BioOne COMPLETE

Molecular phylogeny and taxonomy of the genus Disocactus (Cactaceae), based on the DNA sequences of six chloroplast markers

Authors: Cruz, Miguel Ángel, Arias, Salvador, and Terrazas, Teresa

Source: Willdenowia, 46(1) : 145-164

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: https://doi.org/10.3372/wi.46.46112

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Willdenowia
Annals of the Botanic Garden and Botanical Museum Berlin-Dahlem

MIGUEL ÁNGEL CRUZ[1], SALVADOR ARIAS[1*] & TERESA TERRAZAS[2]

Molecular phylogeny and taxonomy of the genus *Disocactus* (*Cactaceae*), based on the DNA sequences of six chloroplast markers

Version of record first published online on 1 April 2016 ahead of inclusion in April 2016 issue.

Abstract: The genus *Disocactus* is native to Mexico and Central America and includes 11 species in four subgenera: *D.* subg. *Ackermannia*, *D.* subg. *Aporocactus, D.* subg. *Disocactus* and *D.* subg. *Nopalxochia*. Phylogenetic reconstruction was carried out with data from DNA sequences using the maximum parsimony and Bayesian inference criteria to explore the monophyly of the genus, its subgenera and its position within *Hylocereeae*. Six chloroplast markers (*matK*, *psbA-trnH*, *rpl16*, *trnL-F*, *trnQ-rps16* and *ycf1*) were sequenced in ten species of *Disocactus*, 17 representatives from the remaining genera of *Hylocereeae* and five members of outgroups (*Acanthocereus*, *Lemaireocereus* and *Pereskia*). Our phylogenetic analysis supports neither the monophyly of *Disocactus* as it is currently defined nor that of the subgenera. The clade *Disocactus* s.str. was recovered for 13 species, including *Epiphyllum anguliger*, *E. crenatum* and *E. lepidocarpum*. Three subclades were observed within this clade, and three well-supported sister-species relationships were recovered: *D. eichlamii* and *D. quezaltecus*; *D. biformis* and *D. nelsonii*; and *D. ackermannii* and *D. phyllanthoides*. *Disocactus speciosus* subsp. *aurantiacus* was not recovered in the clade of *D. speciosus*. *Epiphyllum* and *Pseudorhipsalis* are identified as sister clades of *Disocactus*. Based on the obtained results, a new circumscription for *Disocactus* is proposed.

Key words: *Aporocactus*, Bayesian inference, *Cactaceae*, chloroplast DNA, *Disocactus*, *Epiphyllinae*, *Epiphyllum*, epiphytic cacti, *Hylocereeae*, *Hylocereinae*, maximum parsimony, molecular phylogeny, *Pseudorhipsalis*, taxonomy

Article history: Received 14 October 2015; peer-review completed 25 November 2015; received in revised form 30 January 2016; accepted for publication 3 February 2016.

Citation: Cruz M. Á, Arias S. & Terrazas T. 2016: Molecular phylogeny and taxonomy of the genus *Disocactus* (*Cactaceae*), based on the DNA sequences of six chloroplast markers. – Willdenowia 46: 145–164. doi: http://dx.doi.org/10.3372/wi.46.46112

Introduction

The genus *Disocactus* Lindl. (*Cactaceae*, *Cactoideae*, *Hylocereeae*) includes species with terrestrial, climbing and epiphytic growth forms. These species exhibit diurnal, brightly coloured flowers and may be found from central Mexico to Central America (Barthlott & al. 2015). However, as in other groups of cacti, the delimitation of the genus has been modified over the years, as a reflection of an oscillating taxonomic history. In 1845, Lindley proposed *Disocactus* as a monotypic genus for *Cereus biformis* Lindl., a species native to Guatemala, with morphological characteristics that were sufficiently distinct from other cacti, such as flat stems and small pink flowers with few perianth segments. Later, Britton & Rose (1923) added another species, which was also from Guatemala (Table 1). In subsequent years, four species were added to *Disocactus*: two corresponded to taxa that were transferred from other genera (*Rhipsalis* Gaertn. and *Pseudorhipsalis* Britton & Rose), and two represented monotypic genera (*Bonifazia* Standl. & Steyerm. and *Chiapasia* Britton & Rose). Therefore, Bravo-Hollis (1978) recognized six species in *Disocactus* (Table 1) based on the classification of Buxbaum (1958).

In the 1990s, the genus *Disocactus* underwent two major modifications in terms of its delimitation and the

1 Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, México D.F. 04510, Mexico; *e-mail: sarias@ib.unam.mx (author for correspondence).
2 Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, México D.F. 04510, Mexico.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Table 1. Summary of specific placement in *Disocactus* and other related genera based on previous taxonomic contributions.

