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## **Phylogenetic relationships and morphological evolution in *Opuntia* s.str. and closely related members of tribe Opuntieae**

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*Abstract.* Phylogenetic analyses of tribe Opuntieae DC., have in recent years, refined the way that we delimit *Opuntia* s.str. Seven segregate genera within tribe Opuntieae have been recognized based on previous morphological studies, as well as results from phylogenetic data (i.e. *Brasiliopuntia*, *Consolea*, *Miqueliopuntia*, *Opuntia*, *Salmiopuntia*, *Tacinga*, and *Tunilla*). Here we underscore the major clades of tribe Opuntieae, with a specific focus on the genus *Opuntia*, analyze morphological evolution throughout the clade, and discuss morphological synapomorphies of individual clades, when known. Polyploidy and hybridization have been major drivers of speciation within the Opuntieae, where polyploids account for nearly 61% of recognized species. Taxonomic implications regarding phylogenetic and cytological studies also are discussed.

### **Introduction**

Tribe Opuntieae DC. consists of seven currently recognized genera, *Brasiliopuntia* A. Berger, *Consolea* Lem., *Miqueliopuntia* Frič ex F. Ritter, *Opuntia* Mill., *Salmiopuntia* Frič, *Tacinga* Britton & Rose, and *Tunilla* D.R. Hunt & Iliff, all of which contain taxa that at one time were circumscribed within a very broad *Opuntia* s.l. (Britton & Rose 1920). Molecular phylogenetic studies have significantly reduced the size of *Opuntia* s.l., showing that many of those previously recognized genera make *Opuntia* s.str. paraphyletic thus requiring the recognition of many of those taxa (Wallace & Dickie 2002, Griffith & Porter 2009, Majure et al. 2012a, Ritz et al. 2012). Hunt (2002) discusses the arbitrary nature of the delineation of genera in this group (i.e. recognizing *Opuntia* s.l. or splitting the genus into numerous genera), however, Wallace & Dickie (2002) argued that the recognition of more, as opposed to fewer genera in Opuntioideae was on par with work done elsewhere in the family Cactaceae. Hunt et al. (2006) recognize most of the above genera based on what was known regarding the phylogeny at that time. More recent phylogenetic analyses have further refined the delimitation of *Opuntia* s.str. by demonstrating that other taxa previously circumscribed under *Opuntia* s.l. actually belong to other clades within tribe Opuntieae (Griffith & Porter 2009, Majure et al. 2012a, Majure et al. 2013a).

The *Opuntia* clade (i.e. *Opuntia* s.str.) most likely originated in southern South America during the late Miocene (Arakaki et al. 2011, Majure et al. 2012a) and from there expanded north into Peru and Ecuador, and then into North America, where it became most diverse. Most northern South American species of *Opuntia* (e.g. *O. boldinghii* Britton & Rose, *O.*

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*caracassana* Salm-Dyck, *O. curassavica* Mill., *O. schumannii* F.A.C. Weber ex A. Berger) and other widespread species in South America (i.e. *O. pubescens* Wendl.) were derived from the diverse North American clade, and made their way back to South America through the Caribbean region and also likely through Central America. Several species endemic to South America were actually derived from North American clades and are suggested to be the products of hybridization (e.g. *O. bisetosa* Pittier, *O. boldinghii*; Majure et al. 2012a).

Species originating from hybridization and polyploidy account for a large part of the species diversity in *Opuntia* s.str. and Opuntieae in general (see below). Not surprisingly, many species that have been used as ornamentals and domesticated over the millennia (e.g. *O. ficus-indica* (L.) Mill.; Kiesling 1998a) are products of hybridization and polyploidy (Griffith 2004, Majure et al. 2012a-b).

Members of the genus *Opuntia* are widely variable regarding resistance to varying temperatures. Although the genus is primarily tropical to subtropical in its distribution, numerous species can survive under extremely cold temperatures. For example, certain members of the Xerocarpa and Humifusa clades (fig. 1) exhibit the northernmost ranges of any other Opuntioideae cacti. *Opuntia fragilis* (Nutt.) Haw. (Xerocarpa clade) is the northernmost distributed cactus, occurring in areas that experience annual low temperatures of -40°C (Loik & Nobel 1993) and *O. humifusa* (Raf.) Raf. s.l. (Humifusa clade) has been recorded to withstand temperatures as low as -20°C (Nobel & Bobich 2002).

Species delimitation within Opuntieae (especially *Opuntia*) is notoriously problematic taxonomically. This is the result of: 1) high amounts of hybridization resulting in mosaics of morphological features expressed by hybrid progeny, 2) morphologically variable species, where morphological characters are oftentimes dependent upon environmental variables, 3) poor specimen preparation and the lack of general collecting of species throughout their ranges, 4) the lack of basic biological data (e.g. chromosome counts), 5) the lack of detailed studies regarding morphology across the distribution of species, and 6) the lack of phylogenetic data. It is clear in numerous instances that species of *Opuntia* may be cladospecies (e.g. *O. strigil* Engelm., fig. 1; see Donoghue 1985). However, owed to the high level of hybridization and subsequent speciation via polyploidy, species often are polyphyletic, and young species in certain clades are likely to form metaspecies complexes, therefore, increasing taxonomic complexity. Cryptic species exist in this clade, and are likely more numerous than is currently known. However, they are often overlooked without incorporating all biological and phylogenetic data needed to detect them.

To date, very few taxonomic revisions have been completed for groups of species in Opuntieae (see Parfitt 1991, Leuenberger 2001, Iliff 2002, Taylor et al. 2002, Puente 2006a, Majure 2012). Most of the work done so far has mostly been regional in scope, not including the entire number of species within a given clade (e.g. Kiesling 1998b, Leuenberger 2001, Majure & Ervin 2008) or the entire distribution of given species (e.g. Madsen 1989, Majure & Ervin 2008). Also, very few treatments have been based on phylogenetic information, a result of the lack of phylogenetic data that was available for the group until recently, and most treatments incorporate very little but very important biological information (e.g. chromosome number) about species.

### **Phylogeny of *Opuntia* s. str.**

Majure et al. (2012a) using the chloroplast genes (*matK* and *ycf1*), plastid intergenic spacers (*atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, *trnL-F*), and nuclear markers (*ppc* and ITS), reconstructed a phylogeny of tribe Opuntieae with particular focus on the genus *Opuntia*. We expand upon that phylogeny here (fig. 1), also with an emphasis on *Opuntia* s. str., with data from Majure et al. (2012a, 2013b) and include only the diploid representatives of *Opuntia* that have been sampled for the above-mentioned loci, as well as members of the genus *Consolea*. The use of ITS for phylogenetic reconstruction in Opuntioideae has been analyzed in detail by Ritz et al. (2012), where it was demonstrated that occasional paralogous copies have been incorporated into analyses potentially leading to aberrant results. This is especially problematic when incorporating taxa from reticulate evolution into phylogenetic analyses (Soltis et al. 2008). The potential problems with the use of ITS for phylogenetic reconstruction are well known, thus great care must be taken when utilizing ITS for this purpose (Feliner & Roselló 2007). For this reason in particular, only diploid taxa were used here to reconstruct the phylogeny of *Opuntia*, which is well known for hybridization. The phylogeny presented here was built by combining all data (see Majure et al. 2012a regarding data combination) and using maximum likelihood (ML) in RAxML (Stamatakis 2006) under 25 rate categories implementing the GTR + gamma model of molecular evolution and carrying out 10000 bootstrap replicates. Both *Salmiopuntia* (Majure et al. 2012a) and the *Miqueliopuntia-Tunilla* clade (here) have been used as outgroup taxa based on results from Griffith & Porter (2009) and Majure et al. (2012a), as the placement of *Opuntia* s. str. and other related genera (*Brasiliopuntia*, *Consolea*, *Nopalea*, *Tacinga*) were the the primary foci of those analyses. The resultant topology of the rest of the tribe Opuntieae (*Brasiliopuntia*, *Consolea*, *Nopalea*, *Opuntia*, *Tacinga*) is the same using either *Salmiopuntia* or *Miqueliopuntia+Tunilla* as outgroups, of course with the exception of the outgroups themselves. It should be noted that when combined in larger datasets with taxa outside of tribe Opuntieae that the *Miqueliopuntia-Tunilla* clade is sister to the rest of tribe Opuntieae (including *Salmiopuntia*, data not shown). Twelve major clades were recovered in this analysis and are discussed below in more detail. Estimates of numbers of species belonging to clades, as well as putative morphological synapomorphies and/or identifying characters are given for each clade where available. Chromosome number, or ranges of ploidy are also noted when known.

