), Volusia Co., LCM 3232 (FLAS). *Opuntia austrina* Small; $2n$**

= **22, Florida**, Charlotte Co., *KS 45* (FLAS), Highlands Co., FL *KS 64* (FLAS), Highlands Co., *LCM 3450* (FLAS), Highlands Co., *LCM 3975* (FLAS), Highlands Co., *LCM 3976* (FLAS), Highlands Co., *LCM 3978* (FLAS), Okeechobee Co., *KS 29* (FLAS), Okeechobee Co., *KS 42* (FLAS), Palm Beach Co., *LCM 3970* (FLAS), Palm Beach Co., *LCM 3973* (FLAS), Polk Co., *KS s.n.* (FLAS), Polk Co., *LCM 3979* (FLAS). **Opuntia lata Small; 2n = 22, Alabama**, Autauga Co., *LCM 2043* (MISSA), Mobile Co., *LCM 4194* (FLAS), **Florida**, Alachua Co., *LCM 3991* (FLAS), Alachua Co., *LCM 4061* (FLAS), Alachua Co., *LCM 4064* (FLAS), Hernando Co., *LCM 3948* (FLAS), Highlands Co., *LCM 3977* (FLAS), Lafayette Co., *LCM 2795* (FLAS), Lake Co., *KS 15* (FLAS), Lake Co., *LCM 4117* (FLAS), Levy Co., *LCM 3645* (FLAS), Manatee Co., *LCM 4065* (FLAS), Okaloosa Co., *LCM 3954* (FLAS), Okeechobee Co., *LCM 4187* (FLAS), Okeechobee Co., *LCM 4188* (FLAS), Orange Co., *LCM 4174* (FLAS), Palm Beach Co., *LCM 3971* (FLAS), Putnam Co., *LCM 4106* (FLAS), Sumter Co., *LCM 3238* (FLAS), Sumter Co., *LCM 4066* (FLAS), **Georgia**, Charlton Co., *LCM 4190* (FLAS), Crawford Co., *JH s.n.* (FLAS), Irwin Co., *LCM 3785* (FLAS), Houston Co., *LCM 3786* (FLAS), Tatnall Co., *JH s.n.* (FLAS), **Mississippi**, Newton Co., *LCM 938* (MISSA), Wayne Co., *LCM 1290* (MISSA), **South Carolina**, Aiken Co., *LCM 3588* (FLAS), Horry Co., *LCM 3832* (FLAS).

***Opuntia humifusa* (4x) taxa: *Opuntia allairei Griffiths; 2n = 44, Texas**, Liberty Co., *LCM 3504* (FLAS). ***Opuntia cespitosa Raf.; 2n = 44, Alabama**, Bibb Co., *LCM 2042* (MISSA), Colbert Co., *LCM 2610* (MISSA), Lawrence Co., *LCM 2609* (MISSA), **Arkansas**, Garland Co., *LCM 2198* (FLAS), Garland Co., *LCM 4203* (FLAS), Garland Co., *LCM 4205* (FLAS), Saline Co., *LCM 2194* (MISSA), Yell Co., GPJ s.n. (FLAS), **Illinois**, Cass Co., IL *ER s.n.* (FLAS), Jo Daviess Co., IL *ER s.n.* (FLAS), **Kentucky**, Anderson Co., *LCM 3276* (FLAS), **Louisiana**, Caddo Parish, *LCM 4200* (FLAS), Caddo Parish, *LCM 4201* (FLAS), Caddo Parish,