Lindley (1845)	Britton & Rose (1923)	Bravo-Hollis (1978)	Barthlott (1991a, b)	Kimnach (1993)	Hunt & al. (2006)
Disocactus	***Disocactus***	***Disocactus***	***Disocactus***	***Disocactus***	***Disocactus***
D. biformis	*D. biformis*	*D. biformis*	*D. ackermannii*	*D. acuminatus*	*D. ackermannii*
	D. eichlamii	*D. eichlamii*	*D. amazonicus*	*D. alatus*	*D. biformis*
		D. macranthus	*D. auranticus*	*D. amazonicus*	*D. eichlamii*
	Aporocactus	*D. nelsonii*	*D. biformis*	*D. biformis*	*D. flagelliformis*
	A. conzattii	*D. ramulosus*	*D. cinnabarinus*	*D. eichlamii*	*D. macdougalli*
	A. flagelliformis	*D. quezaltecus*	*D. eichlamii*	*D. himantocladus*	*D. macranthus*
	A. flagriformis		*D. flagelliformis*	*D. horichii*	*D. martianus*
	A. leptophis	***Aporocactus***	*D. macdougallii*	*D. lankesteri*	*D. nelsonii*
	A. martianus	*A. conzattii*	*D. macranthus*	*D. macranthus*	*D. phyllanthoides*
		A. flagelliformis	*D. martianus*	*D. nelsonii*	*D. quezaltecus*
	Chiapasia	*A. flagriformis*	*D. nelsonii*	*D. quezaltecus*	*D. speciosus*
	C. nelsonii	*A. leptophis*	*D. phyllanthoides*	*D. ramulosus*	
		A. martianus	*D. quezaltecus*		***Pseudorhipsalis***
	Heliocereus		*D. schrankii*	***Aporocactus***	*P. acuminata*
	H. amecamensis	***Heliocereus***	*D. speciosus*	*A. flagelliformis*	*P. alata*
	H. cinnabarinus	*H. cinnabarinus*		*A. martianus*	*P. amazonica*
	H. elegantissimus	*H. elegantissimus*	***Pseudorhipsalis***		*P. himantoclada*
	H. schrankii	*H. speciosus*	*P. horichii*	***Heliocereus***	*P. lankesteri*
			P. lankesteri	*H. aurantiacus*	*P. ramulosa*
	Nopalxochia	***Nopalxochia***	*P. ramulosa*	*H. cinnabarinus*	
	N. phyllanthoides	*N. ackermannii*		*H. schrankii*	
		N. conzattianum		*H. speciosus*	
	Pseudorhipsalis	*N. macdougallii*			
	P. alata	*N. phyllanthoides*		***Nopalxochia***	
	P. himantoclada			*N. ackermannii*	
				N. macdougallii	
	Wittia			*N. phyllanthoides*	
	W. amazonica				
	W. panamensis				

number of included species. Barthlott (1991a) modified the delimitation by proposing a more inclusive genus that consisted of species that share a distribution in Central America and are characterized by diurnal flowering. Barthlott (1991a) transferred the genera *Aporocactus* Lem., *Heliocereus* Britton & Rose, *Nopalxochia* Britton & Rose and *Wittia* K. Schum. to *Disocactus*, which consisted of a total of 15 species. The diagnostic characteristics of the genus recognized by Lindley (1845) were lost (e.g. flat or ribbed stems, stems with or without spines, large or small flowers with intense or white colouration, a thick pericarpel with ribs and spines or one that is thin and bare), which generated uncertainty about the relationship with *Pseudorhipsalis*. For example, Barthlott (1991a, b) recognized *Pseudorhipsalis* as a separate genus based on its small white flowers and the smaller size of its pollen grains. Kimnach (1993) restricted the limit of the genus and believed that the species included in *Disocactus* should exhibit a flower with a narrow receptacle without conspicuous podaria and without spines or hairs. Therefore, he included *Pseudorhipsalis* because he believed that its morphological characteristics were insufficient to maintain it in a separate genus (Table 1).

The most recent classification by Hunt & al. (2006) revisits Barthlott's proposal (1991a, b) and the taxonomic changes made by Bauer (2003). These authors recognized 11 species of *Disocactus* that exhibit intensely coloured flowers (yellow to red) and diurnal flowering. They transferred *D. amazonicus* (K. Schum.) D. R. Hunt to the genus *Pseudorhipsalis*, and certain species or varieties were classified as subspecies (Bauer 2003).

