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Phylogenetic Relationships and Evolutionary Trends in the Cactus Family

Pablo C. Guerrero, Lucas C. Majure, Amelia Cornejo-Romero, and Tania Hernández-Hernández

From the Departamento de Botánica, Universidad de Concepción, Chile. Casilla 160C, Concepción, Chile (Guerrero); the Department of Research, Conservation and Collections, Desert Botanical Garden, Phoenix, AZ (Majure); the Florida Museum of Natural History, University of Florida, Gainesville, FL (Majure); the Departamento de Botánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, México (Cornejo-Romero); and the Catedrática CONACYT asignada a LANGE BIO-UGA Cinvestav, Irapuato, Gto., México (Hernández-Hernández).

Address correspondence to T. Hernández-Hernández at the address above, or e-mail: tania.hernandez@cinvestav.mx.

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Abstract

Members of the cactus family are keystone species of arid and semiarid biomes in the Americas, as they provide shelter and resources to support other members of ecosystems. Extraordinary examples are the several species of flies of the genus *Drosophila* that lay eggs and feed in their rotting stems, which provide a model system for studying evolutionary processes. Although there is significant progress in understanding the evolution of *Drosophila* species, there are gaps in our knowledge about the cactus lineages hosting them. Here, we review the current knowledge about the evolution of Cactaceae, focusing on phylogenetic relationships and trends revealed by the study of DNA sequence data. During the last several decades, the availability of molecular phylogenies has considerably increased our understanding of the relationships, biogeography, and evolution of traits in the family. Remarkably, although succulent cacti have very low growth rates and long generation times, they underwent some of the fastest diversifications observed in the plant kingdom, possibly fostered by strong ecological interactions. We have a better understanding of the reproductive biology, population structure and speciation mechanisms in different clades. The recent publication of complete genomes for some species has revealed the importance of phenomena such as incomplete lineage sorting. Hybridization and polyploidization are common in the family, and have been studied using a variety of phylogenetic methods. We discuss potential future avenues for research in Cactaceae, emphasizing the need of a concerted effort among scientists in the Americas, together with the analyses of data from novel sequencing techniques.

Subject areas: Molecular systematics and phylogenetics, Population structure and phylogeography

Keywords: Cactaceae, cactophilic *Drosophila*, Caryophyllales, convergence, succulents

Cactaceae are one of the most charismatic plant families on earth, and several species are constitutive members of the arid land vegetation in the American continent. The most popular cactus species

are the highly succulent members, which show some of the most dramatic modifications observed in the plant kingdom. These evolutionary changes occurred at all phenotypic levels: morphological,

anatomical, cellular, and metabolic (Gibson and Nobel 1986); and together compose what we know as the succulent syndrome (Edwards and Ogburn 2012). These adaptations allow succulent cacti to survive in some of the driest environments on earth, relying on the water they store in their tissues to avoid desiccation. Although the anatomical modifications of cacti have been studied over several decades (Gibson 1973; Mauseth 1995, 2006; Nobel 2002; and references therein), there are still modifications with an unclear evolutionary origin. Moreover, research on the metabolic and genetic bases of cactus strategies to inhabit dry environments is a promising avenue to be able to implement cactus species as food, forage, or biofuel cultivars (Inglese et al. 2017), as well as to generate bioengineering tools to genetically modify common crops to withstand future predicted conditions of increased drought, desertification, and heat (Yang et al. 2015).

The cactus family comprises around 1400 succulent and non-succulent species distributed throughout the entire American continent (Figure 1). However, the highest richness and endemism can be found in Mexico, the Andean region comprising northern Argentina, Bolivia, and Peru; as well as in eastern Brazil (Figure 1; Ortega-Baes and Godínez-Alvarez 2006; Hernández and Gómez-Hinostrosa 2011, 2015). Cacti are the fifth most endangered of any major taxonomic group, with 31% of species under threat (Goettsch et al. 2015).

The cultivation and use of several species of cacti provide important sources of income for large populations as well as local

communities mostly in Latin America, only recently expanding into the United States. In Mexico, more than 150 species of cacti are used by indigenous people, and at least 50 of them are cultivated (Casas and Barbera 2002), representing reservoirs of diversity for crop alternatives. Cacti play a central role in the culture of Mexico and South American human populations where their presence is more conspicuous. Species of *Opuntia* were already used by hunter-gatherer communities of southwestern United States and Mexico in 9000 BC, and the process of domestication started as far back as 6000 BC (Callen 1965; Soberon et al. 2001). Currently millions of hectares are planted with *Opuntia ficus-indica* for food, forage, or cochineal red dye production in Mexico (3 million ha) and tropical areas of Argentina (1650 ha), Brazil (500 000 ha), Chile (1000 ha), Peru (10 000 ha), and minor regions in sub-Saharan Africa and South Asia (Inglese et al. 2017).

In arid environments of the Americas, succulent cacti play fundamental ecological roles. Given their water storage capabilities, they provide a source of water and moisture for many other species, allowing them to survive and/or fulfill entirely or at least in part, their life cycles, especially during the driest periods (Bailey 1923; Wolf and del Rio 2003; Orr et al. 2015; Delgado-Fernández et al. 2017). Perhaps the most remarkable examples are the cactophilic species of *Drosophila*. Moisture present in cactus stems allows an uncommon process in arid regions, the putrescence of tissue. Different *Drosophila* species are associated with the decaying tissues of different species of columnar or opuntoid cacti both in North and South America (Ruiz and Heed 1988; Manfrin and Sene 2006; Matzkin et al. 2006; Pfeiler et al. 2009). Adults feed from the yeast and bacteria-laden exudate, while larvae ingest the rotting pulp, sometimes in a host-specific manner (Ruiz and Heed 1988). The diversification of cactophilic *Drosophila* has resulted in an ideal model to understand speciation and adaptation (Markow and O'Grady 2008). Unfortunately, there is still a partial understanding of the evolution of the columnar and opuntoid cacti hosting them (see below), which is crucial to understand the radiation of more than 120 *Drosophila* species that specialize on the necrotic cacti.

Although members of the cactus family are slow growing plants, with long generation times and relatively small population sizes, the family and sublineages within it show some of the highest diversification rates observed in the plant kingdom (Hernández-Hernández et al. 2014; Magallón et al. 2015). In recent decades, a continuously growing number of phylogenetic studies of Cactaceae both at the family level, as well as at the subfamilial, tribal, or generic level, have been published, and have clarified the evolutionary relationships within the family and among its outgroups. We also have a better understanding of the ecology, reproductive strategies, population structure, and genetic diversity of several species due to case studies both in North and South America (see sections below). Members of the cactus family have shown to be great model systems to understand evolutionary processes, because they are relatively easy to identify and monitor in the field.

Here, we review the current knowledge about the phylogenetic relationships and evolutionary patterns in the cactus family and mention some of the most relevant and recent findings on other areas of research, such as ecology, reproductive biology, and genomics. Most of our contemporary knowledge comes from the accumulation of phylogenetic information derived from molecular data, and from the use of phylogenies to elucidate biogeography and the evolution of morpho-ecological characters, as well as to define ages of specific clades. We hope this review will be useful as a general framework for people working with the family Cactaceae but likewise to people

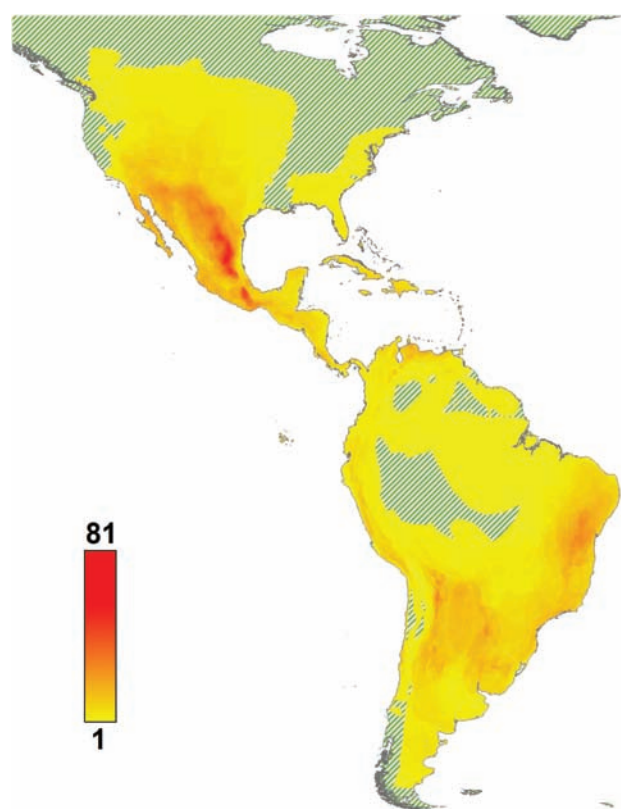


Figure 1. Generalized distribution of Cactaceae based on species richness from the IUCN evaluation of Cactaceae (adapted from Goettsch et al. 2015). Species richness is on a sliding scale from yellow ($n = 1$) to red ($n = 81$). The centers of highest diversity of Cactaceae, western North America, eastern Brazil, the Andes, and southern South America are shown here in red (taken and modified from Goettsch et al. 2015). See online version for full colors.

interested in the evolution of the numerous ecological interactions of cacti with many other organisms. Although the Cactaceae have been studied for many years by a large group of scientists and aficionados, there is still much to learn about their anatomy, basic biology, ecology, evolutionary relationships, physiology, as well as many other areas of research.

Origin and Diversification of Cactaceae

The cactus family belongs to the order Caryophyllales, a large clade (ca. 11 600 spp.) of ecologically diverse and extreme plants with a large representation of halophytes and xerophytes showing C4 or CAM metabolism (Edwards and Ogburn 2012; Stevens 2017). Inside Caryophyllales, Cactaceae form a monophyletic group highly supported both with morphological and molecular data (Barthlott and Hunt 1993; Wallace 1995; Nyffeler 2002; Edwards et al. 2005; Barcenás et al. 2011; Hernández-Hernández et al. 2011; Walker et al. 2018). Three clades within Caryophyllales evolved extreme succulence, Cactaceae, Aizoaceae, and Didiereaceae. Both Cactaceae and Aizoaceae have been shown to have the fastest diversification rates in the plant kingdom (Klak 2004; Hernández-Hernández et al. 2014; Magallón et al. 2015).