LCM 4202 (FLAS), **Massachusetts**, Dukes Co., *BC s.n.* (FLAS), **Mississippi**, Lee Co., MS *JH s.n.* (FLAS), Lowndes Co., *LCM 755* (MISSA), Oktibbeha Co., *LCM 1380* (MISSA), Scott Co., *LCM 2563* (MISSA), **Tennessee**, Bledsoe Co., *LCM 1938* (MISSA), Cannon Co., *LCM 2072* (MISSA), Davidson Co., *JH s.n.* (FLAS), Fayette Co., *LCM 1956* (MISSA; note *O. cf. cespitosa*), Fayette Co., *JH s.n.* (note *O. cf. cespitosa* FLAS), Franklin Co., *BLS 2061* (FLAS), Lewis Co., *JH s.n.* (FLAS), Marshall Co., *JH s.n.* (FLAS), Rutherford Co., *JH s.n.* (FLAS), **Texas**, Lamar Co., *BS 2069* (FLAS), **Virginia**, Fredrick Co., *LCM 3806* (FLAS). ***Opuntia humifusa* (Raf.) Raf.; 2n = 44**, **Alabama**, Marion Co., AL *JH s.n.* (FLAS), **Delaware**, Sussex Co., *LCM 3824* (FLAS), **Georgia**, Dekalb Co., GA *LCM 3787* (FLAS), Jackson Co., *LCM 3789* (FLAS), Marion Co., *JH s.n.* (FLAS), **Maryland**, Alleghany Co., *LCM 3810* (FLAS), **Massachusetts**, Barnstable Co., MA *LCM 3814* (FLAS), **Mississippi**, Calhoun Co., MS *JH s.n.* (FLAS), Carroll Co., *LCM 799* (MISSA), Choctaw Co., *KP 499* (MMNS), Grenada Co., *LCM 1833* (MISSA), Marion Co., *J. Hill s. n.* (FLAS), Marshall Co., *LCM 1293* (MISSA), Montgomery Co., *LCM 768* (MISSA), Stone Co., *TM s.n.* (FLAS), Webster Co., *KP 498* (MMNS), Yalobusha Co., *LCM 767* (MISSA), **New Hampshire**, Rockingham Co., *B. Nichols s.n.* (FLAS), **New Jersey**, Atlantic Co., *V. Doyle s.n.* (FLAS), Burlington Co., *LCM 3821* (FLAS), **North Carolina**, Bladen Co., *JH s.n.* (FLAS), Currituck Co., *LCM 3825* (FLAS), Dare Co., *LCM 3827* (FLAS), Onslow Co., *LCM 3829* (FLAS), Rowan Co., *LCM 3793* (FLAS), Surry Co., *JH s.n.* (FLAS), **South Carolina**, Pickens Co., *LCM 3790* (FLAS), York Co., *LCM 3791* (FLAS), **Virginia**, Fredrick Co., *LCM 3807* (FLAS), Page Co., *LCM 3799* (FLAS), Warren Co., *LCM 3800* (FLAS), **West Virginia**, Hampshire Co., *LCM 3808* (FLAS), Mineral Co., *LCM 3809* (FLAS), Pendleton Co., *ER s.n.* (FLAS). ***Opuntia nemoralis* Griffiths, 2n = 44**, **Arkansas**, Garland Co., *LCM 2192* (MISSA), Garland Co., *LCM 2196* (MISSA), Garland Co., *LCM 4204*

(FLAS); **Louisiana**, Beauregard Parish, *CR s.n.* (FLAS), Cameron Parish, *LCM 4196* (FLAS), DeSoto Parish, *LCM 4198* (FLAS), DeSoto Parish, *LCM 4199* (FLAS), Winn Parish, *BLS 2053* (FLAS). **Opuntia cf. nemoralis* Griffiths, *2n = 44*, **Arkansas**, Pulaski Co., *BLS 2131* (FLAS), Yell Co., *TW s.n.* (FLAS). **Opuntia pollardii* Britton & Rose; *2n = 44*, **Alabama**, Baldwin Co., *LCM 1082* (MISSA), **Florida**, Santa Rosa Co., *LCM 1075* (MISSA), Walton Co., *LCM 1067* (MISSA), Walton Co., *LCM 1070* (MISSA), **Louisiana**, Washington Parish, *CR s.n.* (FLAS), **Mississippi**, Forrest Co., *LCM 806* (MISSA), Hancock Co., *LCM 748* (MISSA), Jackson Co., *LCM 1921* (MISSA), Jackson Co., *LCM 1297* (MISSA), Jackson Co., *LCM 4057* (FLAS), Jackson Co., *LCM s.n.* (MMNS), Neshoba Co., *LCM 1201* (MISSA), Noxubee Co., *LCM 1156* (MISSA), Stone Co., *TM s.n.* (FLAS), Winston Co., *LCM 769* (MISSA).