Various subgenera or sections have been recognized within *Disocactus.* Barthlott (1991a) grouped the species in *D.* subg. *Ackermannia* (K. Schum.) Barthlott, *D.* subg. *Aporocactus* (Lem.) Barthlott, *D.* subg. *Disocactus*, *D.* subg. *Nopalxochia* (Britton & Rose) Barthlott and *D.* subg. *Wittia* Barthlott (Table 2; Fig. 1). Among these subgenera, *D.* subg. *Disocactus* and *D.* subg. *Wittia* are characterized by flat stems and a bare or nearly bare pericarpel; the difference between these two subgenera is the size of the flower. In *D.* subg. *Wittia* the flowers are short in length (2.5–3 cm) and the stamens are inserted in two series, whereas in *D.* subg. *Disocactus* the flowers are larger (3–6 cm), and the stamens are inserted in one series. In the remaining subgenera, the pericarpel exhibits spines, scales or visible areoles. In particular, *D.* subg. *Aporocactus* exhibits stems with 8–11 ribs, areoles

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Table 2. Summary of infrageneric groups in *Disocactus* according to recent taxonomic studies.

Barthlott (1991a)	Kimnach (1993)	Hunt & al. (2006)
Subgenera:	Sections:	Subgenera:
Ackermannia	***Disocactus***	***Ackermannia***
D. ackermannii	*D. biformis*	*D. ackermannii*
D. aurantiacus	*D. eichlamii*	*D. macdougallii*
D. cinnabarinus	*D. nelsonii*	*D. speciosus*
D. schrankii	*D. quezaltecus*	
D. speciosus		***Aporocactus***
	Macranthi	*D. flagelliformis*
Aporocactus	*D. macranthus*	*D. martianus*
D. flagelliformis		
D. martianus	***Pseudorhipsalis***	***Disocactus***
	D. acuminatus	*D. biformis*
Disocactus	*D. alatus*	*D. eichlamii*
D. biformis	*D. himantocladus*	*D. macranthus*
D. eichlamii	*D. horichii*	*D. nelsonii*
D. macranthus	*D. lankesteri*	*D. quezaltecus*
D. nelsonii	*D. ramulosus*	
D. quezaltecus		***Nopalxochia***
	Wittia	*D. phyllanthoides*
Nopalxochia	*D. amazonicus*	
D. macdougallii		
D. phyllanthoides		
Wittia		
D. amazonicus		

with spines and slightly zygomorphic flowers measuring 7–10 cm. Conversely, the stems in *D.* subg. *Ackermannia* have fewer ribs (3–7), are rarely flat, and exhibit areoles with spines and actinomorphic or slightly zygomorphic flowers measuring 8–20 cm. *Disocactus* subg. *Nopalxochia* displays flat stems, areoles with or without spines, and pale pink actinomorphic flowers measuring 8–10 cm. Kimnach (1993) recognized four sections: *D.* sect. *Disocactus*, *D.* sect. *Macranthi* Kimnach, *D.* sect. *Pseudorhipsalis* (Britton & Rose) Kimnach and *D.* sect. *Wittia* Kimnach. Both *D.* sect. *Disocactus* and *D.* sect. *Wittia* exhibit erect or expanded tepals up to the middle section of the flower and the stamens are inserted in two series. However, the apex of the tepals in *D.* sect. *Wittia* is tinted blue and the stamens are nearly exerted, with thin filaments, whereas the tepals in *D.* sect. *Disocactus* range from pink to purple and the stamens are exerted, with thick filaments. Conversely, *D.* sect. *Macranthi* and *D.* sect. *Pseudorhipsalis* share tepals that are expanded from the base, with stamens inserted in a series. Nevertheless, *D.* sect. *Macranthi* may display more than one flower (5–6 cm) per areole, and the nectary occupies two-thirds of the floral length, whereas in *D.* sect. *Pseudorhipsalis* the flowers are small (less than 3 cm), with one flower emerging per areole and a small nectary (one-third of the floral length). Finally, Hunt & al. (2006) recognized four of the subgenera (*D.* subg. *Ackermannia*, *D.* subg. *Aporocactus*, *D.* subg. *Disocactus* and *D.* subg. *Nopalxochia*) proposed by Barthlott (1991a) (Table 2; Fig. 1).

The relationship between *Disocactus* and the remainder of cacti from the tribe *Hylocereeae* has not yet been examined in depth. Britton & Rose (1923) proposed that *Disocactus* and *Epiphyllum* Haw., along with seven other genera (*Chiapasia*, *Eccremocactus* Britton & Rose, *Epiphyllanthus* A. Berger, *Nopalxochia*, *Schlumbergera* Lem., *Wittia* and *Zygocactus* K. Schum.), comprise the subtribe *Epiphyllinae*, which, according to these authors, is characterized by the presence of phylloclades (with a cylindrical base) that are primarily free of spines and develop regular flowers. However, Buxbaum (1958) proposed that *Disocactus*, *Pseudorhipsalis* and five other genera (*Bonifazia*, *Chiapasia*, *Lobeira* Alexander, *Nopalxochia* and *Wittia*) comprise the subtribe *Disocactinae*, which is characterized by phylloclades that branch from cylindrical stems, brightly coloured flowers with a diurnal anthesis, a scaly pericarpel that is nearly bare and a generally scaly receptacle tube. Under the most recent classification (Hunt & al. 2006), *Disocactus* is a member of the tribe *Hylocereeae*, which also includes *Epiphyllum*, *Hylocereus* (A. Berger) Britton & Rose, *Pseudorhipsalis*, *Selenicereus* (A. Berger) Britton & Rose and *Weberocereus* Britton & Rose. The results of recent phylogenetic studies are partial and ambiguous (Nyffeler 2002; Arias & al. 2005; Bárcenas & al. 2011; Hernández-Hernández & al. 2011). However, all agree that *Disocactus* is resolved within a clade that corresponds to the tribe *Hylocereeae*, but its exact position is uncertain.