Unfortunately, Cactaceae, as in most clades inhabiting dry environments, lack a fossil record. Our current knowledge about the possible dates of origin of the family and divergence of lineages is based on the implementation of relaxed molecular clocks using molecular phylogenies. Recent estimates place the origin of the family around 30–35 Mya, in the Late Eocene (Arakaki et al. 2011; Hernández-Hernández et al. 2014; Magallón et al. 2015). These dates are coincident with the origin of other succulent arid-adapted lineages, such as succulent euphorbs (36 Mya, Bruyns et al. 2011), *Agave* (20–26 Mya, Good-Avila et al. 2006), or succulent asclepiads (Ceropegieae + Marsdenieae, Asclepiadae: 30–35 Mya, Rapini et al. 2007). It has been shown that the world experimented a global drop in CO₂ during these dates, corresponding to the Late Eocene (Zachos et al. 2001). These pose a plausible scenario for the origin of these succulent lineages, which possess crassulacean acid metabolism (CAM), highly efficient in the use of CO₂ (Arakaki et al. 2011; Hernández-Hernández T, Vásquez-Cruz M, in preparation).

Although succulent plant lineages might have originated during the Late Eocene, estimated dates for subclades within Cactaceae, as well as other succulent families, indicate the diversification of the most speciose lineages occurred later, most importantly during the late Miocene (Hernández-Hernández et al. 2014), when the climatic conditions where these plants occur were already arid. It has been proposed that cactus subclades (e.g., tribes and genera) diversified and accumulated a high number of species in response to various biotic and abiotic enablers, for example, the interaction with novel pollinators or the expansion of the Sonoran Desert (Hernández-Hernández et al. 2014), or the high prevalence of hybridization and polyploidization (Machado 2008; Majure et al. 2012b).

Very early hypotheses based on morphological and geographical observations placed the possible center of origin of the family in South America, with the Andes as the major biodiversity hotspot (Buxbaum 1969; Gibson and Nobel 1986; Leuenberger 1986; Mauseth 1990; Edwards et al. 2005). Modern estimations of biogeographic centers of origin based on current distributions and molecular phylogenies lend further evidence to support these hypotheses (Majure et al. 2012b; Hernández-Hernández et al. 2014). The central Andes have been proposed also as a center of diversity for other

species-rich lineages, and its uplift likely increased diversification in the region (Antonelli et al. 2009).

The availability of molecular data for the cactus family has helped to elucidate the phylogenetic relationships among closely related out-group lineages, as well as major clades within the family (Figure 2). Although Portulacaceae (i.e., *Portulaca*) has most commonly been resolved as sister to Cactaceae (Brockington et al. 2011), more recent analyses with better taxon sampling and using transcriptome or hybrid enrichment data suggest that the worldwide distributed succulent Portulacaceae and Anacampserotaceae form a clade sister to Cactaceae (Figure 3; Moore et al. 2017; Walker et al. 2018; Wang et al. 2018). Anacampserotaceae and Portulacaceae form wood in certain members, and all cacti are woody. Anacampserotaceae exhibit facultative CAM photosynthesis (Winter and Holtum 2017), while Portulacaceae exhibit both C4 and C3–C4 intermediates (Ocampo et al. 2013), and likewise Cactaceae show C3, CAM, and facultative CAM photosynthesis. Anacampserotaceae and Portulacaceae have dry, capsular fruits, while Cactaceae have mostly berry fruits or in some cases dehiscent berries or berry-like capsules (e.g., *Pilosocereus*, *Stenocereus*) or even dry, indehiscent “pods” in certain Opuntioideae (both dry pods and dehiscent berries are derived in the family). Anacampserotaceae, Portulacaceae, and Cactaceae share the character of having trichomes in the axils of the leaves, these being produced from the areoles (modified short shoots; Stevens 2017) in Cactaceae. Although Portulacaceae and members of *Pereskia* s.s. have basal placentation (Edwards et al. 2005), that character appears to be derived separately in *Pereskia*, as parietal placentation is found throughout the rest of Cactaceae. Likewise, fully inferior ovaries appear to have been derived twice, once in *Leuenbergeria* and once in the core Cactaceae (Maihuenioideae, Cactoideae, Opuntioideae). A more detailed analysis of morphological characters of all 3 major clades will be necessary to further clarify those characters that can be used to recognize the ([Anacampserotaceae + Portulacaceae] [Cactaceae]) clade.

Early Diverging Lineages: “Pereskioideae,” Opuntioideae, Maihuenioideae

It is common that classical taxonomy within Cactaceae is incongruent with the results obtained with molecular phylogenies (Figure 2; Nyffeler 2002; Hernández-Hernández et al. 2011). For example, molecular phylogenies showed members of subfamily “Pereskioideae” (species within “*Pereskia*” s.l.) conformed to an early diverging grade of 2 clades that were subsequent sisters to the rest of the succulent and more morphologically-derived members of Cactaceae (Figure 2; Edwards et al. 2005; Walker et al. 2018). The rest of the subfamilies, however, are monophyletic in molecular phylogenies: Maihuenioideae, Opuntioideae, and Cactoideae. The Maihuenioideae have been recovered with good support as sister to the Cactoideae in several analyses (Figure 2; Edwards et al. 2005; Moore et al. 2017; Walker et al. 2018; Majure LC, Baker MA, Puente Martínez R, Salywon A, Fehlberg S, in preparation), although the taxon has been difficult to place in other analyses. Cactoideae + Maihuenioideae are sister to Opuntioideae, and the monophyly of both Opuntioideae and Cactoideae is highly supported with molecular data (Edwards et al. 2005; Griffith and Porter 2009; Bárcenas et al. 2011; Hernández-Hernández et al. 2011; Moore et al. 2017; Walker et al. 2018).

Species within the former “Pereskioideae” (included in “*Pereskia*” s.l.) have long been considered to exhibit the plesiomorphic morphological states of cacti, with their large, photosynthetic leaves

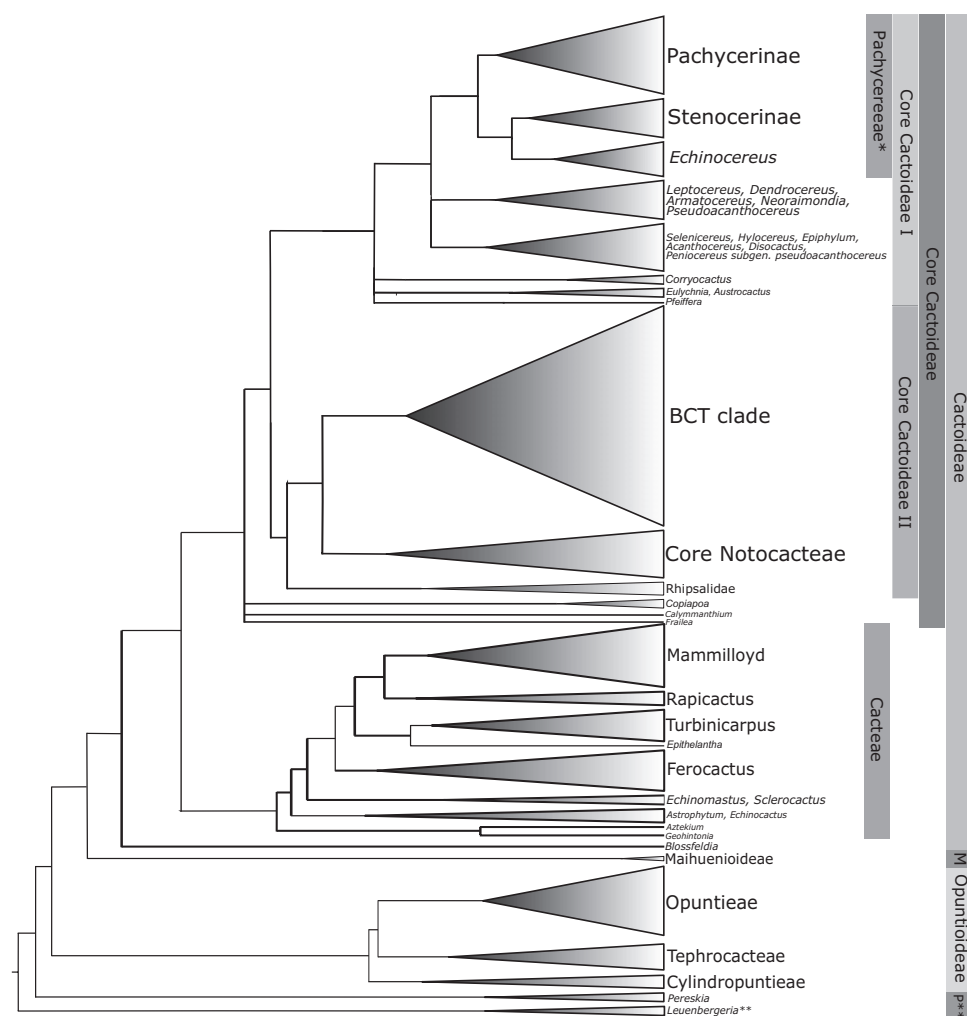


Figure 2. General draft of the current knowledge of phylogenetic relationships among major clades in Cactaceae. M = Maihuenioideae, P = Pereskioideae. In Barcenas et al. (2011), Core Cactioideae I corresponds to Echinocereae; and the clade comprising *Selenicereus*, *Hylocereus*, *Epiphyllum*, *Acanthocereus*, *Disocactus*, and *Peniocereus* subgen. *pseudoacanthocereus* corresponds to tribe Hylocereeae. *According to recent studies in the group, Sánchez et al. (2014) suggests subtribe Stenocercinae, together with *Echinocereus* s.s., (both within Tribe Pachycereeae), should now be circumscribed as Echinocereinae. **According to recent studies, former *Pereskia* s.l. species included now in the *Leuenbergeria* clade (*Leuenbergeria* s.s.) should be circumscribed in their own tribe, *Leuenbergerioideae* (Arakaki M, personal communication).

and mostly nonsucculent stems (Figure 3). Most taxa are woody shrubs or small trees, inhabiting more humid, tropical environments. However, recent phylogenetic analyses using nuclear, chloroplast, and mitochondrial data (Edwards et al. 2005), multigene family, nuclear loci derived from hybrid enrichment (Moore et al. 2017) and transcriptome data (Walker et al. 2018) have shown that what was formerly considered to represent one group, actually is composed of 2 separate clades, *Leuenbergeria* and *Pereskia* (Figure 2). Edwards et al. (2005) showed that *Leuenbergeria* does not develop stem stomata and maintains relatively rapid bark formation, while *Pereskia* does exhibit stem stomata and delayed bark formation, showing the gradation from an ancestral morphotype that uses its leaves as the primary photosynthetic organ to a plant more derived for stem photosynthesis, as is the case in most highly succulent, derived cacti.