### **Major Clades of Opuntieae**

*Miqueliopuntia miquelii* (Monv.) F. Ritter and *Tunilla* form a well-supported clade that is sister to the *Salmiopuntia-Consolea-Tacinga + Brasiliopuntia + Opuntia schickendantzii* F.A.C. Weber – *Opuntia* clade. *Miqueliopuntia* is monotypic and restricted to Chile (Iliiff 2002), while *Tunilla* consists of ca. 5 species (Hunt 2006) that are more widespread throughout western and southern South America (Iliiff 2002). Both *Miqueliopuntia* and *Tunilla* are clump-forming shrubs with determinate stems, with *Miqueliopuntia* having mostly cylindrical stems and *Tunilla* exhibiting cylindrical to flattened stems. *Tunilla* exhibits fleshy fruit that split longitudinally upon maturation (Hunt & Iliiff 2000) and has seeds with a rugose surface and lacking an evident funicular girdle (Stuppy 2002). *Miqueliopuntia*

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*miquelii* has the highest chromosome number of any other member of *Opuntieae*, as far as is known. However, only one chromosome number has been documented for the species, where it was recorded as 20-ploid ( $2n = 220$ ; Majure et al. 2012b). Very few chromosome counts have been carried out for species of *Tunilla*, but all reports thus far are of tetraploid individuals (Majure et al. 2012b).

*Salmiopuntia salmiana* (J. Parm ex Pfeiff.) Frič has been placed in other genera, such as *Austrocylindropuntia* Backeb. and *Cylindropuntia* (Engelm.) F.M. Knuth (see Anderson 2001), but it is now clear that the species belongs in tribe *Opuntieae* (Griffith & Porter 2009). Compared to other members of *Opuntieae*, *Salmiopuntia* is recognized by a combination of terete stems, pinkish or salmon-colored flower buds, oftentimes, sterile and proliferous fruit, and its highly branched, shrubby growth form. The species is distributed throughout parts of southern South America (Britton & Rose 1920, Anderson 2001). All previous reported chromosome counts of *S. salmiana* were polyploid either at the tetraploid (Bowden 1945, Katagiri 1952, 1953; Baker et al. 2009) or pentaploid (Yuassa et al. 1973) levels (see Majure et al. 2012b). However, curiously, Realini et al. (2014, this volume) produced three new counts of the species at the triploid level. The generic status of the species has recently come into question, and due to its frequent sterility and polyploid nature some researchers suggest that it may be of hybrid origin (Realini et al. 2014; D.R. Hunt, pers. comm.). It is clear, however, that the species does not belong in *Opuntia* s.str. (fig. 1; also see Griffith & Porter 2009, and Majure et al. 2012a), and the basic biology of *S. salmiana* needs to be investigated further to determine how best to treat the species.

The polyploid genus *Consolea* (Negrón-Ortiz 2007, Majure et al. 2012b) forms a strongly supported clade; plastid data place *Consolea* as sister to *Brasiliopuntia* + *O. schickendantzii* + *Tacinga-Opuntia* s.str. clade (BSTO clade), and ITS data resolve *Consolea* as nested within *Opuntia* s.str. (although this is poorly supported; see Griffith & Porter 2009, Majure et al. 2012a). Thus, *Consolea* possibly originated from a polyphyletic (allopolyploid) ancestor derived from *Opuntia* and another member of *Opuntieae*, or ITS data may exhibit retained ancestral polymorphism or homoplasmy leading to the placement of *Consolea* within *Opuntia* (Majure et al. 2012a). Combined plastid and nuclear ITS and *ppc* data strongly support the placement of *Consolea* as sister to the BSTO clade (see fig. 1). More nuclear gene sampling may be necessary to further test this placement, but considering morphological synapomorphies and biogeography, the resolution of *Consolea* as sister to the BSTO clade appears most likely. *Consolea* is clearly not synonymous with *Opuntia* s.str. as suggested by Nyffeler & Egli (2010).

The genus *Consolea* is restricted to the Caribbean region (Areces-Mallea 1996, 2001) and consists of nine species (Areces-Mallea 2001, Negrón-Ortiz 2007), which can be grouped morphologically into two species groups (Areces-Mallea 2001). Phylogenetic analyses suggest that certain species may be polyphyletic (fig. 1). Putative synapomorphies for the *Consolea* clade are monopodial (indeterminate) trunks with horizontal, often falcate branches, these also demonstrating a degree of indeterminate growth, giving species a semaphore-like growth pattern; cladodes may or may not possess a strongly reticulate epidermis (Areces-Mallea 2001). Species in the clade also are recognized by their small, red, pinkish or orange flowers and spiny pericarpel. Numerous species of *Consolea* are known to be

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functionally dioecious (Negrón-Ortiz 2007). Certain species abort immature fruit after flowering, which presumably aid in vegetative dispersal (Majure pers. obs.). Species of *Consolea* are either hexaploid or octoploid (Negrón-Ortiz 2007, Majure et al. 2012b).

*Tacinga* is a well-supported clade sister to the *Brasiliopuntia-Opuntia schickendantzii* clade and is composed of 8 species (Majure et al. 2013a) and one putative hybrid taxon (Taylor et al. 2002). Morphologically the clade is composed of three species groups (*T. palmadora* (Britton & Rose) N.P. Taylor & Stuppy, *T. lilae* (Trujillo & Ponce) Majure & R. Puente and *T. wernerii* (Eggli) N.P. Taylor & Stuppy; *T. funalis* Britton & Rose and *T. braunii* Esteves; *T. saxatilis* (F. Ritter) N.P. Taylor & Stuppy, *T. inamoena* (K. Schum.) N.P. Taylor & Stuppy and *T. subcylindrica* (M. Machado & N.P. Taylor) M. Machado & N.P. Taylor; Taylor et al. 2002, Lambert 2009, Majure et al. 2013a), but only two major clades are recovered in our phylogeny (fig. 1). This is most likely a result of incomplete taxon/locus sampling, as only *matK* was available for *T. funalis*. Larger data sets including all species of *Tacinga* and more loci would most likely reinforce these species groups as clades. Synapomorphies of the *Tacinga* clade are raised stomata, deep, narrow fruit umbilici, hooked embryos, and reduced endosperm (Majure et al. 2013a). Ploidal levels in species of *Tacinga* range from diploid to hexaploid (Majure et al. 2012b).

*Brasiliopuntia brasiliensis* (Willd.) A. Berger and *Opuntia schickendantzii* form a well supported clade (fig. 1), although morphological studies have never before suggested this relationship (see Britton & Rose 1920). For example, Britton & Rose (1920) actually considered *O. schickendantzii* to be part of ser. *Aurantiacae*. However, Taylor (2008) did mention that *O. schickendantzii* resembles a miniature *Brasiliopuntia*, referring to its cylindrical trunk. The two species occur in close proximity to one another in northern Argentina based on collections of the two species, however, *B. brasiliensis* is found most commonly in dry forests or on xerophytic substrates in eastern Brazil (Taylor et al. 2002), but also in Peru, Bolivia and Paraguay (Taylor & Zappi 2004), and *O. schickendantzii* is restricted to Argentina and Bolivia (if *O. cochabambensis* Cárdenas is considered conspecific). *Brasiliopuntia* forms trees that have monopodial trunks with horizontal, branches that often bare ephemeral cladodes (Taylor et al. 2002). *Opuntia schickendantzii* forms small shrubs to treelets also demonstrating a degree of indeterminate growth (i.e., trunks are mostly monopodial) with horizontally spreading or mostly ascending cladodes; these are sometimes whorled (Iliff 2002). Scanning electron micrographs of pollen reveal that the two species have similar pollen, with polyporate grains exhibiting spinulose-perforate (or bullate-perforate) surfaces (see fig. 3 here; Leuenberger 1976, Taylor et al. 2002, Garalla & Cuadro 2007), although the spinulae are better developed in *Brasiliopuntia*. This pollen character is most likely a synapomorphy of the clade. It should also be noted that *O. schickendantzii* is very poorly studied, and other morphological features may be discovered that link these two species.