Recently, phylogenetic analyses based on DNA sequences have tested the relationships between the taxa and, consequently, their actual delimitations. Increasing evidence based primarily on chloroplast molecular markers indicates that some tribes and genera that had been delimited using only morphological data are not monophyletic, such as *Grusonia* Rchb. f. ex Britton & Rose (Griffith 2002), *Pachycereus* (A. Berger) Britton & Rose (Arias & al. 2003), *Mammillaria* Haw. (Butterworth & Wallace 2004), *Peniocereus* (A. Berger) Britton & Rose (Arias & al. 2005), *Pereskia* Mill. (Edwards & al. 2005), *Rebutia* K. Schum. (Ritz & al. 2007), *Pfeiffera* Salm-Dyck (Korotkova & al. 2010), *Echinopsis* Zucc. (Schlumpberger & Renner 2012), *Ferocactus* Britton & Rose and *Turbinicarpus* Buxb. & Backeb. (Vázquez-Sánchez & al. 2013). Phylogenetic analyses including sequences from *Disocactus* have been unable to clearly establish the monophyly of the genus or its relationships with the other members of *Hylocereeae* because these analyses were not intended to discern the phylogenetic relationships of this genus (Nyffeler 2002: three spp.; Bárcenas & al. 2011: ten spp.; Hernández-Hernández & al. 2011: eight spp.), and sample sizes were insufficient. In the topologies produced by Nyffeler (2002) and Bárcenas & al. (2011), only one sister relationship was recovered between *Hylocereus* and *Selenicereus,* whereas *Disocactus* formed part of the polytomy of the tribe *Hylocereeae*. However, in the analyses performed by Hernández-Hernández & al. (2011), *Epiphyllum* diverged early within *Hylocereeae*, whereas

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use



Fig. 1. Selected species of *Disocactus* and related genera – A: *Disocactus ackermannii*, Mexico, Oaxaca, *M. Á. Cruz 10* (MEXU); B: *D. speciosus* subsp. *heterodoxus*, Mexico, Chiapas, *S. Arias 1666* (MEXU); C: *D. aurantiacus* El Salvador, Santa Ana, *G. Cerén 2580* (MHES); D: *D. speciosus* subsp. *speciosus*, Mexico, cultivated at Jardín Botánico UNAM; E: *D. speciosus* subsp. *bierianus*, Mexico, Guerrero, *G. A. Salazar* 6564B (MEXU); F: *D. crenatus* subsp. *crenatus*, Guatemala, Quetzaltenango, *S. Arias 1178* (MEXU); G: *D. macranthus*, Mexico, Veracruz, *M. Á. Cruz 7* (MEXU); H: *D. biformis*, Guatemala, Sacatepequez, *M. Veliz 23600* (BIGU); I: *D. phyllanthoides*, Mexico, Veracruz, *S. Arias* 2201 (MEXU); J: *D. lepidocarpus*, Costa Rica, Alajuela, *B. Hammel 25624* (INB); K: *Pseudorhipsalis amazonica*, Costa Rica, Guanacaste, *B. Hammel 24524* (INB); L: *Aporocactus flagelliformis*, Mexico, Veracruz, *M. Á. Cruz 1* (MEXU).

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Disocactus was a sister to the clade that includes the genera *Hylocereus*, *Selenicereus* and *Weberocereus*.

Due to the above issues and the discrepancy between taxonomists regarding the number of species within *Disocactus* (Table 1) caused by poor morphological delimitation of the genus, the question arises of whether *Disocactus* is monophyletic. The objectives of this study were threefold: (1) to carry out a phylogenetic reconstruction of the genus *Disocactus* using six chloroplast molecular markers (*matK*, *psbA-trnH*, *rpl16*, *trnL-F*, *trnQ-rps16* and *ycf1*) to evaluate whether *Disocactus* and the subgenera recognized by Hunt & al. (2006; *D.* subg. *Ackermannia*, *D.* subg. *Aporocactus*, *D.* subg. *Disocactus* and *D.* subg. *Nopalxochia*) are monophyletic groups; (2) to understand the position of *Disocactus* within *Hylocereeae*; and (3) to generate a taxonomic proposal for *Disocactus* based on these results.