Maihuenioideae (Figure 3), composed of 2 species (*Maihuenia patagonica* and *M. popoegii*), are densely caespitose shrubs that occur in Patagonian cold semi-arid regions of southern South America (Chile, Argentina). Phylogenetic analyses with both sufficient data and taxon sampling have resolved Maihuenioideae

as sister to Cactioideae (Edwards et al. 2005; Moore et al. 2017; Walker et al. 2018; Wang et al. 2018). Although some molecular phylogenies recover *Maihuenia* in an early diverging position within the family, the members possess highly specialized traits, showing adaptations to cold and dry environments. This taxon is atypical for most derived cacti in that it exhibits C_3 photosynthesis, and its stomata are restricted to the areoles of the plant (Anderson 2001). Maihuenioideae, like *Pereskia*, have large (although cylindrical), persistent leaves, which based on most well-resolved phylogenetic analyses would be interpreted as a retained ancestral character (i.e., plesiomorphy). The long branch subtending the *Maihuenia* clade suggests it might be a recent radiation derived from an ancient lineage (Arakaki et al. 2011; Hernández-Hernández et al. 2011).

Opuntioideae (Figures 2 and 3) are a well-supported (Wallace and Dickie 2002; Edwards et al. 2005; Barcenas et al. 2011; Hernández-Hernández et al. 2011; Moore et al. 2017; Walker et al. 2018), major clade in Cactaceae that are found in almost all major habitats in the Americas (deserts, savannas/grasslands, temperate zones, tropical dry forests), from sea level to more than 4500 m in

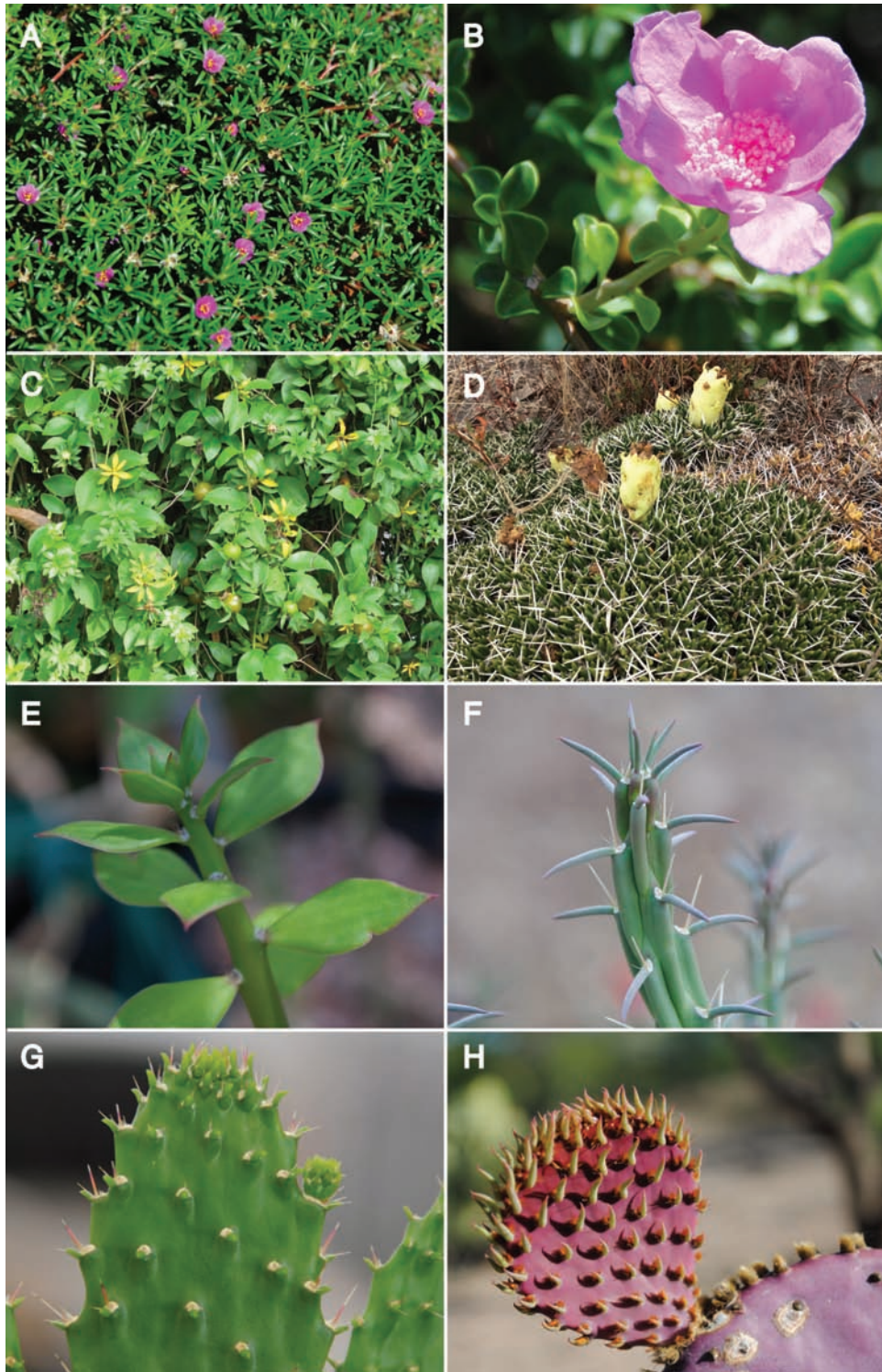


Figure 3. Members of *Portulaca*, *Leuenbergeria*, *Pereskia*, *Maihuenia* and leafy opuntoids. (A) *Portulaca pilosa* (Portulacaceae) showing succulent leaves and herbaceous growth form, (B) *Leuenbergeria portulacifolia* showing leaves, spines and staminate flower, (C) *Pereskia aculeata* with berry fruit and showing large, photosynthetic leaves, notice the large leaves arising from the pericarpel, (D) *Maihuenia poeppigii* showing persistent, cylindrical leaves and spines, and large pericarpel leaves, (E) *Pereskopsis porteri* showing persistent, large, flattened leaves, (F) *Cyllindropuntia imbricata* var. *spinitecta* showing large, cylindrical leaves on developing stem, (G) *Consolea microcarpa* showing conical leaves on developing cladode, (H) *Opuntia santa-rita* showing conical leaves on developing cladode. Photos A–C, E–H taken by L.C. Majure, photo D taken by P. Guerrero. See online version for full colors.

elevation in the Peruvian Andes (Anderson 2001). There are roughly 16 genera in the clade and 3 tribes, Cyllindropuntieae, Opuntieae, and Tephrocacteae (Figure 2). Numerous phylogenetic analyses have

focused on Opuntioideae sublineages, although very few analyses have attempted to resolve relationships within the entire clade. Griffith and Porter (2009) have produced the most comprehensive

phylogeny of the group to date, although, it is still unclear how the 3 tribes are related to one another, as most phylogenetic analyses have been unresolved or very poorly supported (Wallace and Dickie 2002; Crozier 2005; Edwards et al. 2005; Bárcenas et al. 2011; Hernández-Hernández et al. 2011) or lacked sufficient taxon sampling to test those relationships (Majure et al. 2012b; Ritz et al. 2012; Bárcenas 2015). Using transcriptomic data, Walker et al. (2018) resolved tribe *Cylindropuntiae* as sister to a *Tephrocacteae* + *Opuntiae* clade, although based on very poor taxon sampling. Phylogenomic analyses based on plastome data with ample taxon sampling are proving to be very useful for resolving relationships among these 3 subclades (Majure LC, Baker MA, Cloud-Hughes M, Salywon A, Neubig KM, in review).

The *Opuntioideae* are renowned for hybridization and polyploidization. There are reports of cases in which hybridization associated with polyploidy (allopolyploidy) and vegetative propagation gave rise to new species capable of invading habitats different from those occupied by both putative parental species (e.g., *Cylindropuntia prolifera*; see Mayer et al. 2000), or which can survive environmental conditions unfavorable for the parental taxa (e.g., *Opuntia* × *occidentalis*; Benson and Walkington 1965; Bobich and Nobel 2001). Although the most dramatic examples are in *Opuntioideae*, hybridization might have played a role in the evolution of several other cactus lineages as well. Hybrids among cactus species are common in cultivation and in the wild, and cases of species produced from interspecific or even intergeneric hybridization events have been documented for members of the *Cactoideae* as well (Machado 2008 and references therein). Majure et al. (2012c) found that nearly 60% of all species in tribe *Opuntiae* are polyploid, and Majure et al. (2012b) described intergeneric hybridization events between *Consolea* and *Opuntia*. Baker and Pinkava (1987), Pinkava (2002), and Majure et al. (2012c) outlined the presence of polyploidy across both *Cylindropuntiae* and *Opuntiae*, showing its prominence throughout those 2 clades. There are little data for tribe *Tephrocacteae* regarding ploidy, however, most species analyzed to date have been polyploid (Pinkava 2002). Polyploidy and reticulate evolution have played a major role in speciation in tribe *Opuntiae* (Majure and Puente 2014), and taxonomically difficult species complexes often arise from those processes (Majure et al. 2012a; Majure et al. 2017). Majure et al. (2012a) showed that polyploids often become more geographically widespread than their putative parental (diploid) taxa, perhaps as a result of their ability to cope with adverse environmental conditions not supported by their diploid relatives.

Although there is a preponderance of allopolyploidy in *Opuntioideae*, there are numerous examples of putative autopolyploids (genome duplication within one species) as well, such as in *Cylindropuntia* and *Opuntia*. *Cylindropuntia alcabes*, *C. bigelovii*, *C. fulgida*, and *C. munzii* have both diploid and triploid populations (Pinkava 2002), *C. cholla* and *C. leptocaulis* are represented by diploid, triploid, and tetraploid populations (Pinkava 2002; Powell and Weedin 2004), and *C. davisii*, *C. ramosissima*, and *C. whipplei* are represented by both diploid and tetraploid populations (Pinkava 2002; Powell and Weedin 2004; Baker and Pinkava, in press). In the case of *C. whipplei*, the tetraploid populations are morphologically distinguishable and are circumscribed as a separate variety, *C. whipplei* var. *enodis* (Baker 2006). *Opuntia drummondii* is known from diploid, triploid, and tetraploid populations, which are virtually indistinguishable (Majure et al. 2012a, 2017), and *O. strigil* is known from diploid and tetraploid populations (Powell and Weedin, 2004). There are other examples where a single species may have

multiple ploidal levels (e.g., *Opuntia humifusa*), however, they are not true autopolyploids and indeed those multiple levels of ploidy represent products of hybridization and cryptic speciation (Majure et al. 2017).