3) *Opuntia macrorhiza* Engelm

Opuntia macrorhiza (2x) taxa: * *Opuntia xanthoglochla* Griffiths, *2n = 22*, **Texas**, Bastrop Co., *LCM 1982* (MISSA), Bastrop Co., *MJM 949* (FLAS), Fayette Co., *LCM 1983* (MISSA), Harris Co., *BLS 2089* (FLAS), Milam Co., *TX MJM 947* (FLAS), Smith Co., *BLS 2082* (FLAS).

Opuntia macrorhiza (4x) taxa: **Opuntia fusco-atra* Engelm.; *2n = 44*, **Texas**, Fayette Co., *LCM 3505* (FLAS). **Opuntia grandiflora* Engelm.; *2n = 44*, **Arkansas**, Miller Co., *BLS 2062* (FLAS), **Mississippi**, Bolivar Co., *LCM 1680* (MISSA), Holmes Co., *HS s.n.* (FLAS), Yazoo Co., *LCM 2366* (MISSA), **Texas**, Anderson Co., *BLS 2077* (FLAS), Austin Co., *BLS 2091* (FLAS), Henderson Co., *BLS 2081* (FLAS), Jack Co., *LCM 3536* (FLAS), Leon Co., *BLS 2074* (FLAS), Marion Co., *BLS 2086* (FLAS), Smith Co., *LCM 3540* (FLAS), Van Zandt Co., *BLS 2083* (FLAS). *Opuntia macrorhiza* (4x) taxa: **Opuntia fusco-atra* Engelm.; *2n = 44*, **Texas**, Fayette Co., *LCM 3505* (FLAS). **Opuntia grandiflora* Engelm.; *2n = 44*, **Arkansas**,

Miller Co., *BLS* 2062 (FLAS), **Mississippi**, Bolivar Co., *LCM* 1680 (MISSA), Holmes Co., *HS s.n.* (FLAS), Yazoo Co., *LCM* 2366 (MISSA), **Texas**, Anderson Co., *BLS* 2077 (FLAS), Austin Co., *BLS* 2091 (FLAS), Henderson Co., *BLS* 2081 (FLAS), Jack Co., *LCM* 3536 (FLAS), Leon Co., *BLS* 2074 (FLAS), Marion Co., *BLS* 2086 (FLAS), Smith Co., *LCM* 3540 (FLAS), Van Zandt Co., *BLS* 2083 (FLAS).

4) *Opuntia pusilla* (Haw.) Haw.

* *Opuntia pusilla*, **2n = 22**, **Alabama**, Lamar Co., *JH s.n.* (FLAS), **Florida**, Alachua Co., *LCM* 4003 (FLAS), Bay Co., *KS* 307 (FLAS), Bay Co., *KS* 309 (FLAS), Columbia Co., *LCM* 4191 (FLAS), Escambia Co., *KS* 328 (FLAS), Franklin Co., *KS* 301 (FLAS), Franklin Co., *KS* 330 (FLAS), Gulf Co., *KS* 325 (FLAS), Hamilton Co., *LCM* 4192 (FLAS), Hamilton Co., FL *LCM* 4193 (FLAS), Levy Co., *LCM* 2819 (FLAS), **Mississippi**, Clarke Co., *LCM* 1270 (MISSA), Forrest Co., *LCM* 756 (MISSA), Jasper Co., *LCM* 766 (MISSA), Lamar Co., *LCM* 1548 (MISSA), Lauderdale Co., *LCM* 2094 (MISSA), Lauderdale Co., *LCM* 3919 (MISSA), Lowndes Co., *LCM* 843 (MISSA), Newton Co., *LCM* 828 (MISSA), Newton Co., *LCM* 937 (MISSA), Newton Co., *LCM* 4211 (FLAS), Perry Co., *LCM* 757 (MISSA), Smith Co., *LCM* 753 (MISSA), Wayne Co., *TM s.n.* (FLAS), Wayne Co., *TM s.n.* (FLAS). * *Opuntia pusilla*, **2n = 33**, **Alabama**, Baldwin Co., *LCM* 1091 (MISSA), **Florida**, Flagler Co., *LCM* 3221 (FLAS), St. Johns Co., *LCM* 3219 (FLAS), Walton Co., *LCM* 1066 (MISSA), **Mississippi**, Hancock Co., *LCM* 1033 (MISSA), **South Carolina**, Horry Co., *JH s.n.* (FLAS), Horry Co., *LCM* 3833 (FLAS). *Opuntia pusilla*, **2n = 44**, **Florida**, Duval Co., *LCM* 3700 (FLAS), Nassau Co., *CJ s.n.* (FLAS), St. Johns Co., *LCM* 3218 (FLAS), St. John's Co., *KS* 9.4.10 (FLAS), **Georgia**, Dekalb Co., *LCM* 3788 (FLAS), Glynn Co., *TM s.n.* (FLAS), **Mississippi**, Jackson Co., *LCM* 955 (MISSA), Jackson Co., *LCM* 1920 (MISSA), **North Carolina**, Dare Co., *LCM* 3828 (FLAS),