*Opuntia* s.str. is composed of seven subclades, and the genus *Nopalea* is completely nested within *Opuntia*. Two of those subclades, *Elatae* and *Macbridei*, are entirely South American except for where they have been introduced, and the other five subclades, *Scheerianae*, *Macrocentra*, *Humifusa*, *Nopalea*, and *Basilares*, are mostly restricted to North and Central America and the Caribbean region. The *Opuntia* clade can be recognized by the

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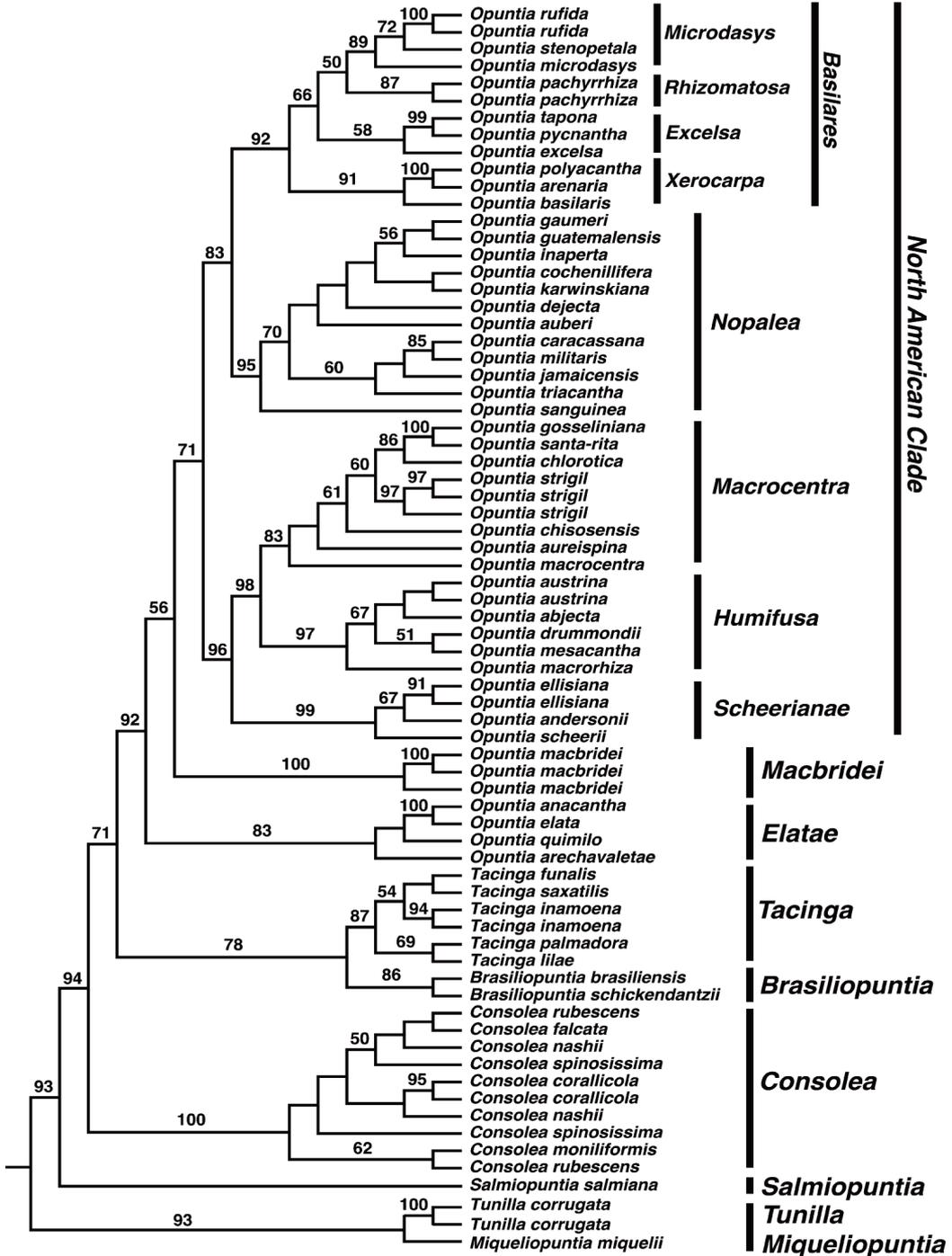


Fig. 1. Most likely phylogenetic reconstruction of tribe Opuntieae (based on data from Majure et al. 2012a and Majure et al. 2013b) produced using RaxML under 25 rate categories implementing the GTR + gamma model of molecular evolution and carrying out 10000 bootstrap replicates. Bootstrap values are given above branches.

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combination of sympodial growth, superficial stomata (in those species observed so far; Majure unpubl. data), polyporate pollen exhibiting a reticulate exine (in most but not all species; Leuenberger 1976, Taylor et al. 2002, Puente 2006b, Garalla & Cuadro 2007; fig. 3), and a strongly coiled embryo with greatly reduced perisperm (Stuppy 2002, Majure et al. 2013a). The seeds of *Opuntia* may or may not be covered in unicellular trichomes (Stuppy 2002).

The South American Elatae clade is sister to the rest of *Opuntia* s.str. and is composed of roughly 20 species. Leuenberger (2001) produced a monograph for several members of this clade, *Opuntia* ser. *Armatae*, but did not include many species that are also closely related (e.g. *O. anacantha* Speg., *O. quimilo* K. Schum., *O. sulphurea* G. Don) and that have been treated in different series. He did mention, however, that *O. quimilo* may belong in *O.* ser. *Armatae*. It is clear from phylogenetic analyses that ser. *Armatae* and ser. *Aurantiacae* are not real biological entities, as species from both clades are interdigitated in this clade (e.g. *O. elata* is sister to *O. anacantha* and *O. schickentdantzii* is actually more closely related to *Brasiliopuntia*; fig. 1). Although superficially resembling North American taxa, *O. sulphurea* is clearly part of the Elatae clade, contrary to the statement made by Taylor (2008) that it did not belong to the Elatae group. The emblematic Galapagos Island *Opuntia* radiation, an ancestor of which apparently dispersed to the islands from mainland South America, also is part of the Elatae clade (Majure et al. 2012a). The Elatae clade is restricted to Argentina, Bolivia, Brazil, Paraguay, Uruguay, and Ecuador (including the Galapagos Islands). Very little is known regarding chromosome number for species in this clade, but members that have been studied have been reported as diploid, triploid, tetraploid, and hexaploid (Majure et al. 2012b). Putative synapomorphies for this clade are not entirely clear, but the seed funicular envelope of most species within this clade is densely covered in trichomes (Leuenberger 2001, Majure unpubl. data), a likely symplesiomorphy that has been lost in most other species of *Opuntia* (Majure et al. 2013a) and some species of the clade itself (Majure unpubl. data).

The Macbridei clade consists of one species, is sister to the North American *Opuntia* clade and shares some morphological characters with members of the Elatae clade (*O. quimilo*, *O. quitensis* F.A.C. Weber; see Majure et al. 2012a) and the North American Microdasys clade (*O. stenopetala* Engelm.). In fact, some treatments have considered *O. macbridei* Britton & Rose and *O. quitensis* to be conspecific (Madsen 1989), but the entire ranges of the two taxa were not studied. Phylogenetic (Majure et al. 2012a) and cytogenetic data (Majure et al. 2012b) suggest that the two taxa are distinct and resolve them in two separate clades. Madsen (1989) does mention two growth forms of the species in his treatment. *Opuntia macbridei* occurs from central Peru to southern Ecuador. *Opuntia macbridei* is dioecious (Madsen 1989), has orange-red flowers with acute or obovate tepals, and the pericarpels are sometimes dimorphic, having ovaries embedded into large (normal) sized, flattened cladodes or embedded into reduced cladodes (as in most *Opuntia* species).