Tribe *Opuntiae* (ca. 230 spp.) is the most diverse and widespread clade among the *Opuntioideae*, with the largest genus *Opuntia* (ca. 200 spp.), ranging from Alberta, Canada to Patagonia, Argentina and likewise which has been introduced by man throughout the world (Anderson 2001; Casas and Barbera 2002). Majure et al. (2012b) showed that *Opuntia* (i.e., the prickly pears, nopales) originated in southern South America and from there, dispersed into dry regions of western North America, where they became most diverse. There are 8 major clades within the group, 2 of which are South American and 6 that are North American in origin. A number of northern South American taxa (e.g., *Opuntia bisetosa*, *O. schumannii*) originated from hybridization among North American taxa. Majure et al. (2012b) clearly showed that the hummingbird-pollinated genus “*Nopalea*” is deeply nested within *Opuntia* and is closely related to other Central American and Caribbean species, and merely represents a pollinator switch with correlating morphological changes. Switches to hummingbird pollination have occurred several times in the clade (e.g., *Consolea*, *Tacinga*, *Opuntia quimilo*, *O. quitensis*, *O. stenopetala*). Tribe *Opuntiae* also is the most economically important among the 3 clades, with prickly pears being used for biofuel production, fodder for livestock, foodstuffs, ornamentals, pharmacological products, as well as for producing cochineal dye from *Dactylopius* spp. (Hemiptera) (Inglese et al. 2002; Nefzaoui and Ben Salem 2002; Sáenz-Hernández et al. 2002).

Tribe *Cylindropuntiae* is the second-most diverse clade (ca. 70 spp.) in the *Opuntioideae* and are known commonly as the chollas and relatives (Figure 3F). This group occurs primarily in the western North American desert region, although, the leafy genera *Pereskopsis* and *Quiabentia* are found in tropical dry forest of Mexico/northern Central America and South America, respectively. Griffith and Porter (2009) showed the clade to be highly supported based on nuclear and plastid data, although, the genus *Grusonia* was nested within *Cylindropuntia* in their phylogeny. The use of ITS in their phylogenetic reconstruction likely resulted in the nested placement of *Grusonia* (Majure LC, Baker MA, Cloud-Hughes M, Salywon A, Neubig KM, in review). Bárcenas (2015) reconstructed a phylogeny of *Cylindropuntiae* based on plastid loci and showed the clade to be well supported, however, several clades lacked resolution at the species level. Majure et al. (Majure LC, Baker MA, Cloud-Hughes M, Salywon A, Neubig KM, in review) reconstructed a phylogeny of the group using plastome data derived from genome skimming and resolved species relationships, as well as major clade relationships.

Tribe *Tephrocacteae* was analyzed by Griffith and Porter (2009) in their comprehensive phylogeny of the *Opuntioideae*, however, they recovered a polyphyletic *Tephrocacteae*, which may also be a result of the use of ITS in their phylogeny. Ritz et al. (2012) reconstructed the phylogeny of the group using the plastid gene *matK* and the low copy nuclear gene *phyC* and recovered a well-supported, monophyletic group. Walker et al. (2018) also recovered the group as a clade with strong support using transcriptome data, albeit with very poor taxon sampling. Majure et al. (Majure LC, Baker MA, Cloud-Hughes M, Salywon A, Neubig KM, in review) likewise recovered a strongly supported clade using plastome data.

Members of the *Opuntioideae* subfamily exhibit interesting morphological features, especially with regards to their phylogenetic

placement. Several species have “large” (macroscopic) leaves, as compared with the more highly-derived members of Cactoideae. These large leaves are likely plesiomorphic (i.e., retained ancestral character) considering that the group is sister to Maihuenioideae + Cactoideae, although this has not been rigorously tested. Two members of tribe Cyliotropuntieae, *Peresklopsis* and *Quiabentia*, have large, flattened leaves (Figure 3), which have mostly been considered to represent the ancestral condition in Cactaceae, although the lack of a well-resolved phylogeny precludes the ability to test this hypothesis. Members of Opuntioideae also exhibit the synapomorphy of small, hairlike spines called glochids. These are normally produced in the adaxial portion of the areole but can be heterogeneous in their size, shape, and development from the areole. They differ from normal spines in being caducous, that is, the cells at the base of the glochid undergo programmed death at the time of maturation, thus rendering the glochids loose and easily dislodged.

Cactoideae: The North American Clades

From the geographic origin of the family in the Central Andes in South America, biogeographic estimations suggest several lineages colonized the north of the American continent independently at different times, in some cases radiating profusely (Hernández-Hernández et al. 2011; Majure et al. 2012b). Two members of the early diverging clades *Leuenbergeria* and *Pereskia* (*L. lychnidiflora* and *P. aculeata*), reach the more humid portions of southern Mexico, and *L. lychnidiflora* also occurs in Central America. Epiphytic cacti belonging to *Rhipsalis* and *Epiphyllum* inhabit similar humid forest regions as well, together with some columnar species of *Stenocereus*, and some *Opuntia* species that are mostly restricted to tropical dry forest (members of the *Nopalea* clade; sensu Majure et al. 2012b). However, lineages that underwent the fastest and largest radiations of species occurred in the arid regions of northern Mexico and the southwestern United States (Hernández-Hernández et al. 2014; Hernández and Gómez-Hinostrosa 2015).

Mexico is the center of origin of a hyperdiverse clade corresponding to the tribe Cacteae, including around 27 genera (Figure 2; Vázquez-Sánchez et al. 2013; Hernández-Hernández et al. 2014). These are the globose and generally small cacti like *Mammillaria*, *Astrophytum*, *Thelocactus*, or *Turbiniacarpus*, but some species reach very large sizes like *Echinocactus platyacanthus* or *Ferocactus cylindraceus* (Anderson 2001). Tribe Cacteae has long been recognized as a cohesive monophyletic group, which was confirmed with molecular data (Vázquez-Sánchez et al. 2013). This tribe is sister to a clade including all remaining members of subfamily Cactoideae (Figure 2). After an event of long range dispersal from South America, Cacteae started their diversification in the Sierra Madre Oriental around 15 Mya, with a subsequent dispersal to the Mexican Plateau, where it reached its maximum diversity (Hernández and Gómez-Hinostrosa 2005, 2015; Vázquez-Sánchez et al. 2013; Hernández-Hernández et al. 2014). One of the largest cactus genera occurs here, *Mammillaria*, which includes around 155 small globose species distributed mostly in Mexico (Hernández and Gómez-Hinostrosa 2015). Virtually half of the species are microendemics, which together with the accelerated habitat destruction and illegal trade makes them one of the most threatened groups of cacti (Hernández and Gómez-Hinostrosa 2015). Molecular phylogenies have shown that *Mammillaria* is paraphyletic, with members of *Coryphantha* (ca. 40 spp) and *Escobaria* (ca. 17 spp) embedded in the same clade (Butterworth and Wallace 2004; Hunt 2006). Several *Coryphantha* and *Escobaria* species are distributed in the southern arid portions

of the United States, and one species even reaches Canada (Dicht and Luthy 2006). A multinational effort is required to better understand this large radiation of small globose cacti, which may be one of the fastest radiations in the plant kingdom.

The clade sister to tribe Cacteae includes both north and south American taxa (Core Cactoideae; Figure 2). Molecular phylogenies show this clade clearly split into 2 major clades around 15–13 Mya (Figure 2; Hernández-Hernández et al. 2011, 2014), with some taxa with uncertain positions (e.g., south American *Copiapoa*, *Frailea*, *Calymmanthium*, etc.; see figures 3 and 4 in Hernández-Hernández et al. 2011). North American epiphytic and columnar cacti evolved within one of those clades (Core Cactoideae I). The early diverging members of Core Cactoideae I clade are south American, generally columnar cacti, such as *Corryocactus* or *Neoraimondia* (Hernández-Hernández et al. 2011), followed by a clade composed of mostly shrubby, epiphytic or prostrate cacti (*Epiphyllum*, *Selenicereus*, *Leptocereus*, *Acanthocereus*, *Disocactus*, etc.) with flowers already differentiated toward novel pollination by bats or hummingbirds. This last clade, corresponding to the tribe Hylocereeae, has been studied in more detail using chloroplast sequences showing that traditionally recognized genera, such as *Hylocereus* and *Selenicereus*, were nonmonophyletic (Cruz et al. 2016; Korotkova et al. 2017). These lineages are found through tropical regions of the Caribbean, México, Central America, and Florida (Anderson 2001). Sister to this shrubby cactus clade are the giant North American columnars (former Pachycereeae tribe, now circumscribed as Echinocereae, see below), composed of 2 clades (Figure 2), corresponding to subtribes Pachycerinae (*Pachycereus*, *Cephalocereus*, *Carnegiea*, etc.) and Stenocerinae (*Stenocereus*, *Echinocereus*, *Myrtillocactus*, etc.); that diverged very recently, around 4–6 Mya (Hernández-Hernández et al. 2011, 2014). In spite of their long life cycles and generation times, the ca. 10 genera and 60 species of north American columnars (Hunt 2006), in particular members of Stenocerinae, together with the independent lineages of south American columnars (see below), show some of the fastest diversification rates observed in the plant kingdom (0.8–1 sp. per million year, Hernández-Hernández et al. 2014).

North American columnars (tribe Pachycereeae, in part) are some of the most thoroughly studied cacti, especially *Carnegiea* (saguaro) and *Stenocereus* (e.g., Fleming and Valiente-Banuet 2002; and see below). Hypotheses about phylogenetic relationships appeared early, first using anatomical and phytochemical characters (Gibson and Horak 1978; Unger et al. 1980), and later with molecular data (Wallace 2002; Hartmann et al. 2002), ecology and even genomics (Copetti et al. 2017; see below). Within Pachycereeae, columnars included in the Pachycerinae subtribe (*Pachycereus*, *Neobuxbaumia*, *Cephalocereus*, and *Carnegiea*) are usually large and ramified tree cacti found in arid, warm, and sub-humid regions of Mexico and Central America, as well as in the arid southwestern United States. The estimated age of the clade is around 5 Mya (Hernández-Hernández et al. 2014). The highest species richness is found in the southern part of Mexico, including the Balsas Depression, Tehuacan-Cuicatlan Valley, and Southern Mountains. A phylogeny reconstructed with chloroplast and nuclear regions with limited sampling (30 species) representing the 4 genera of Pachycerinae show the group is not monophyletic, unless 2 species of *Stenocereus* from Central America are included within it (Arias et al. 2003). Within the subtribe, relationships remain elusive (Hernández-Hernández et al. 2011). *Pachycereus* (13 species sensu Hunt 2006) is clearly a polyphyletic assemblage (Arias et al. 2003; Arias and Terrazas 2009; Hernández-Hernández et al. 2011).

Based on structural and molecular data, Arias and Terrazas (2009) propose *Pachycereus* as a monophyletic genus including only 5 species (*P. grandis*, *P. pecten-aboriginum*, *P. pringlei*, *P. tepamo*, and *P. weberi*), with *Carnegiea* as its sister taxon (Arias and Terrazas 2009). *Cephalocereus* and *Neobuxbaumia* are not monophyletic either, and both conform to a clade named *Cephalocereus* (Arias et al. 2003; Tapia et al. 2017). The prostrate or climbing *Peniocereus* s.str. species, that were thought to belong to the Hylocereae tribe, were shown to belong to Pachycereinae with molecular data (Arias et al. 2005; Hernández-Hernández et al. 2011), showing the high levels of plasticity in the evolution of cacti growth form within lineages.