Material and methods

Taxon sampling

The present study included ten of the 11 species of *Disocactus* according to the classification proposed by Hunt & al. (2006) and incorporated a comprehensive sampling of ten terminals for five subspecies of *D. speciosus* (Cav.) Barthlott and two for *D. ackermannii* (Haw.) Ralf Bauer, *D. biformis* (Lindl.) Lindl., *D. macranthus* (Alexander) Kimnach & Hutchinson and *D. phyllanthoides* (DC.) Barthlott. Representatives from *Epiphyllum*, *Hylocereus, Pseudorhipsalis*, *Selenicereus* and *Weberocereus* were included to test the monophyly of the genus because they were recovered as part of a single clade in previous phylogenies (Nyffeler 2002). Outgroups were sampled based on previous phylogenetic analyses of the family (Nyffeler 2002; Arias & al. 2005; Hernández-Hernández & al. 2011) and included representative species of *Acanthocereus* Britton & Rose (Lodé 2013), *Lemaireocereus* Britton & Rose and *Pereskia*. This study involved 315 new sequences; their voucher information and GenBank accession numbers are listed in the Appendix.

Amplification and sequencing

Phylogenetic reconstructions for *Cactaceae* have mainly employed chloroplast molecular markers. Nuclear markers suitable for cacti have yet to be developed because the existing nuclear markers provide a lower number of informative sites compared with chloroplast markers, and they do not exhibit polymorphism as has been shown for ITS in other groups of cacti, e.g. *Lophocereus* (A. Berger) Britton & Rose (Hartmann & al. 2001), *Mammillaria* (Harpke & Peterson 2006), *Hylocereus* and *Selenicereus* (Plume & al. 2013). A combination of different chloroplast markers is necessary to increase the resolution of the phylogeny (Korotkova & al. 2010). Within the diverse chloroplast markers, it has been proposed that introns outperform spacers and coding regions in terms of phylogenetic utility (introns > spacer sequences > coding regions) (Korotkova & al. 2011). Six molecular markers were used: two introns (*rpl16* and *trnL-F*), two spacers (*psbA-trnH* and *trnQ-rps16*) and two coding regions (*matK* and *ycf1*).

Collected samples were dried with silica gel and stored at −20 °C. Total DNA was extracted using the EZ-10 Spin Column Plant Genomic DNA Minipreps kit (Bio Basic Inc., Ontario, Canada) according to the manufacturer's protocol, but the incubation time in tissue lysis buffer was modified to 120 minutes. Total DNA was stored at −20 °C. Specific regions were amplified using a standard polymerase chain reaction (PCR) protocol in a total volume of 25 µL containing 2.5 µL of 10× buffer, 0.5 µL of dNTPs (10 mM), 1 µL of BSA, 0.3 µL of each primer (10 mM), 0.75 µL of $MgCl_2$, 0.125 µL of Taq, 19.125 µL of DEPC-treated water and 0.75 µL of DNA. The *matK* exon was amplified using the primers matK_3F_kim and matK_1R_kim (CBOL Plant Working Group, 2009) by initially denaturing the DNA for 2 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 40 s at 48 °C and 40 s at 72 °C, with a final extension of 7 min at 72 °C. The *rpl16* intron was amplified using the primers rpl161F and rpl163R (Hernández-Hernández & al. 2011) by initially denaturing the DNA for 5 min at 94 °C, followed by 26 cycles of 1 min at 94 °C, 50 s at 55 °C and 2 min at 72 ºC, with a final extension of 4 min at 72 °C. The *trnL-trnF* region was amplified using the primers "C" and "F" designed by Taberlet & al. (1991). The PCR programme employed for this region consisted of 2 min at 94 °C, 29 cycles of 30 s at 94 °C, 30 s at 52 °C and 1 min at 72 ºC, and a final extension of 7 min at 72 °C. The *psbA-trnH* intergenic spacer was amplified using the primers psbA (Sang & al. 1997) and trnH (Tate & Simpson 2003), with initial denaturation of the DNA for 2 min at 94 °C, followed by 29 cycles of 30 s at 94 °C, 30 s at 52 °C and 1 min at 72 °C, and a final extension of 7 min at 72 °C. The *trnQ-rps16* intergenic spacer was amplified using the primers trnQ2 (Korotkova & al. 2010) and rps16x1 (Shaw & al. 2007), with initial denaturation of the DNA for 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 1 min at 55 °C and 1 min at 72 °C, and a final extension of 10 min at 72 °C. The *ycf1* region was amplified using the primers ycf1-4182F and ycf1-5248R (Franck & al. 2012), with initial denaturation of the DNA for 3 min at 94 °C, followed for 35 cycles of 1 min at 94 °C, 1 min at 50 °C and 2 min at 72 °C, and a final extension of 5 min at 72 °C. The unpurified PCR products were sequenced at the University of Washington (UW-High Throughput Genomics Center, http://www.htseq.org/). All regions were sequenced using amplification primers. Sequence assembly and editing was carried out using Sequencher 4.8 (GeneCode).