The Nopalea clade is sister to the Basilares clade and consists of those species previously recognized within the genus *Nopalea*, as well as other, mostly Caribbean species. Numerous phylogenetic analyses have shown that *Nopalea* is nested within *Opuntia* s.str. (Wallace & Dickie 2002, Griffith & Porter 2009, Hernández-Hernández et al. 2011, Majure et al. 2012a) and those species represent a shift to hummingbird pollination with concomitant floral mor-

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phological changes (Puente 2006b, Majure et al. 2012a). However, most other members of the clade have open, entomophilous flowers (such as *O. repens* Bello and *O. triacantha* (Willd.) Sweet). The Nopalea clade consists of roughly 22 species of which about half are diploid. The Nopalea clade is one of the most widespread clades in North America extending throughout the Greater and Lesser Antilles to northern South America (although *O. pubescens* is more widespread in South America; Madsen 1989) and throughout Mexico and Central America. Considering the broad morphological diversity in the Nopalea clade, it is most likely that more data would better resolve relationships among subclades within the group. It is clear that most hummingbird-pollinated taxa (e.g. *Nopalea*) are closely related, as are most of those taxa occurring throughout the Greater and Lesser Antilles (*O. caracasana*, *O. jamaicensis* Britton & Harris, *O. militaris* Britton & Rose, *O. triacantha*; fig. 1). Likewise, the polyploid species, *O. puberula* Hort. Vindob. ex Pfeiff., *O. decumbens* Salm-Dyck, *O. pumila* Rose and *O. pubescens* are all closely related, while *Opuntia repens* forms a clade by itself (Majure et al. 2012a). Synapomorphies for the Nopalea clade are not obvious, and much more work is necessary to determine relationships among subclades, as well as morphological characters that link all of these taxa. However, it should be noted that all of these taxa have strongly acute floral buds.

The Scheerianae clade is a species-rich clade that is sister to the Macrocentra-Humifusa clade (fig. 1). With approximately 15 species, the Scheerianae clade is widely distributed throughout North and Central America and the Caribbean, and members have also been introduced into many other parts of the world. For example, *Opuntia stricta* (Haw.) Haw. has been introduced into Australia, South Africa and Spain, where it becomes weedy and often invasive (Britton & Rose 1920, Dodd 1940, Foxcroft et al. 2004, Guillot Ortiz & Der Meer 2006). *Opuntia stricta* also has been introduced into parts of South America (Britton & Rose 1920). It is commonly used as an ornamental in and around Lima, Peru (Majure pers. obs.). In Mexico, *O. stricta* has been planted along the Veracruz coast for dune stabilization (R. Puente pers. obs.). Most members of the Scheerianae clade are polyploid ranging from tetraploids to hexaploids. Only *O. andersonii* H.M. Hern., Gómez-Hin., & Bárcenas, *O. scheeri* F.A.C. Weber, *O. ellisiana* Griffiths and *O. engelmannii* Salm-Dyck ex Engelm. var. *cuija* are diploid (Majure et al. 2012b). The Scheerianae clade contains several very complicated species complexes mostly composed of polyploid taxa (e.g. the *O. engelmannii* species complex and the *O. stricta* species complex), and certain members of the clade have been involved in numerous interclade hybridization events (see below).

The Macrocentra clade is sister to the Humifusa clade and is most diverse in Northern Mexico and the southwestern United States. The clade consists of approximately 10 species and has a high number of diploid species compared to most other clades. Most species within this clade form large shrubs that have noticeably purple cladodes, even when not under stressful conditions, making them highly prized as ornamentals. This dark purple cladode color is likely a synapomorphy of the clade. Species within this clade have entirely yellow flowers or yellow flowers with red centers. Species range from diploid to tetraploid (Powell & Weedon 2004, Majure et al. 2012b).

The Humifusa clade is widely distributed from northern Mexico throughout the United States (mostly east of the Rocky Mountains) and into eastern Canada (Ontario). Diploid

species are restricted to the southeastern and southwestern United States, but their polyploid products are much more widespread from the southern United States to Canada (Majure et al. 2012c). The Humifusa clade consists of between 10–15 species, but species limits within the clade are still being investigated (Majure, in progress). Members of the Humifusa clade have either entirely yellow flowers (e.g. *O. austrina* Small), flowers with red centres (e.g. *O. macrorhiza* Engelm.), or pink flowers (e.g. *O. pottsii* Salm-Dyck; although Arizona populations often have yellow flowers with red centres). Most species are low, spreading to slightly ascending shrubs, although *O. austrina* can become a large, erect shrub or treelet to 1.5 m tall. Species either have fibrous or tuberous roots. Chromosome counts reveal diploids, triploids, and tetraploids in this clade (Majure et al. 2012c).

The Basilares clade consists of four well-supported subclades (i.e., Xerocarpa, Excelsa, Rhizomatosa and Microdasys clades; covered below). Many of the species within the Basilares clade have pubescent cladodes (e.g. *O. basilaris* Engelm. & J.M. Bigelow, *O. microdasys* (Lehm.) Pfeiff., *O. pycnantha* Engelm. ex J.M. Coult., *O. rufida* Engelm.), a likely synapomorphy of the clade, although pubescence has been lost in numerous species. This is also the oldest group of species in the North American clade (Majure et al. 2012a). Members of this clade have been involved in the production of multiple hybrid, polyploid species as well (Majure et al. 2012a).

The Xerocarpa clade is supported as sister to the rest of the members of the Basilares clade. Members of the Xerocarpa clade include the most, cold tolerant species of cacti and those with very large distributions in North America (i.e. *O. fragilis*). The Xerocarpa clade is mostly distributed from northern Mexico and throughout the southwestern United States; however, *O. fragilis* exists from the southwestern United States northwest to Manitoba, Canada and east to Ontario, Canada (Parfitt 1991). The wide distribution of *O. fragilis* is likely the result of vegetative dispersal via migrating bison (see Majure & Ribbens 2012 and refs. therein). Other well-known members of the Xerocarpa clade are *O. basilaris* and *O. polyacantha* Haw. (fig. 1). There are roughly 5–10 species within the Xerocarpa clade depending on whether certain taxa are considered conspecific with *O. polyacantha* (e.g., *O. arenaria* Engelm., *O. erinacea* Engelm. & J.M. Bigelow). All members of this clade are characterized by having dry fruit when mature (Benson 1982, Parfitt 1991, Pinkava 2003), a synapomorphy of the clade. Species in this clade have flowers with yellow, pink or whitish tepals and dark green stigma lobes. Ploidal levels in this clade range from diploid to octoploid (Parfitt 1991, Parfitt 1997, Pinkava 2002, Majure et al. 2012b).

The Excelsa clade consists of three diploid species, which are mostly distributed in Baja California and along the southern Pacific Coast of Mexico. No clear morphological features link *O. excelsa* Sánchez-Mej. with *O. pycnantha* and *O. taponia* Engelm., however, the latter two species are both pubescent and have similar spine morphology. All three species have entirely yellow flowers.

The Rhizomatosa clade is composed of three species, *O. chaffeyi* Britton & Rose, *O. megarrhiza* Rose, and *O. pachyrrhiza* H.M. Hern., Gómez-Hin. & Bárcenas. These three species are very restricted in their distribution in central Mexico and are known from very few populations (Parfitt 1985a, Hernandez et al. 2001a-b). *Opuntia pachyrrhiza* is diploid, whereas *O. chaffeyi* is tetraploid and *O. megarrhiza* is hexaploid (Majure et al. 2012b). All

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three species possess large rhizomes from which are produced ephemeral cladodes that are often shed under unfavorable environmental conditions (i.e., extreme cold, drought; Parfitt 1985a, Hernandez et al. 2001a-b). These characters are undoubtedly synapomorphies for the clade, as no other known species of *Opuntia* exhibit those features.