Several North American columnars from the Sonoran Desert were recently sequenced for genomic data (Copetti et al. 2017). Phylogenies built for *Carnegiea gigantea* (saguaro), *Pachycereus pringlei* (cardón, sahuaso), *Pachycereus schottii* (senita), and *Stenocereus thurberi* (organ pipe, pitaya) using 458 genes were congruent with our current partial understanding of evolutionary relationships within and among tribes. Gene phylogenies showing more than 90% bootstrap support showed there is high discordance among gene trees and the species tree (37% gene trees were discordant), attributed to the long generation times and moderately large effective population sizes of these species, leading to extensive incomplete lineage sorting (Copetti et al. 2017).

Unfortunately, we still lack phylogenetic studies either within or among members of the Stenocereinae subtribe. This group includes *Stenocereus* (ca. 23 species), and the small or monotypic genera *Polaskia* (2 spp.), *Myrtillocactus* (ca. 5 spp.), and *Escontria* (1 spp.) (Gibson 1982). The genus *Echinocereus* is the third most species-rich in the cactus family, with around 44–71 species (Sánchez et al. 2014, 2018). It has been placed in its own subtribe, Echinocereinae (Anderson 2001). However, molecular data showed *Echinocereus* is a strongly supported, monophyletic group sister to a clade including Stenocereinae members. In accordance with the principle of priority, Sánchez et al. (2014) proposes that *Echinocereus*, together with remaining members of the Stenocereinae subtribe should be called Echinocereinae (Figure 2). *Echinocereus* is a very young clade (diversification estimated around 4 Mya), showing the fastest diversification rate within Cactaceae (1–0.6 species per My; Hernández-Hernández et al. 2014).

The iconic saguaro (*Carnegiea gigantea*) is possibly the most popular and well-studied species of cactus (e.g., Brum 1973; Pierson and Turner 1998; Drezner 2003; and for a review, see Drezner 2014). However, species within the genus *Stenocereus* have also been studied in depth within the last few decades, with reports on anatomy (Terrazas and Loza-Cornejo 2003), seed germination and seedling physiology (Rojas-Aréchiga 2001; Loza-Cornejo et al. 2003; Naranjo et al. 2003; Ayala-Cordero et al. 2004, 2006; Arroyo-Cosultchi et al. 2006), extensive work on domestication and the use of certain species (Pimienta-Barrios and Nobel 1994; Casas et al. 1997, 1998, 1999a, 1999b; Pimienta-Barrios et al. 2000; Parra et al. 2010), physiology (De la Barrera and Nobel 2003), diversity and conservation (Clark-Tapia and Molina-Freaner 2003; Clark-Tapia et al. 2006; Casas et al. 2006), reproductive biology (Clark-Tapia and Molina-Freaner 2004; Ibarra-Cerdeña et al. 2005; Arias-Cóyotl et al. 2006; Bustamante and Búrquez 2008; Bustamante et al. 2010), and studies about their relationships with cactophilic *Drosophila* (Fogleman 1989).

Core Cactoideae, the South American Clades

Although a higher number of cactus species are found in North America, morphological and ecological diversity is higher in South

America, even though modern lineages on both sides of the continent are estimated to be relatively contemporaneous (Hernández-Hernández et al. 2014). Of the 9 traditionally recognized tribes of the Cactoideae, 5 are endemic to South America (Browningieae, Calymmantheae, Cereeae, Notocactaeae, and Trichocereae), while the rest are distributed both in South and North America (Cactaeae, Hylocereae, Pachycereae, and Rhipsalideae) (Anderson 2001). Recent phylogenies resulting from the analyses of molecular data reveal that particularly in South America, cacti show a complex taxonomic scenario at the suprageneric level, where most tribes are not monophyletic and several genera remain unplaced (Nyffeler and Eggli 2010; Hernández-Hernández et al. 2011). Molecular phylogenies also show that the nonmonophyly of traditionally recognized genera is very common across all lineages within the cactus family. However, the problem of lack of resolution in cactus phylogenies at all levels is most frequent in the South American lineages (within the Core Cactoideae II clade; Figure 2). This might be a result of insufficient information provided by traditional molecular markers. However, and most importantly, this could also be a result of the lack of studies and scarcity of taxonomic work, particularly of taxa from the Central Andes (i.e., Peru, Bolivia, northern Argentina, and Chile).

In spite of this complex taxonomic scenario, some South American lineages are morphologically cohesive and strongly supported in phylogenetic analyses. One of the most enigmatic groups is the genus *Copiapoa*, comprising about 32 species endemic to coastal Atacama Desert (Larridon et al. 2015). This genus has been considered “orphan,” because its tribal and phylogenetic placement remains uncertain within Cactoideae (Nyffeler and Eggli 2010). This monophyletic group shows very low genetic variation both with plastid and nuclear markers (Larridon et al. 2015). The genus *Eulychnia* (7 species *sensu* Hunt 2006) is also considered an “orphan,” or with uncertain phylogenetic position within Cactoideae. Species occur along the western slopes of the Andes, between the latitudes of 32°S (Los Molles, Chile) and a single species at 16°S (Chala, Peru) (Larridon et al. 2014). Allopatric speciation seems to be a common process in *Eulychnia* and other Cactaceae lineages as well, such as *Eriosyce* subgen. *Neoporteria* (Guerrero et al. 2011a), *Mammillaria* (Hernández and Gómez-Hinostrosa 2015), and columnar cacti in North America (Gibson and Horak 1978), and might be a common speciation mechanism in the family. Detailed studies at the generic and population levels, as well as field work, are needed to evaluate speciation mechanisms in the family more accurately.

Considered part of the tribe Trichocereae, *Gymnocalycium* is another of the few examples of a well-supported South American group. It is a genus of globose cacti comprising about 50 species (Anderson 2001). Demaio et al. (2011) reconstructed a molecular phylogeny with 4 chloroplast markers. They found recurrent conflicts with the traditional taxonomic classification based on stem morphology. However, fewer conflicts were detected when using seed morphology. Interestingly, their analyses shed light on the tight associations of morphological evolutionary trends with the habitats occupied by species. For example, stem size reduction seems to be correlated with the adaptation from warmer habitats to cooler conditions. Also, their findings support the evolution of napiform roots to increase water and starch storage (Nobel 2002).

Tribe Notocactaeae, composed mainly of solitary or shrubby cacti, were shown to be paraphyletic in molecular phylogenies (Nyffeler and Eggli 2010). However, there is a core Notocactaeae clade that has been consistently recovered as monophyletic with good support values (Figure 2; Nyffeler 2002; Hernández-Hernández et al. 2011). The

core Notocactaceae comprise the widespread and species-rich genera *Eriosyce* s.l. (ca. 31 species) and *Parodia* s.l. (ca. 60 species), together with the bitypic genus *Neowerdermannia*, plus the 2 monotypic and locally distributed genera *Rimacactus* and *Yavia* (Nyffeler and Eggli 2010). These lineages include mostly globose species that diverged about 12 million years ago (Hernández-Hernández et al. 2011, 2014). The origin of the tribe is inferred to be from the east-central Andes, in southern South America (18–22°S), where some basal genera diverged, such as *Yavia* and *Neowerdermannia* (Hernández-Hernández et al. 2014). In the western Andes, the highest diversity of Notocactaceae is southward displaced (22–34°S) (Guerrero et al. 2011b), since the hyper arid Atacama Desert severely constrains the colonization of major groups of plants and animals (Guerrero et al. 2013). Phylogenies with limited sampling suggest *Parodia* is monophyletic while *Eriosyce* s.l. is not (Nyffeler 2002; Nyffeler and Eggli 2010; Guerrero et al. 2011a; Hernández-Hernández et al. 2011).

The need for taxonomic recircumscription at different levels based on phylogenetic analyses with several loci is very common within subfamily Cactoideae, particularly in South American tribes. Tribe Trichocereae harbors dozens of genera and hundreds of species, but phylogenetic relationships in this group have been extremely difficult to elucidate. As traditionally circumscribed, molecular phylogenies show this group to be polyphyletic, because early diverging taxa (e.g., *Discocactus*, *Rebutia*, *Sulcorebutia*) are mixed with taxa classified within the Bowningieae and Cereeae tribes, and molecular phylogenies show they conform to a clade that has been called the BCT clade (Figure 2; Nyffeler 2002; Hernández-Hernández et al. 2011). This clade is characterized by the labile evolution of morphologically diverse vegetative traits. Within this clade, and closely related, we can find species with huge, columnar growth forms, such as *Echinopsis atacamensis*, to small globose cacti, such as *Matucana pujupatii*. It has been shown that the evolution of the gigantic tree-like growth form is convergent, occurring independently from ancestors with different growth forms both in North and South America (Figure 3; Hernández-Hernández et al. 2011). Reproductive traits are also exceptionally labile among members of the former, morphologically circumscribed tribe Trichocereae. Most species have mixed pollination systems (e.g., birds, bees), while others exhibit a more specialized system, such as *Cleistocactus baumannii*, which is predominately hummingbird pollinated (Gorostiague and Ortega-Baes 2016). This morphological lability increases the difficulty of taxonomic circumscription of genera, since many species show high levels of convergence of similar vegetative and reproductive morphology. There is further need for more robust phylogenies obtained with additional loci or genomic data to improve the low support for groups within the BCT clade, since traditional markers have been typically invariant and provide no resolution (Albesiano 2012; Albesiano and Terrazas 2012).

An iconic example of taxonomic conflicts in South American members of the BCT clade is the genus *Echinopsis* sensu lato. *Echinopsis* s.l. includes more than 100 species, and exhibits a great diversity in architecture and in pollination systems (bee, hummingbird, or sphingid pollinated) (Schlumberger and Renner 2012). Molecular phylogenies have shown *Echinopsis* s.l. to be nonmonophyletic (Schlumberger and Renner 2012; Ritz et al. 2007). In this genus, distant species may exhibit similar morphological traits, while close relatives may present very different morphological traits (Schlumberger and Renner 2012; Ritz et al. 2007). Interestingly, morphological homoplasy was triggered by convergence to similar selective forces rather than hybridization, which seems to be rare in the group (Schlumberger and Renner 2012). Also within the BCT,

the genus *Haageocereus*, a sprawling, shrubby or tree-like columnar cactus, is distributed along the western slope of the Peruvian Andes, with 2 species extending into northern Chile (Arakaki 2008). Microsatellite markers suggest a complex scenario of reticulate evolution within *Haageocereus* and *Espostoa* (Arakaki 2008). Ritz et al. (2007) studied the evolutionary relationships of several montane Andean genera within BCT using plastid markers. In line with other studies, they found that the traditional delimitation of tribes and of large genera need to be revised. For example, the small Andean genus of globose cacti *Aylosteria* was re-established and separated from *Rebutia* (Ritz et al. 2007). Further studies supported the existence of 3 lineages within *Aylosteria*, characterized by distinctive combinations of morphological character states (Ritz et al. 2016). Interestingly, analysis of AFLP fragments strongly suggests levels of hybridization among species (Ritz et al. 2016).