Phylogenetic analysis

The sequences were aligned using MAFFT (Katoh & al. 2002), and the alignments were then manually inspected. In all of the alignments, the extremes were eliminated

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Table 3. Phylogenetic analysis characteristics and numerical results for each sequence data set and the combined data set.

	rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*matK*	*ycfl*	all regions
No. of characters in matrix	1210	1129	543	659	814	1378	5853
% of A+T	70	69	70	69	66	69	69
% of G+C	30	31	30	31	34	31	31
No. of variable characters	128	97	53	61	33	353	725
No. of parsimony-uninformative characters	88	54	30	39	26	151	388
No. of parsimony-informative characters	40	43	23	22	7	202	411
No. of hotspots	3	7	4	4	0	0	18
No. of inversions	1	0	0	0	1	0	2
No. of coded indels	21	30	24	13	0	34	122

due to the presence of ambiguities. Insertions and deletions (indel) were coded following the simple indel coding method suggested by Simmons & Ochoterena (2000) using the Indel Coder option of SeqState v. 1.40 (Müller 2005). Maximum parsimony (MP) phylogenetic analyses were carried out for each of the markers to demonstrate their congruence (trees with similar topologies) and concatenate them in a single matrix and to identify markers that maximize the resolution of the tree. MP phylogenetic analysis with the concatenated marker matrix without hotspots (highly variable regions) was carried out using PAUP (Swofford, 2002). A heuristic search with 15 000 replicates was conducted; the tree bisection and reconnection (TBR) branch-swapping algorithm was employed, and *Pereskia lychnidiflora* DC. was designated as an outgroup. A second MP phylogenetic analysis was performed with the concatenated marker matrix and coded indels and without hotspots using the above parameters. For branch support, 10 000 jackknife (JK) replicates and 10 000 bootstrap (BS) replicates were employed. Bayesian inference (BI) analysis was carried out using MrBayes 3.2 (Huelsenbeck & Ronquist 2001). The data were partitioned, and each partition used GTR+I+G as a substitution model, pursuant to the results obtained with jModeltest2 (Darriba & al. 2012). Two independent runs were conducted with four chains each, and six million generations were run, with the chains sampled every 1000 generations. Chain convergence was observed using Tracer (Rambaut & Drummond 2009). The first 20 % of trees were discarded as the burn-in. The remaining trees were then used to build a 50 % majority rule consensus tree. The alignment is available at http://purl.org/phylo/treebase/phylows/study/TB2:S18804.

Results

Six markers were amplified for all ingroup and outgroup species, with two exceptions: *rpl16* was not obtained for *Epiphyllum lepidocarpum* (F. A. C. Weber) Britton & Rose, and *ycf1* was not obtained for *E. pumilum* Britton & Rose and *E.thomasianum* (K. Schum.) Britton & Rose. The aligned matrix of the six concatenated plastid sequences and the coded indels was 5853 bp in length, only 14 % of which consisted of variable sites, and half of those variable sites were parsimony-informative (PI) sites (411). The cytosine-guanine content was lower than the adenine-thymine content. Four markers presented regions that were difficult to align; these regions corresponded to polyA or polyT sequences. In the *rpl16* intron, an inversion was identified in the sequences of *Disocactus flagelliformis* (L.) Barthlott, *D. martianus* (Zucc.) Barthlott and all of the sequences of *Acanthocereus*, *Hylocereus*, *Lemaireocereus*, *Peniocereus*, *Selenicereus* and *Weberocereus*. The *ycf1* region contributed 41 % of the observed indels, whereas the *psbA-trnH* spacer contributed 29 % of the indels, despite being a short region. This characteristic is directly related to the disparity in the length of the marker sequence (Table 3). The results obtained from the MP and BI analyses were congruent; the same number of clades was recovered with high branch support. MP and BI yielded trees with similar, although not identical, topologies, due to the degree of resolution that each method provides.