The Microdasys clade consists of four species, *O. microdasys*, *O. rufida*, *O. stenopetala*, and *O. ×carstenii* R. Puente & C. Hamann. Based on previous reports, all species are diploid (Majure et al. 2012b). *O. ×carstenii* was described as a hybrid between *O. stenopetala* and *O. microdasys* (Puente & Hamann 2005) and thus represents a homoploid hybrid species, not a commonly reported phenomenon in *Opuntia*, where hybrids are often at the polyploid level (Parfitt 1980, Pinkava & Parfitt 1988, Majure et al. 2012a). The clade is distributed throughout parts of the Chihuahuan Desert and eastern Mexico mostly west of the Sierra Madre Oriental. Both *O. microdasys* and *O. rufida* are spineless and pubescent. *Opuntia stenopetala* is a dioecious species (Parfitt 1985b) with glabrous, spiny cladodes and very narrow, reddish-orange tepals and has even been compared to *O. quitensis* (Ilf 2002; see below). Morphological synapomorphies of this clade need to be investigated further.

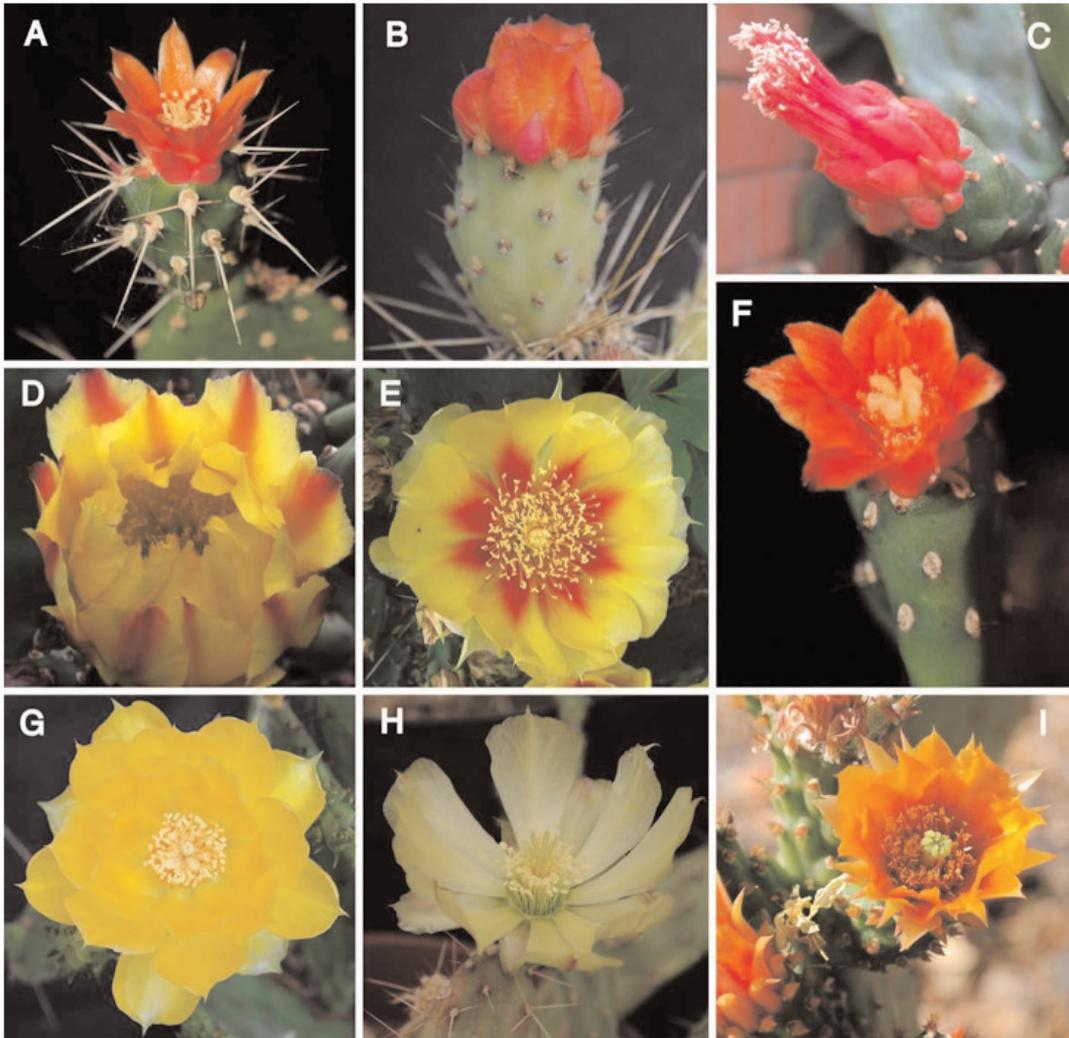
There are many other species of *Opuntia* that do not belong to one clade or another, as they are derived from two or more different clades via hybridization, and those species have not been included in species estimations for the above clades. Those species often obscure morphological synapomorphies of clades as well, from their demonstration of a mosaic of morphological characters that often span the clades they were derived from. It seems most judicious in revisionary studies to treat these taxa within one clade or another from which they were derived (see *O. ochrocentra* Small; Majure 2012).

### **Morphological Evolution**

Inner tepal color is quite variable in tribe Opuntieae, with colors ranging from red, yellow, pink, orange, and even white or green. Many species of *Opuntia* have multicolored flowers as well, such as in *Opuntia engelmannii* var. *lindheimeri* (Engelm.) B.D. Parfitt & Pinkava, which sometimes has yellow and orange flowers on the same plant or *Opuntia phaeacantha* Engelm. with flower color ranging from yellow to pink. Species with yellow tepals with abaxially pink (e.g. *O. triacantha*) or red (e.g. *O. monacantha* Haw.) midveins are common in certain species. Other species may have yellow tepals with red or reddish brown bases on the adaxial surfaces (e.g. *O. macrocentra* Engelm., *O. macrorhiza*; see fig. 2).

Flower color is often attributed to pollination syndrome, although several studies have shown that, like in most angiosperms, pollination syndrome is very plastic in Opuntieae as well. For example, the large red flowers of *O. quimilo* are either hummingbird or insect pollinated (Díaz & Cocucci 2003).

The ancestral inner tepal color of *Opuntia* was most likely red to red-orange according to ancestral state reconstruction (Majure unpubl. data). The shift to yellow flowers was most pronounced in the North American clade, although several members of the South American Elatae clade also show shifts to mostly yellow flowers. Although the hummingbird pollinated members of the Nopalea clade show a shift from insect to bird pollination along with floral morphological changes (Majure et al. 2012a), the red- to pinkish color of most of those species most likely represents a retained ancestral character (i.e., plesiomorphy). The



**Fig. 2.** Examples of the diversity of floral color and morphology in tribe Opuntieae. A) *Consolea millspaughii* showing acute, reddish tepals, B) *Tacinga lilae* showing acute tepals forming a tubular corolla, C) *Opuntia cochenillifera* showing erect, pinkish tepals and greatly exerted stamens, D) *O. monacantha* showing yellow tepals with red midveins abaxially, E) *O. cespitosa* showing yellow tepals tinged red at the base adaxially, F) *O. macbridei* showing acute, red-orange tepals, G) *O. austrina* showing obovate, yellow tepals, H) *O. megarrhiza* showing obovate greenish-yellow tepals, and I) *O. carstenii* showing acute, orange tepals. (Photos by L.C. Majure and M. Pajuelo)

shift to red or red-orange tepals in *O. stenopetala* is derived in that species, as are the pink tepals of *O. basilaris*. Thus, tepal color in *Opuntia* is homoplasious in certain instances.