It has been suggested that the Andean uplift directly influenced the speciation of globose cacti within the BCT clade (Ritz et al. 2007), as well as Andean opuntias (Ritz et al. 2012). Marine incursions into the continent (caused by orogenesis) were responsible for the separation of the 2 centers of South American cactus diversification (northeastern Brazil and the central Andes) (Ritz et al. 2007, 2012, 2016). However, insufficient research on molecular systematics of South American cacti makes it difficult to understand the ecological and evolutionary factors leading to current diversity patterns. The essentials for understanding the radiation of Andean cacti are robust phylogenetic data and a sound taxonomic background (Ritz et al. 2016).

The former tribe Cereeae comprises several columnar cacti with a phylogenetic position related to Browningieae and Trichocereae members (Nyffeler 2002; Hernández-Hernández et al. 2011). Molecular phylogenies have been published for genera of Cereeae such as *Cereus*, *Cipocereus*, and *Praecereus* (Franco et al. 2017), as well as *Pilosocereus* (Calvente et al. 2016). Similar to the North American genera such as *Mammillaria* or *Echinocereus* (Hernández-Hernández, unpublished data; Sánchez et al. 2018), most diversification events in *Cereus* in the eastern Brazilian hotspot of cactus diversity (Figure 1) occurred very recently, during the mid to late Pleistocene (<1 Mya) (Franco et al. 2017). Calvente et al. (2016) focused their analyses on the morphological evolution of *Pilosocereus*. *Pilosocereus* also shows high lability in vegetative and floral traits, which may be related to recent diversification, morphological plasticity, and possible hybridization and introgression events. *Pilosocereus* also diversified extensively in the eastern Brazilian hotspot, but the *P. leucocephalus* s.s. group dispersed and diversified in central and north America (Calvente et al. 2016). The biogeographic history and diversification of *Pilosocereus* seems to be strongly related with the dry diagonal of seasonal forests (from deciduous thorn woodland Caatinga to Paraguayan Chaco), and to a northern core area, from northern Brazilian Amazon to Mexico and the southern United States (Calvente et al. 2016).

Even though most cacti have growth forms and habits that can be classified as columnar or globose, some other morpho-anatomical specializations appeared in cactus species living in humid tropical and subtropical forests. That is the case of the epiphytic and lithophytic growth forms, which account for 10% of the diversity of the whole family (Korotkova 2011). The tribes Rhipsalideae and Hylocereeae are the 2 largest epiphytic and lithophytic groups in the family (Korotkova 2011). Rhipsalideae members are mainly South American, while Hylocereeae are predominantly Mesoamerican and Caribbean (Barthlott 1983; Taylor and Zappi 2004; Korotkova et al. 2011). These represent 2 independent lineages where the

epiphytic and lithophytic growth form appeared convergently, since Hylocereeae is in the Core Cactoideae I (related to the North American members of Core Cactoideae), and Rhipsalideae is in the Core Cactoideae II (more closely related to South American members) (Figure 2; Nyffeler 2002; Hernández-Hernández et al. 2011). Calvente et al. (2011) reconstructed the phylogeny of Rhipsalideae using molecular data. Four well-supported clades were recovered, supporting the recognition of genera that had been placed in synonymy with other taxa: *Lepismium*, *Rhipsalis*, *Hatiora*, and *Schlumbergera*. Comparative analyses in Rhipsalideae showed higher phylogenetic consistency in the evolution of vegetative and reproductive traits compared with other tribes where more morphological homoplasy is more pervasive (Calvente et al. 2011). A potential explanation is that the specialized epiphytic and lithophytic life form constrains morphological evolution, compared with other cacti that occupy a wide range of habitats such as tribe Trichocereae. Also, in Rhipsalideae, hybridization and introgression (which promote a complex taxonomic scenario and trait lability) are thought to be rare or absent in the wild (Calvente et al. 2011).

Studies dealing with evolutionary trends in South American cacti have increased substantially in the last 2 decades. They suggest the Andean uplift had an influence in the diversification of taxa in central Andes; the putative center of origin of the family. It also promoted the split and emergence of a second center of origin in eastern Brazil (Ritz et al. 2007, 2012, 2016). Although, the radiation of small globose cacti in Chile and Argentina has not been investigated in depth, the study of Hernández-Hernández et al. (2014) supports the independent origin and diversification of different lineages, both in space and time. For example, *Copiapoa* occurs in coastal western Andes (stem group age 12 Ma, crown group age 3.38 Ma), core Notocactaeae species grow on both sides of the Andes (stem group 11.92 Ma, crown group age 8.78 Ma) and *Gymnocalycium* occurs in the eastern Andes (stem group 6.12 Ma, crown group age 5.08 Ma).

The convergence and lability in reproductive and vegetative traits is quite dramatic in South American members of the Core Cactoideae. Several groups have undergone many nomenclatural changes, leading to problematic generic and species taxonomic circumscriptions. Cacti with similar globose or arborescent growth forms are found both in South and North America, evolving independently (Figure 4; Hernández-Hernández et al. 2011). However, the ecological equivalence of these forms and their habitats remains to be tested. Furthermore, convergent traits are present at the intra-generic level in South American lineages, where a single genus can present the same growth forms and pollination syndromes nested in different subclades (e.g., *Echinopsis*; see Schlumberger and Renner 2012).

Ecology and Reproductive Biology

Although members of the cactus family are found over a wide range of habitats, including cold climates at high elevations and tropical rain forests at sea level (Anderson 2001), succulent species are more prominent in semi-arid regions. There they play important ecological roles and are part of a web of ecological interactions during their often extremely long life cycles. The demography of species in Cactaceae has been reviewed extensively (Godínez-Alvarez et al. 2003). The distribution of cacti is determined by environmental heterogeneity and by species-specific physiological requirements, and in general, it seems that temperature extremes pose distributional limits (Godínez-Alvarez et al. 2003 and references therein).

Besides the remarkable ecological interaction among microbial communities, flies and opuntoid or columnar cacti that characterize the cactophilic *Drosophila* model system (Markow and O'Grady 2008), large succulent cacti are fundamental functional pieces in desert ecosystems as sources of food and shelter for numerous animal species (Drezner 2014; Franklin et al. 2016). Some of those biological interactions shaped the evolution of certain lineages. For example, it has been shown that the evolution of columnar cacti both in North and South America was possibly driven by the interaction with bird, bat or moth pollinators (Valiente-Banuet et al. 1996, 2002; Munguía-Rosas et al. 2009; Hernández-Hernández et al. 2014). In addition, the dispersion of seeds mainly by bats and birds (Bregman 1988; Godínez-Alvarez et al. 2002; Pérez-Villafañá and Valiente-Banuet 2009), plays an important role in shaping the genetic structure of populations (Nassar et al. 2002; Figueredo et al. 2010; Bustamante et al. 2016).

A remarkable characteristic of the cactus family is the reproductive versatility of its members, shown in the vast variation in floral morphology, together with an assortment of reproductive and pollination systems (Almeida et al. 2013). Species within the family are generally self-incompatible, with bisexual flowers. Gametophytic self-incompatibility has been identified as the most common type of self-incompatibility in selected species of the genera *Schlumbergera* (Boyle 1997, 2003), *Hylocereus* and *Selenicereus* (Lichtenzweig et al. 2000), *Echinopsis* (Boyle and Idnurm 2001) and *Hatiora* (Boyle 2003), as well as in 7 species of *Ariocarpus*, with 6 species being partially and one completely self-incompatible (Martínez-Peralta et al. 2014). However, there are reports of autogamy, cleistogamy, functional dioecy, trioecy, and androdioecy (Arias 2007; Gutiérrez-Flores et al. 2017; Sánchez and Vázquez-Santana 2018). Within the Pereskioideae family, *Pereskia marcanoi*, *P. potulacifolia*, *P. quiqueyana*, and *P. zimmerflora*, and the Caribbean members of *Leuenbergeria* have been reported as dioecious (Leuenberger 1986; Sánchez and Vázquez-Santana 2018). Functional dioecy is relatively common in Opuntioideae, where it has been recorded in *Consolea*, *Cylindropuntia*, and *Opuntia* (Sánchez and Vázquez-Santana 2018 and references therein). Interestingly, the majority of dioecious or subdioecious species within the Opuntioideae clade are polyploid (Negrón-Ortiz 2007; Baker and Cloud-Hughes 2014), although several species of *Opuntia* show dioecy at the diploid (*O. quitensis*, *O. quimilo*, *O. stenopetala*; Díaz and Cocucci 2003; Majure et al. 2012c; Flores-Rentería et al. 2013) and polyploid levels (*O. robusta*; Del Castillo and Trujillo 2009). Dioecy is also correlated with polyploidy in *Echinocereus* (Cactoideae; Baker 2006; Hernández-Cruz et al. 2018 and references therein), *Mammillaria* (Parfitt 1985; Ashman et al. 2013; Glick et al. 2016), as well as within *Pachycereus pringlei* (Gutiérrez-Flores et al. 2018) having only diploid populations that are hermaphroditic in that species. On the other hand, the population variation in the sex ratios and breeding systems suggests that the polymorphism in the reproductive attributes of the family has evolved as an adaptive response to environmental factors (Gutiérrez-Flores et al. 2017). According to the sex ratios in *Pachycereus pringlei*, mainly gynodioecy populations prevail in northern Baja California, trioecy and mainly dioecy in the south, and hermaphrodite in surrounding islands. This biogeographic pattern is attributed to past expansions towards the north, ecological and climatic variation, as well as geographical isolation, and highlights the driving role of these factors in the reproductive versatility of cacti (Gutiérrez-Flores et al. 2017). The correlation of polyploidy and dioecy has been recorded in numerous plant groups, although, it seems clear in Cactaceae that this transition has been from a clear monomorphic gender to gender dimorphism rather than from diploid progenitors already possessing gender dimorphism (Ashman et al. 2013).