Dare Co., *LCM* 3836 (FLAS), New Hanover Co., *LCM* 3830 (FLAS), **South Carolina**, York Co., *LCM* 3792 (FLAS).

5a) *Opuntia stricta* (Haw.) Haw.

Opuntia dillenii (Ker-Gawl.) Haw., 2n = 66, Florida, Charlotte Co., *LCM* 3949 (FLAS), Flagler Co., *LCM* 3220 (FLAS), Monroe Co., *LCM* 3319 (FLAS), Hillsborough Co., *LCM* 3952 (FLAS), **Puerto Rico**, Cabo Rojo, *LCM* 3843 (FLAS). *Opuntia stricta* (Haw.) Haw., 2n = 66, Alabama, Mobile Co., *LCM* 823 (MISSA), **Florida**, Clay Co., *LCM* 3701 (FLAS), Levy Co., *LCM* 2820 (FLAS), Monroe Co., *LCM* 3320 (FLAS), St. Johns Co., *LCM* 3217 (FLAS), Seminole Co., *LCM* 2083 (MISSA), **Mississippi**, Jackson Co., *LCM* 1922 (MISSA).

5b) Putative hybrids involving *Opuntia stricta*.

Opuntia alta Griffiths 2n = 66, Louisiana, Cameron Parish, *LCM* 4195 (FLAS), LaFourche Parish, *CR s.n.* (FLAS). * *Opuntia ochrocentra* Small, 2n = 55, Florida, Monroe Co., *LCM* 3907 (FLAS), Monroe Co., *LCM* 3968 (FLAS), Monroe Co., *LCM* 3969 (FLAS).

6) *Opuntia tortispina* Engelm. & J.M. Bigelow.

Opuntia tortispina, 2n = 44, New Mexico, Quay Co., *LCM* 3531 (FLAS), *Opuntia tortispina*, 2n = 66, New Mexico, Benalillo Co., *LCM* 3528 (FLAS), Sierra Co., *LCM* 3521 (FLAS), **Oklahoma**, Cimarron Co., *ER s.n.* (FLAS), **Texas**, Carson Co., *LCM* 3532 (FLAS), Hutchinson Co., *LCM* 3533 (FLAS), Hutchinson Co., *LCM* 3535 (FLAS).

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BIOGRAPHICAL SKETCH

Lucas C. Majure was born in Jackson, MS and lived in Pisgah, MS until the age of four, when he and his family moved to Decatur, MS. He attended Newton County School, where he graduated in 1999. He then attended East Central Community College, where he received his associate's degree in Spring 2001. He completed undergraduate studies at Mississippi State University (MSU) in Starkville in the fall of 2003 with a major in biology and minor in Spanish. After graduation he worked at the Mississippi Museum of Natural Science for one year as an herbarium assistant and also performed work with the Natural Heritage Program locating and monitoring rare and endangered plant populations in the state of MS. In spring 2004, he started master's work at MSU with Dr. Gary N. Ervin, where he focused on the ecology and morphological variation of *Opuntia* in the mid-south United States. In the fall of 2007, he began doctoral work at the University of Florida under the direction of Drs. Doug and Pam Soltis and Dr. Walter Judd, where he studied the evolution and systematics of the *Opuntia humifusa* complex, as well as broad-scale evolution of tribe Opuntieae. Lucas graduated in the summer of 2012 with a degree in botany and began post-doctoral research in August 2012 with Walter Judd studying the species limits of a clade in the genus *Miconia* (Miconiae: Melastomataceae).