Stamen movement is prevalent throughout *Opuntia*, although this character has not been well documented for most species. Most hummingbird pollinated or both insect and bird pollinated species (e.g. red-flowered species), however, do not show stamen movement (Nopalea clade members, *O. quimilo*; Díaz & Cocucci 2003), or different sexes show differential stamen movement (e.g. only staminate flowers show stamen movement in *O. stenopetala*; R. Puente pers. obs.). Most other members of Opuntieae also do not produce

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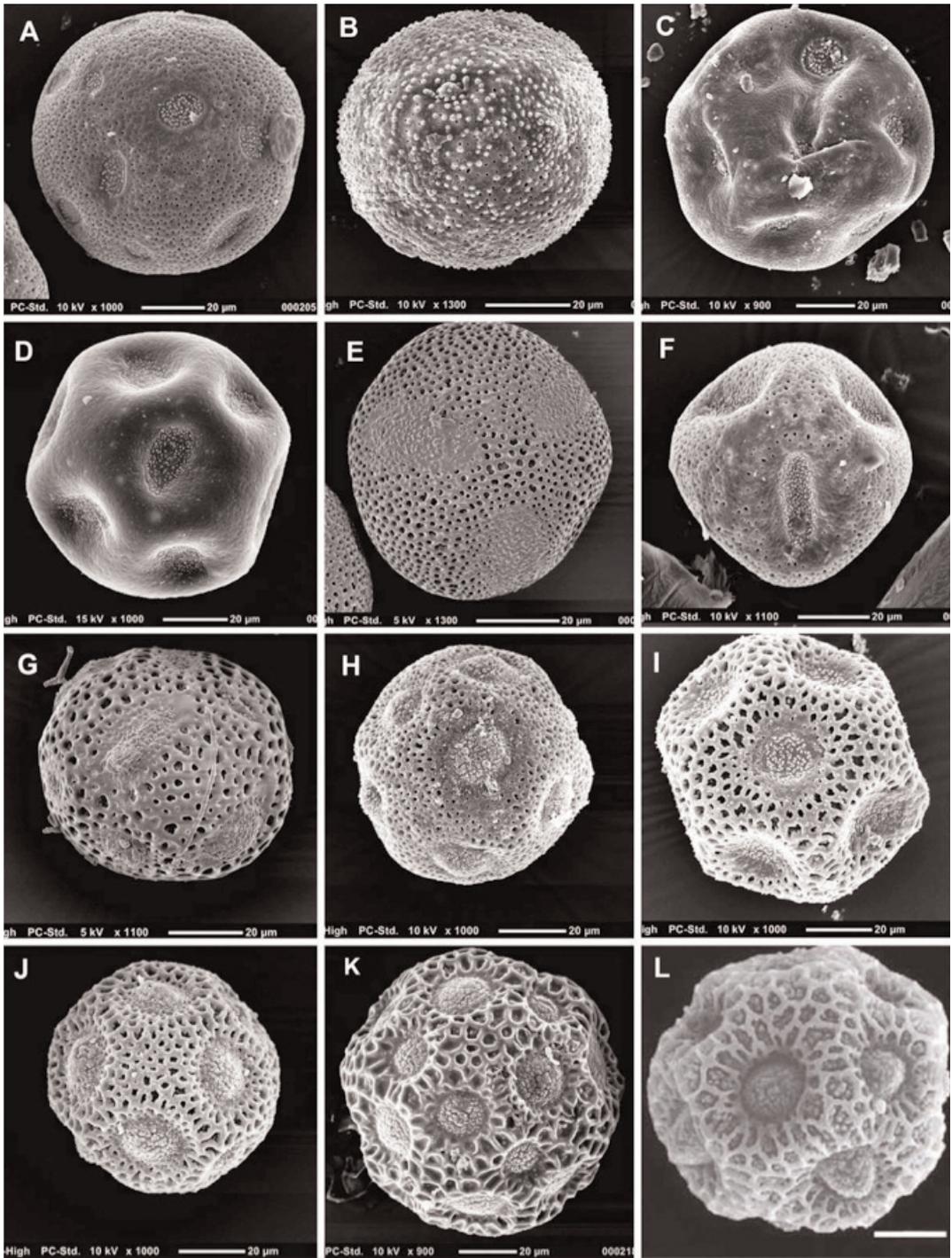
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stamens that move upon touch (e.g. *Brasiliopuntia*, *Consolea*, *Tacinga*). On the contrary most yellow-flowered species of *Opuntia* exhibit stamens that move upon any type of manipulation of the filaments, i.e. thigmonasty, as the stamens move toward the stigmatic region of the style (Díaz & Cocucci 2003, Majure pers. obs.). This type of movement has recently been described anatomically by Cota-Sanchez et al. (2013). This feature appears to be derived in most species of *Opuntia* and serves as a selfing mechanism, but likely has been lost in some Nopalea clade members and carpellate *O. stenopetala*. However, much more data will be needed to fully test this hypothesis.

Pollen surface structure in Opuntieae is highly homoplasious and appears to be related to pollination syndrome and/or flower structure (Majure et al. 2013). For instance, *O. macbridei*, *O. quimilo* and *O. stenopetala* exhibit pollen intermediate between colpate and porate grains (figs 3G, H; Leuenberger 1976, Garalla & Cuadro 2007). All three of these species have flowers with reddish or orange-red tepals, presumably are bird-pollinated to some degree, and are dioecious (Parfitt 1985b, Madsen 1989, Díaz & Cocucci 2003) or sometimes trioecious in *O. quimilo* (Nattero & Malero 2011). If we consider that *O. macbridei* and *O. quitensis* are likely not conspecific, then these taxa are in different clades as well, so it is clear that this “unique” pollen morphology, as well as floral morphology has evolved more than once.

Pollen exine varies from finely tectate punctate, in those taxa that show extreme morphological changes for bird pollination (e.g. *Tacinga funalis*, bird-pollinated members of the Nopalea clade, fig. 3D; Taylor et al. 2002, Puente 2006b), to moderately tectate punctate in other presumably bird pollinated taxa with more open flowers (*Consolea*, other members of *Tacinga*, *O. macbridei*, *O. stenopetala*, fig. 3A, F, G; Leuenberger 1976, Taylor et al. 2002; fig. 3). Primarily yellow flowered and presumably insect pollinated species of *Opuntia* have a strongly reticulate exine (fig. 3K, L, M). Unfortunately however, very little observation data regarding pollinators is available for most species within Opuntieae. It is known, however, that pollination syndromes are “leaky,” and certainly “bird-pollinated” species could occasionally be pollinated by insects (see Díaz & Cocucci 2003, R. Puente pers. obs.). For example, *O. inaperta* in the Yucatán Peninsula is visited and most likely pollinated by stingless bees of the genus *Melipona* (R. Puente pers. obs.). Likewise, “insect-pollinated” species are on occasion pollinated by birds (Majure pers. obs.).

Cladodes in Opuntieae are most commonly glabrous, although several species demonstrate pubescence. Pubescence appears to have originated in the Basilares clade in *Opuntia*, with subsequent losses in some species, based on phylogenetic analyses of diploid species only (fig. 1). However, pubescence can be seen in other taxa that apparently are not part of the Basilares clade (e.g. *O. decumbens*, *O. pubescens*, *O. pumila*). These polyploid taxa make up part of the Nopalea clade, and no evidence has been found suggesting that they originated in any part from the Basilares clade. So it is possible that pubescence actually originated in the most recent common ancestor of the Nopalea + Basilares clade, or otherwise, pubescence has evolved more than once in *Opuntia*. There are other polyploid taxa, such as *O. tomentosa* Salm-Dyck, with pubescent cladodes, but these species were most likely derived from hybridization between the Nopalea and Basilares clades (Majure et al. 2012a).



**Fig. 3.** Pollen types of selected taxa of tribe Opuntieae. A) *Consolea moniliformis*, B) *Brasiliopuntia brasiliensis*, C) *Opuntia schickendantzii*, D) *Opuntia dejecta*, E) *Opuntia quitensis*, F) *Opuntia stenopetala*, G) *Opuntia quimilo*, H) *Opuntia elatior*, I) *Opuntia schumannii*, J) *Opuntia durangensis*, K) *Opuntia megarrhiza*, L) *Opuntia pachyrrhiza*. Scale bar 20 µm.