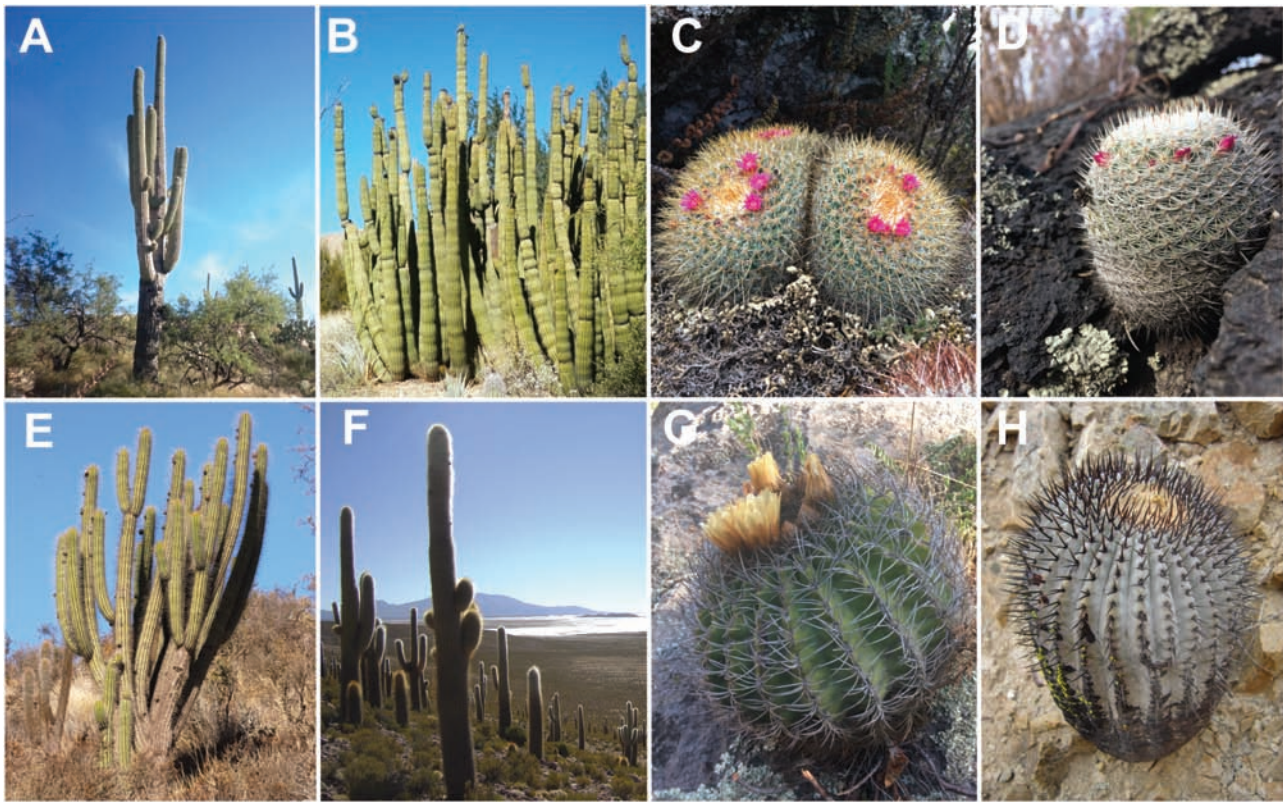


Figure 4. Members of Cactoideae from North and South America with columnar (treelike) or globular growth forms. (A) *Carnegiea gigantea* distributed along the Sonoran Desert in northwestern Mexico and southwestern United States, (B) *Pachycereus pringlei* distributed in Baja California and Sonora in northwestern Mexico, (C) *Mammillaria rhodantha* distributed in central Mexico, (D) *Mammillaria haageana* subsp. *san-angelensis* distributed in south-central Mexico, (E) *Eulychnia acida* distributed in north-central Chile, (F) *Echinopsis atacamensis* distributed in the altiplano along the border of Argentina, Bolivia, and Chile, (G) *Eriosyce marksiana* subsp. *lissocarpa* distributed in the Andes Range in south-central Chile, and (H) *Copiapoa cinerea* subsp. *columna-alba* distributed in the coastal Atacama Desert (Chile). Photos A and B taken by T. Hernández-Hernández, photos C and D taken by A. Cornejo-Romero, photos E–H taken by P.C. Guerrero. See online version for full colors.

Mating systems also vary widely among cactus lineages. Species within the former “Pereskioideae” tend to outcross (and also contain some dioecious species, as mentioned above, thus forcing outcrossing), but selfing is more widespread in Opuntioideae and Cactoideae (Reyes-Agüero et al. 2006; Mandujano et al. 2013). According to outcrossing rate values obtained by means of genetic markers, t and pollination experiments, t_e (0 in species with complete selfing, 0.5 for species with mixed mating and 1 in species with outcrossing system), *Pereskia guamacho* lend to outcrossing ($t = 0.61$ – 0.8) and Opuntioide members show outcrossing rate values ranging from 0 to 1 (Mandujano et al. 2010). Likewise, many species of the Opuntioideae show thigmonasty (movement of the stamens toward the stigma upon stimulation; Díaz and Cocucci 2003), which promotes selfing in that group. Within Cactoideae, most of the Cereaceae and Cactaceae members show mixed mating systems tending toward selfing with outcrossing rate values ≤ 0.5 ; while Pachycereaceae are mainly outcrossing with values > 0.5 and Cactaceae outcrossing rate (Mandujano et al. 2010). At the family level, the long-lived species with arborescent, shrubby or columnar life form are usually outcrossers; while species with shorter lifespans, such as globose taxa, tend to have mixed and selfing mating systems (Mandujano et al. 2010 and references therein). Mating systems in the family show an evolutive tendency from a mixed toward mainly either outcrossing or selfing (Mandujano et al. 2010).

Several floral traits vary widely within the family: color, shape, size, and time of anthesis (Bravo-Hollis 1978; Anderson 2001). The spatial separation of anthers and stigma, that is, herkogamy,

is common in cactus flowers and acts as mechanism that reinforces outcrossing (Cota-Sánchez and Crouch 2008). Floral diversity can be associated with a wide range of pollination syndromes. Chiropterophily, ornithophily, and phalaenophily (birds, bats, and sphingid moths) are observed in species with arborescent, shrubby, or columnar life forms, and evolved as a derived state independently in various lineages (Fleming and Valiente-Banuet 2002; Hernández-Hernández et al. 2014). Melitophily is the ancestral condition, and is more frequent in Opuntioideae, “Pereskioideae” and globose cacti (Reyes-Agüero et al. 2006; Mandujano et al. 2010). The variety of fertilization and breeding systems within the family highlights the plasticity of the reproductive response to the spatially and temporally unpredictable habitats in which species occur (Mandujano et al. 2010; Gutiérrez-Flores et al. 2017), and might be important for the accelerated diversification of the family, since it promotes genetic variability and evolution of the genome (Charlesworth 2006; Cota-Sánchez and Crouch 2008; Hernández-Hernández et al. 2014). However, the relationship between reproductive versatility within the family and speciation or diversification rates remains to be tested. There is a great need for further detailed studies of the reproductive biology of species from all lineages.

Population Genetics and Phylogeography

Genetic studies at the population level have been carried out in several cactus species, and show evolutionary patterns associated with

life history traits, such as growth form, mating, and pollination systems (Hamrick et al. 2002). These studies have also highlighted the effect of biogeographic and climatic events on genetic patterns, particularly during the Plio-Pleistocene (Hershkovitz and Zimmer 1997; Ritz et al. 2007). Other studies have used population genetic data to determine relationships and species status. For example, Baker and Butterworth (2013) used both microsatellite and morphological data to test intraspecific relationships and circumscription in *Coryphantha robustispina*. The scarcity of genetic studies at the population level is remarkable among the “Pereskioideae” and Opuntioideae, which are the least studied (Nassar et al. 2002; Cariaga et al. 2005; Helsen et al. 2011). However, for members of Cactoideae there are studies of approximately 45 species from arid and semiarid regions of North America, especially in the Pachycereeae and Cacteae tribes (for reviews, see Hamrick et al. 2002; Solorzano et al. 2016 and references therein), as well as for some *Pilosocereus* species in South America (Figueredo et al. 2010).

In general, major genetic attributes of populations of cacti with long life spans and columnar growth forms include moderate or high levels of genetic diversity, and low population differentiation (Hamrick et al. 2002; Figueredo et al. 2010; Bustamante et al. 2016). These attributes are also related to an outcrossing mating system and long-distance dispersal of pollen and seeds mainly by bats, which have promoted lower interpopulation differentiation due to their high movility (Hamrick et al. 2002; Nassar et al. 2003). From a biogeographic point of view, in North America, scenarios of vicariance and allopatry linked to the separation of the peninsula of Baja California have been deduced in species like *Lophocereus schottii* (Nason et al. 2002), as well as historical dispersion among peninsular, island and continental populations of *Stenocereus eruca* (Clark-Tapia and Molina-Freaner 2003; Molina-Freaner and Clark-Tapia 2005). In both hemispheres, events of population fragmentation, expansion, contraction, and secondary contact probably caused by quaternary climate change, have been inferred (Nassar et al. 2002; Guerrero et al. 2011a; Bonatelli et al. 2014; Gutiérrez-Flores et al. 2017). In South America, genetically diverse populations of Venezuelan and Brazilian columnar species suggest remnant populations derived from extensive, stable, and continuous arid habitats during the Last Glacial Maximum (Nassar et al. 2003; Moraes et al. 2005; Figueredo et al. 2010). In columnar, bat-pollinated cacti, substantial gene flow has maintained genetic connectivity among populations even though they have experienced changes in overall distribution through time (Figueredo et al. 2010, and references therein). On the other hand, gene flow could be a determining factor in columnar cactus speciation and colonization of arid emergent Neogene environments (Gibson and Nobel 1986; Hernández-Hernández et al. 2014), because in individuals dispersed over long distances, natural selection can favor those capable of surviving in a new environment (Sork et al. 2017; Gineapp et al. 2017).