Using the MP criteria, 57 equally parsimonious trees were obtained, with a length (L) of 1296 steps, showing a consistency index (CI) of 0.72 and a rescaled consistency (RC) index of 0.57. The strict consensus tree recovered three main groups. The H1 group includes clades D (95 % JK, 95 % BS), P (100 % JK, 100 % BS) and E (100 % JK, 100 % BS), whereas the H2 group consists of clades A (100 % JK, 100 % BS) and HSW (100 % JK, 100 % BS), and the H3 group includes three species of *Acanthocereus* (100 % JK, 100 % BS) (Fig. 2). Clade D defines the genus *Disocactus* and contains eight species previously classified therein plus three species of *Epiphyllum*, which are recovered in two subclades with moderate or no support. Subclade 3D (97 % JK, 86 % BS) is sister to the remaining included species, whereas subclade 2D (100 % JK, 100 % BS) forms part of the second divergent event in the genus. Subclade 1D (– JK, – BS) is the last to diverge and includes four species. *Epiphyllum anguliger* (Lem.) H. P. Kelsey & Dayton was recovered as sister species of sub-

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

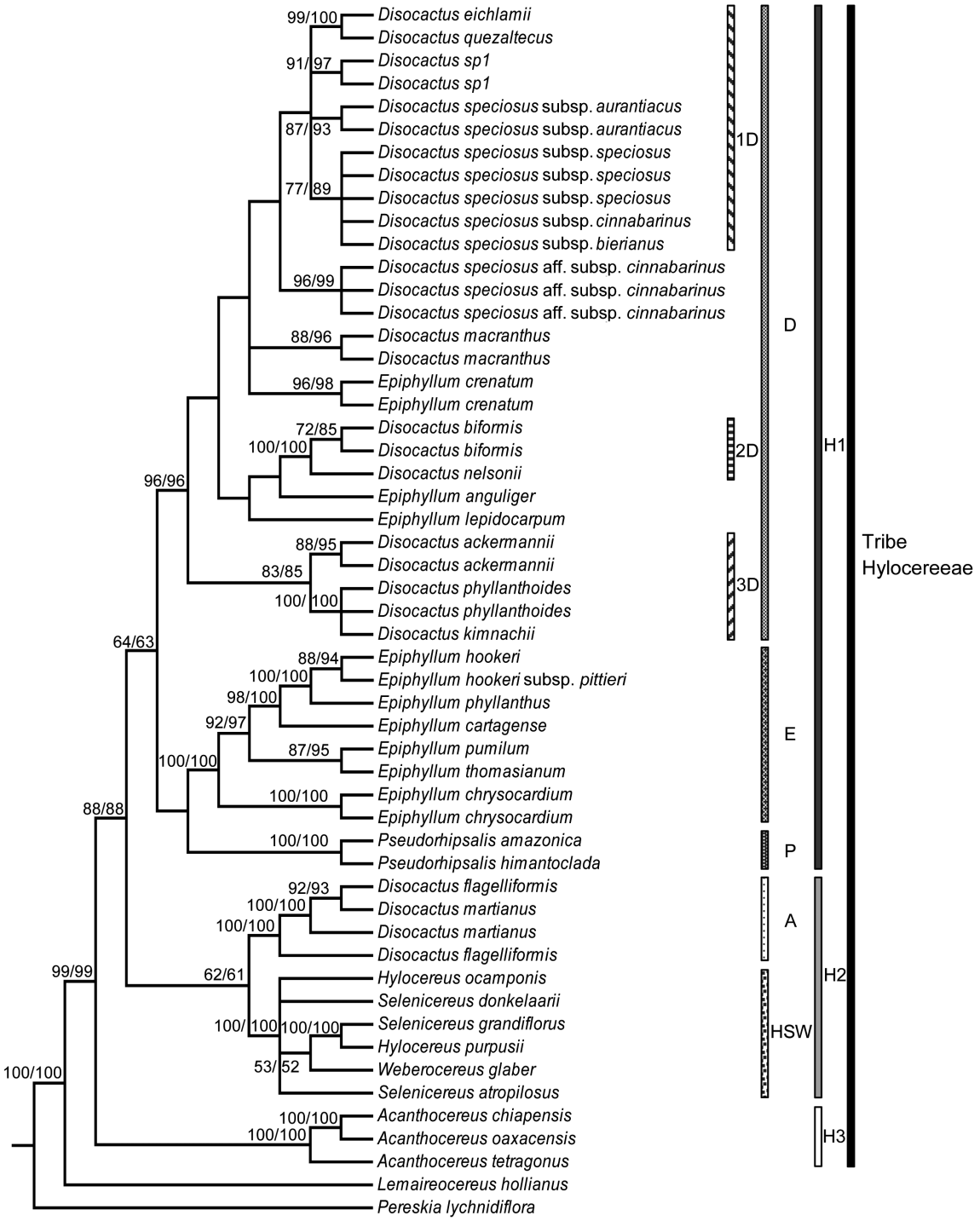


Fig. 2. Strict consensus tree (maximum parsimony analysis) of the combined cpDNA sequence data. Numbers given at branches are jackknife (JK) and bootstrap (BS) support values. Bars and acronyms indicate the main groups discussed in the text.

clade 2D and *E. lepidocarpum* was recovered as a sister taxon to this relationship; however, both hypotheses lack support. *Epiphyllum crenatum* (Lindl.) G. Don is nested between the previous relationship and subclade 1D. *Disocactus speciosus* (ten terminals) was the only species recovered as non-monophyletic. Clade P, which includes two species of *Pseudorhipsalis*, and clade E, which consists of six species of *Epiphyllum* whose relationships exhibit moderate-to-high support, form a sister clade to *Disocactus* (Fig. 2).