(Photomicrographs by R. Puente)

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Cladode disarticulation is a common feature in certain species of *Opuntia*, but relatively uncommon in the group as a whole. Cladode disarticulation appears to be a homoplasious character, having arisen in numerous clades. Those species exhibiting easily disarticulating cladodes usually have more strongly retrorsely barbed spines as well (Majure pers. obsv.), presumably to aid in vegetative dispersal. The degree of spine “barbedness,” however, has not been quantified across a range of taxa. *Opuntia fragilis* may be the best well-known member of *Opuntia* for having easily disarticulating cladodes leading to vegetative dispersal. This feature apparently has resulted in the large distribution of the species that we see today, having been dispersed by large mammals (Majure & Ribbens 2012). Easily disarticulating cladodes are also common in certain members of the Nopalea clade, such as *O. caracassana*, *O. jamaicensis*, *O. militaris*, *O. pubescens*, *O. pumila*, and *O. repens*. This feature also developed in the Humifusa clade in *O. drummondii* Graham and *O. abjecta* Small. Many of these taxa are much more restricted in distribution than is typical for species with disarticulating cladodes, suggesting the lack of appropriate dispersal agents.

Hairy seeds are found in *Consolea*, *Miqueliopuntia*, *Tacinga*, *Brasiliopuntia*, and some members of *Opuntia* (e.g. Elatae clade and apparently some members of the Nopalea clade; Stuppy 2002), but this feature appears to have been lost in most species of North American *Opuntia*.

The large rhizomes of both *O. megarrhiza* and *O. pachyrrhiza* have been compared with the tuberous roots of *O. macrorrhiza* (Britton & Rose 1920, Hernandez et al. 2001a-b), and *O. megarrhiza* was even placed in the same series as *O. macrorrhiza* by Britton & Rose (1920), *Opuntia* ser. *Setispinae*. It is clear from the phylogeny, however, that these species are not closely related to the tuber-forming *O. macrorrhiza* or *O. pottsii* (i.e. western North American members of the Humifusa clade; see Majure et al. 2012a), and tubers are likely not homologous to the large, fleshy rhizomes of the Rhizomatosa clade. Members of some of the eastern North American members of the Humifusa clade also produce tuberous roots (e.g. *O. austrina*; Small 1933, Majure 2012). The function of these large potato-sized tubers has not been tested. *Opuntia arenaria* also produces rhizomes, which function in the production of new shoots (Boke 1979, Benson 1982), but they are not large and fleshy as in members of the Rhizomatosa clade. Likewise, *O. pubescens* shows greatly thickened roots when growing in rocky, thin soil (R. Puente pers. obs.). Much more work is needed to determine the genetic and/or environmental factors causing the production of these enlarged, apparently non-homologous (throughout *Opuntia* s.str.) root/rhizome structures.

### **Polyploidy and Reticulate Evolution**

Chromosome numbers are relatively well known in tribe Opuntieae having been evaluated via chromosome counts (Pinkava et al. 1985, Pinkava 2002, Majure et al. 2012b-c) and flow cytometry for numerous species (Negrón-Ortiz 2007, Segura et al. 2007, Helsen et al. 2009). Of those species with counts, 26.2% are diploid, 60.4% are polyploid, and 13.4% have multiple counts (i.e. diploid and polyploid; Majure et al. 2012b). Several clades within Opuntieae are entirely polyploid (i.e. *Salmiopuntia*, *Miqueliopuntia*, *Tunilla* and *Consolea*), while others are diploid or contain both diploids and polyploids. Of the eight species of *Tacinga* (see Majure et al. 2013), only five species have been counted. Two of those species

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are diploid (e.g. *T. funalis* and *T. palmadora*), and the other three are polyploid (*T. inamoena*, *T. lilae*, *T. saxatilis*; Majure et al. 2012b). *Opuntia* s.str. (including *Nopalea*) is composed of 30.4% diploid, 55.8% polyploid, and 13.8% of species with diploid and polyploid counts. *Brasiliopuntia brasiliensis* and *Opuntia schickendantzii* are both diploid (Majure et al. 2012b).

Polyploid products, whether derived from hybridization (i.e., allopolyploidy) or genome duplication within one species (i.e., autopolyploidy), are often reproductively isolated from their progenitors, and thus polyploidy often leads to the formation of new species (Stebbins 1950, 1971; Grant 1981, Judd et al. 2007, Soltis & Soltis 2009). Considering that many “species” in tribe Opuntieae and noticeably *Opuntia* s.str. are composed of numerous ploidal levels, it is most likely that many cryptic species actually exist but have yet to be discovered. Very careful, detailed work is necessary to determine the delimitation of species within the major clades of *Opuntia*, for example. Certain clades within *Opuntia* are composed of polyploid complexes that consist of a series of restricted diploids and mostly, more widespread and abundant polyploids (see Parfitt 1991, Pinkava 2002, Majure et al. 2012c) that are morphologically very similar to their putative diploid progenitors. These groups are notoriously difficult to treat taxonomically, as different higher ploidal levels within a species often are derived from different origins, although, the morphological differences among ploidal levels are either cryptic or mosaics of other species (as may be expected in hybrid origins; see Majure et al. 2012a,c). In other cases involving autopolyploidy, different ploidal levels may be virtually identical. For example, *Opuntia drummondii* is known to be diploid, triploid, and tetraploid but is most likely of autopolyploid origin and plants of different ploidal levels are virtually indistinguishable (Majure 2012, Majure et al. 2012a,c).

Numerous polyploid products within Opuntieae and especially *Opuntia* are derived from hybridization among clades (i.e. interclade hybrids). For example, three taxa, *Opuntia acaulis* Ekman & Werdermann, *O. bahamana* Britton & Rose and *O. lucayana* Britton were derived from hybridizations between species of *Consolea* and *Opuntia* (Majure et al. 2012a). *Opuntia cubensis* Britton & Rose and *O. ochrocentra* were derived from hybridizations among *O. dillenii* (Ker Gawl.) Haw. (Scheerianae clade) and the *Nopalea* and *Humifusa* clades, respectively (Majure et al. 2013b).

Polyploid species derived from hybridization within a given clade also have been documented. For example, *Opuntia aurea* McCabe & E.M. Baxter and *O. pinkavae* B.D. Parfitt are derived from hybridization among *O. basilaris* and other members of the *Xerocarpa* clade (Majure et al. 2012a). *Opuntia cespitosa* Raf. and *O. humifusa* are derived from different species within the *Humifusa* clade (Majure et al. 2012c, Majure et al. unpubl. data).

### **Taxonomic Implications**

Phylogenetic and cytogenetic studies within tribe Opuntieae have numerous taxonomic implications, a few of which are covered here.

*Tacinga lilae* (B. Trujillo & M. Ponce) Majure & R. Puente was formerly placed in *Opuntia* s.l. (Trujillo & Ponce 1990) and believed to be referable to *O. curassavica* by Hunt et al. (2006), but was transferred to *Tacinga* by Majure et al. (2013a). This species exhibits clear morphological affinities with the *T. palmadora*–*T. wernerii* subgroup of the genus

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*Tacinga* (see above), as well as possessing the major synapomorphies of the genus *Tacinga* (raised stomata, reduced persisperm, hooked shaped embryo, and a deep, narrow fruit umbilicus (Majure et al. 2013a). Majure et al. (2013a) suggested that this species represents a polyploid (hexaploid, Majure et al. 2012c) cryptic species that is easily confused with diploid *T. palmadora* but can be separated readily by several morphological characters including seed number (20–30 in *T. lilae* vs 3–5 in *T. palmadora*), funicular pulp colour (pinkish-red in *T. lilae* vs translucent or yellow in *T. palmadora*), the production of proliferous fruit in *T. lilae*, as well as other vegetative and reproductive features (see Majure et al. 2013a).