On the contrary with what happens with long-lived gigantic columnar species, some species with a relatively small, globose life form belonging to the genera *Mammillaria* (in North America) and *Melocactus* (in South America) reveal patterns of moderate or low intrapopulation genetic diversity with a high degree of population differentiation (Nassar et al. 2001; Mota-Lambert et al. 2006a, 2006b; Solorzano et al. 2015). The 9 endemic species of *Mammillaria* with different degrees of rarity of the Tehuacan-Cuicatlán Valley show low diversity attributed to reduced population sizes and genetic drift. As a result of drift, inbreeding and genetic differentiation are high (Solórzano and Dávila 2015). The limited movement of alleles contributes to the marked genetic structure in

those small, globose cacti, since short-range generalist insects, such as bees and wasps, disperse pollen; while seed dispersal probably occurs by gravity (Solórzano et al. 2016). The restricted distribution, geographic isolation, and high edaphic specificity of small cacti probably promote allopatric divergence among populations and diversification by natural selection (Solórzano et al. 2016 and references therein, Maya-García et al. 2017). With regard to some of the South American species of *Melocactus*, they also show very low levels of genetic diversity within populations and high differentiation among populations. This pattern has been explained by mixed mating, self-compatibility, as well as restricted pollination and dispersal carried out by territorial hummingbirds and insects (Nassar et al. 2001; Mota-Lambert et al. 2006a, 2006b). Genetic differentiation is probably the result of contraction and expansion of available habitat during the Quaternary period that isolated populations. Thus, genetic drift, inbreeding, and poor gene flow play an important role in the evolution of *Melocactus* species in parts of South America (Nassar et al. 2001; Mota-Lambert et al. 2006a, 2006b). The few genetic data reported so far support the hypothesis that genetic drift is a relevant factor in the speciation of short-lived globose taxa, particularly in self-mating or mixed species, because they have low effective population sizes, show high levels of endemism and have restricted distributions (Gorelick 2009). Genetic studies indicate that cactus populations can represent remnant, geographically isolated habitats, that maintain unique genetic information and can serve as genetic reservoirs for neotropical xerophytic vegetation (Nassar et al. 2002; Moraes et al. 2005; Gutiérrez-Flores et al. 2016). Therefore, it is crucial to focus on conservation genetic studies to implement strategies that allow for the maintenance of the gene pool and the evolutionary processes that have driven elevated speciation in the family.

The effect of biogeographic events and climate change on cactus population genetic structure, formerly inferred by population genetics, has been tested by phylogeographic methods. During the Pleistocene glacial cycles, global cooling and drying caused the expansion of arid biomes and the diversification of plant species adapted to aridity (Van Devender 1990; Hewitt 2004). Particularly, climatic oscillations of the Quaternary promoted cycles of contraction–expansion of habitat and changes in species’ distributions affecting the genetic structure and speciation of xerophytic plants (Odee et al. 2012; Turchetto-Zolet et al. 2013). The few phylogeographic studies available for cacti have focused on the changes in distribution and demographic dynamics of columnar cacti included in the Pachycereeae and Cereeae tribes, particularly in the arid biomes of tropical and subtropical latitudes, where the greatest number of species are located (Nassar et al. 2002; Clark-Tapia and Molina-Freaner 2003; Cornejo-Romero et al. 2017; Franco et al. 2017). Studies show genetic signatures of population expansion–contraction, fragmentation range, vicariance, and population isolation, during the same palaeoclimatic phase indicating complex biotic responses of cacti to climatic oscillations. In North American subtropical deserts, *Lophocereus schottii* and *Pachycereus pringlei* contracted and diverged in xerophytic refugia during the cold-dry glacial period, and expanded in the current warm and wet interglacial (Nassar et al. 2002; Gutiérrez-Flores et al. 2016). On the other hand, in most of the columnars within the *Pilosocereus* genus distributed in the seasonally dry, tropical forests of South America, the opposite phenomenon is observed (Bonatelli et al. 2014; Perez et al. 2016; Franco et al. 2017). That is, the populations expanded in the glacial period, which was drier and colder, and contracted during the warmer and wetter interglacial periods, posing the hypothesis of existence of interglacial micro-refuges (Haffer 1969; Rull 2009;

Bonatelli et al. 2014). Estimated divergence times of these lineages correspond to the Pleistocene, and some columnars have shown incomplete lineage sorting (Copetti et al. 2017), suggesting recent speciation processes. These findings, as well as shifts in distributions based on species distribution models, provide evidence that supports the hypothesis of cactus diversification under the Quaternary arid environment expansion in both hemispheres, and allows for inferring the influence of regional geotectonic events on lineage divergence occurring throughout the Neogene (Bonatelli et al. 2014; Gutiérrez-Flores et al. 2016; Cornejo-Romero et al. 2017; Quilpidor et al. 2017).

Cactus Genomics

Genomic studies within Cactaceae have appeared only recently and reveal intriguing facts about the evolution of the family. Sanderson et al. (2015) assembled the complete chloroplast genome of the giant saguaro (*Carnegie gigantea*), an emblematic species of the Sonoran Desert. They show the saguaro plastid genome is exceptionally reduced, having lost one copy of the inverted repeat (IR) and most plastid *ndh* genes (or otherwise many *ndh* genes are pseudogenized); it has the smallest plastome among obligate photosynthetic angiosperms (ca. 113 000 bp). The conspicuous occurrence of homoplasy at the morphological level in the family apparently occurs at the genomic level too. Copetti et al. (2017) report draft nuclear genomic sequences at high coverage for saguaro, as well as sequences for other 3 columnar cacti from the Sonoran Desert and an outgroup *Pereskia*, at low coverage. Although assemblies for the nonsaguaro sampled were fragmented, they were able to recover 4436 gene or exon alignments. Among those, only 458 genes had maximum likelihood support values above 90% for all clades. Copetti et al. discovered that almost 60% of amino acid sites in proteins exhibited homoplasy, producing discordance between gene genealogies and species history. Thirty-seven percent of the phylogenies built did not fit hypothesized species relationships. This was attributed to many factors, including divergent population dynamics and long life spans, as well as gene flow via hybridization (introgression) and incomplete lineage sorting of convergent evolutionary changes (hemiplasy) (Copetti et al. 2017).

Transcriptome data acquired for large phylogenomic analyses across Caryophyllales, including Cactaceae, have helped increase our understanding of the phylogeny of the family, as well as have confirmed previous phylogenetic hypotheses (paraphyly of traditionally recognized *Pereskia* s.l.; Walker et al. 2018). Likewise, these data have been used to determine paleopolyploid events in the order, as well as major gene duplications in Cactaceae (Wang et al. 2018). Majure et al. (Majure LC, Baker MA, Puente-Martínez R, Salywon A, Fehlberg S, in preparation) are using plastome data to develop robust phylogenies throughout the Opuntioideae, as well as with several other groups in Cactaceae, including clades within Cactoideae (e.g., *Melocactus*). Several clades that have been very difficult to resolve previously are much better resolved and well-supported (i.e., relationships among the 3 tribes of Opuntioideae). There is great potential for resolving recalcitrant nodes in Cactaceae with whole plastome data, and furthermore, we are at an exciting time for developing comparative phylogenomic datasets with both plastid and nuclear data.

Future Perspectives

Unfortunately, taxonomic uncertainty not only negatively affects our understanding of evolutionary or biogeographic processes in the

cactus family, but can be an important threat to diversity of cacti, because red lists and governmental conservation policies urgently need rigorous and taxonomically informed species check lists (Duarte et al. 2014), otherwise resource allocation for conservation is likely to be inefficient and inappropriate. It is necessary to understand species evolutionary relationships and limits, as compared with close relatives, if we are to properly conserve them through targeted management strategies. From the point of view of conservation genetics, it is of central interest to know both the mechanisms of diversification and the persistence of cacti to predict their response to future climate changes, foreseeing that, if global warming continues, aridity will increase and deserts will possibly expand (IPCC 2007).

An improved sampling and more specific studies at the tribal and generic levels are needed to produce more stable taxonomic classifications and to understand complex evolutionary trends both in North and South American cacti. In particular, many South American groups are still underrepresented in molecular-based systematics studies. It is necessary to boost phylogeographic analyses of poorly studied groups, such as Opuntioideae, *Leuenbergeria* and *Pereskia*, as well as to pay special attention to the main centers of diversification on the continent, areas that also are often threatened from anthropogenic disturbances. Concerted effort of scientists in the Americas is necessary to improve collections, samples, and studies in species rich areas, particularly in the Central Andes and northern México, as well as the southern United States and the Caribbean. In addition, the insufficient phylogenetic resolution in several groups requires the development of new genetic markers, particularly taking advantage of novel sequencing techniques and analyses of genomic data.

There is great promise in incorporating modern sequencing technologies for building phylogenetic datasets to aid in the resolution of recalcitrant nodes in the Cactaceae tree of life. Recent analyses using both transcriptomic data (Walker et al. 2018) and hybrid enrichment of nuclear loci (Moore et al. 2017) have yielded robust datasets that are increasing our current knowledge of the phylogenetic relationships of cacti and relatives. For instance, it is now well-supported that the sister clade to Cactaceae is composed of a clade containing Anacampserotaceae + Portulacaceae. Likewise, phylogenomic analyses based on plastome data derived from genome skimming are proving to be effective in resolving difficult nodes within subfamily Opuntioideae (Majure LC, Baker MA, Puente-Martínez R, Salywon A, Fehlberg S, in preparation). A combination of different next generation sequencing methodologies and data types will revolutionize the way in which we determine evolutionary relationships in Cactaceae. Fortunately, hybrid enrichment, as well as transcriptome sequencing often yield high fractions or nearly entire portions of the plastome as well (Moore et al. 2017; Majure LC, Baker MA, Puente-Martínez R, Salywon A, Fehlberg S, in preparation) while providing hundreds of loci from the nuclear genome. This combination of data will be especially important for resolving reticulate evolution events throughout the Cactaceae and is essential for comparative phylogenetics among separate data types.

The cactus family includes several lineages possessing some of the fastest diversification rates in plants on earth. Understanding the phylogenetic relationships of these radiations, and using the phylogenies as frameworks to understand the evolution of ecological and morphological traits that fostered them would certainly lead to a better understanding of general evolutionary processes generating plant biodiversity. In particular, the cactus family is an ideal system to study the fundamentals of speciation mechanisms. This is due to the apparently common occurrence of antagonistic processes, such

as ecological and allopatric speciation in different lineages inhabiting the same geographic locations (e.g., *Mammillaria* and *Opuntia*). In addition, the prevalence of hybridization, gene introgression, and incomplete lineage sorting in the generation of isolation among cactus populations should be further studied. Due to the lability of reproductive strategies, the role of species interactions (i.e., pollination, seed dispersion) as selection forces toward genetic isolation needs to be more intensely studied in many cactus lineages.

The availability of a reference genome in the family opens the door to a tremendous variety of possibilities to study cactus evolution (Copetti et al. 2017) and to understand general evolutionary processes. Lineages such as the North American columnars are ideal for studying the role of hybridization and gene introgression in the evolution of species. The role of genome duplication and positive selection in shaping the adaptations of cacti can be studied now at the genomic level.

However, to achieve a more complete understanding of the ecological roles of cacti and the role of interactions in their evolution, there is need to increase the number of species studied from an ecological point of view beyond columnar cacti. Future phylogeographic studies should apply the methods based on next generation sequencing (NGS), such as restriction-site-associated DNA sequencing (RAD-seq), as well as integrate models of species distribution, ecological and climatic aspects with the support of robust phylogenies at the species level to better characterize genetic diversity, as well as to elucidate the demographic and biogeographic history and speciation processes that have resulted in one of the most diverse succulent families in the world.

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