The specific status assigned to *Tacinga lilae* was based on the assumption that *T. palmadora* had been well studied and was well understood morphologically (e.g., Taylor et al. 2002, Taylor & Zappi 2004), which apparently is not the case (Nigel Taylor, pers. comm.). *T. palmadora* thus needs further study throughout its range in Brazil to determine whether or not *T. lilae* is actually distinct from it. However, it should be noted that *T. lilae* is most likely reproductively isolated from *T. palmadora*, as it is a hexaploid and *T. palmadora* is diploid (Majure et al. 2012c; Majure & Puente unpubl. data), so the two taxa are biological species by definition (Mayr 2000), although they may be difficult to distinguish. Also, the two taxa are not genetically identical and are only moderately supported as sister taxa in phylogenetic analyses (bs = 69; Fig. 1). *Tacinga lilae* and *T. palmadora* show an interesting disjunction pattern between the Caatinga of Brazil and the Caribbean region of northern South America (Majure et al. 2013a) that is shared with two other species pairs in Cactaceae (i.e. *Pereskia guamacho* F.A.C. Weber / *P. aureiflora* F. Ritter; *Pseudoacanthocereus sicariguensis* (Croizat & Tamayo) N.P. Taylor / *P. brasiliensis* (Britton & Rose) F. Ritter (Taylor & Zappi 2004).

*Opuntia abjecta* (Florida Keys) and *O. militaris* (Guantánamo, Cuba) were considered by Benson (1982) to be synonymous with the widespread Caribbean species *O. triacantha*. Likewise, he considered *O. cubensis* from Guantánamo, Cuba and *O. ochrocentra* from the Florida Keys to be the same species. Majure et al. (2012a) showed that *O. abjecta* and *O. triacantha* actually are in two very different clades (the Humifusa clade versus the Nopalea clade; see fig. 1). Majure et al. (2013b) further showed that *O. militaris* is likely not synonymous with *O. triacantha* (see fig. 1) and that *O. cubensis* and *O. ochrocentra* are two polyploid species derived from separate origins and widely divergent putative diploid, maternal progenitors (*O. militaris* and *O. abjecta*, respectively) and thus should be considered as two distinct species. Morphological characters also clearly separate the two taxa (Majure et al. 2013b).

*Opuntia pachyrrhiza* (Hernandez et al. 2001a) was previously considered to be a synonym of *Opuntia megarrhiza* (Guzman et al. 2007, Baker et al. 2009, although, see Hernandez et al. 2001b); however, Majure et al. (2012b) showed the two taxa to be distinct cytologically with *O. pachyrrhiza* reported as diploid and *O. megarrhiza* recorded as a hexaploid. Also, *O. megarrhiza* is resolved as sister to *O. chaffeyi* in phylogenetic analyses including polyploid taxa (Majure et al. 2012a; the taxonomic affinity as suggested by Hernandez et al. 2001b), thus we suggest *O. pachyrrhiza* and *O. megarrhiza* should be recognized as two separate species.

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Although diploid *O. andersonii* is considered to be of hybrid origin between diploid *O. microdasys* (Microdasys clade) and diploid *O. engelmannii* var. *cuija* Griffiths and Hare (Hernández et al. 2003), no evidence from phylogenetic analyses was found supporting that scenario (Majure et al. 2012a). It is interesting to note that *O. engelmannii* and *O. microdasys* are not closely related, so homoploid hybrids between the two species would presumably be relatively sterile not having undergone genome duplication to overcome sterility barriers as a result of chromosomal pairing incompatibilities (Stebbins 1950, Grant 1981). Even putative hybrid products from closely related species of *Opuntia* within the same clade have undergone genome duplication (Majure et al. 2012c). Likewise, pollen appears to be highly viable in this species, and fruit production apparently is normal as well (Hernández et al. 2003). Justifiably, more, detailed biological studies need to be carried out for *O. andersonii*, as well as for *O. engelmannii* var. *cuija*, which has not been included in any phylogenetic analyses.

As mentioned above, the genus *Nopalea* is deeply nested within *Opuntia* s. str., thus recognizing the genus would make *Opuntia* s. str. paraphyletic. Most species that had been recognized as *Nopalea* by Britton & Rose (1919) were either described as *Opuntia* or have been transferred at some point in their taxonomic history. However, *Nopalea gaumeri* Britton & Rose lacks a combination in *Opuntia*. We provide the new combination here:

***Opuntia gaumeri*** (Britton & Rose) R. Puente & Majure, **comb. nov.** Basionym: *Nopalea gaumeri* Britton & Rose, The Cactaceae 1: 37 (1919).

*Opuntia schickendantzii* makes *Opuntia* s. str. paraphyletic, as currently circumscribed, thus it is necessary to place this species in a different genus. Although *Opuntia schickendantzii* is resolved as sister to *Brasiliopuntia*, there are very few obvious morphological synapomorphies linking the two taxa (see above), and likely for this reason they have never been perceived to be closely related (but see Taylor 2008). However, based on phylogenetic position, pollen morphology and growth form (i.e. monopodial primary stems), *O. schickendantzii* is best circumscribed within the genus *Brasiliopuntia*. We make the new combination below and provide a brief description and distribution of the species.

***Brasiliopuntia schickendantzii*** (F.A.C. Weber) R. Puente & Majure **comb. nov.** Basionym: *Opuntia schickendantzii* F.A.C. Weber in Bois, Dict. Hort., 898 (1898); *Cylindropuntia schickendantzii* (F.A.C. Weber) Backeberg, Kaktus-ABC 122 (1936); *Austrocylindropuntia schickendantzii* (F.A.C. Weber) Backeberg, Cact. Succ. J. (US) 23: 14 (1951).

Shrub 1.0–2.0 m high, primary trunk cylindrical or slightly flattened, 3–5 cm Ø, growth indeterminate; secondary branches ascending to erect, growth determinate, lanceolate to narrowly obovate, 15–25 × 3–5 cm, grayish green, frequently turning purple, somewhat tuberculate; leaves minute, 2 mm, reddish; spines 1–2, 1–2(–6) cm, subulate, thin. Flowers 4 cm Ø, yellow; pericarpel obovate, 2 × 1.5 cm, bright green; tepals yellow fading to pink; filaments white; style white; stigma 6-lobed, light green. Fruit globose to elliptical, spineless, 3–5 × 1.5–2 cm, green ripening

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red, frequently sterile. Seeds few or sterile. Pollen polyporate, with spinulose-perforate exine. Ploidy, diploid,  $2x = 2n = 22$ .

Distribution: Southern Bolivia (Cercado, Cochabamba, Mendez) to Northern Argentina (Córdoba, Catamarca, Jujuy, La Rioja, Salta, Tucumán); 1000–2300 m elevation.

### **Conclusions**

Based on phylogenetic results and morphological data, tribe Opuntieae consists of seven genera, *Salmiopuntia*, *Miqueliopuntia*, *Tunilla*, *Consolea*, *Tacinga*, *Brasiliopuntia*, and *Opuntia*. The *Opuntia* clade is the most speciose group and consists of seven primary subclades. The most diverse group of *Opuntia* is the North American clade. Many taxa have yet to be analyzed phylogenetically and still lack basic biological data, such as chromosome number and morphological data, so much more work will be necessary before we have a clear understanding of the total number of species of *Opuntia*.

We now have a greater opportunity to begin clade-level revisions of the major clades of Opuntieae, especially subclades of *Opuntia*, which is arguably one of the most taxonomically challenging groups within Opuntieae. Through the use of basic biological data (i.e., chromosome counts, ecological data, morphology), as well as phylogenetic tools, we can more confidently assign species to clades and illuminate potential cryptic species that are not immediately obvious based solely on gross morphology (Majure 2012a-c, Majure et al. 2013a,b). However, very tedious fieldwork over the entire distribution of species and careful study of live specimens, where available, will be the key in working out taxonomic problems and species delimitations within this group.

### **Acknowledgments**

We would like to thank the Desert Botanical Garden for access to many of the specimens used in our analyses. We also thank Nigel Taylor for comments on an earlier version of this manuscript. Partial funding for this research was provided by the American Society for Plant Taxonomists, the Botanical Society of America, the Cactus and Succulent Society of America, the Florida Division of Forestry, the New England Botanical Club, and the National Science Foundation (DEB-1011270).

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*Pre-print published 7 April 2014*  
*edited and published by David Hunt*  
*The Manse, Chapel Lane, Milborne Port, DT9 5DL, U.K.*