The BI analysis showed that the H1, H2 and H3 groups were recovered with high support (Fig. 3). Two subclades were recovered in addition to the three subclades observed in the MP analysis. Subclade 4D consists of *Disocactus macranthus* and *Epiphyllum crenatum*, with 0.63 PP support. Subclade 5D includes *E. anguliger*

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

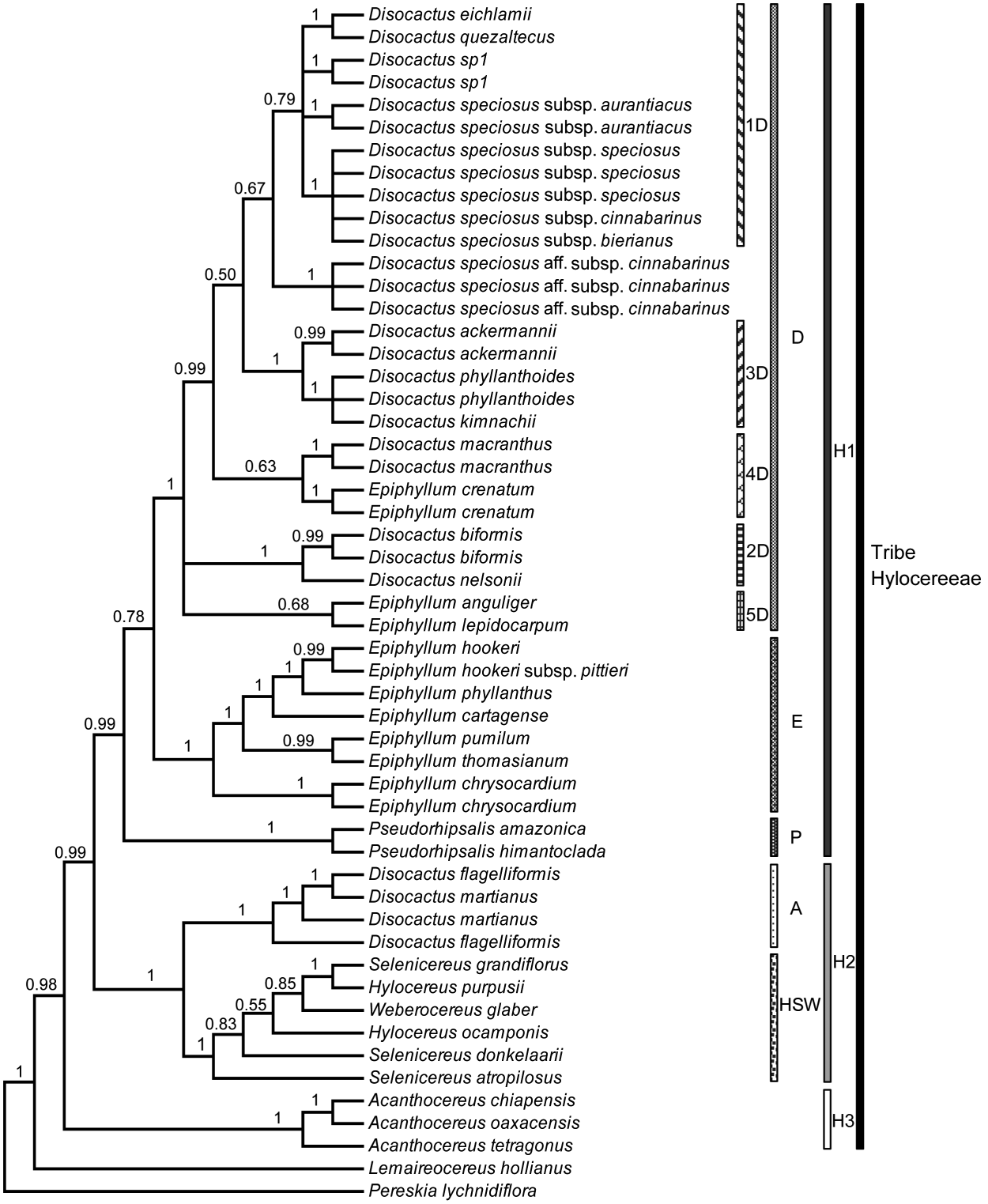


Fig. 3. Majority rule consensus tree (Bayesian MCMC analysis) of the combined cpDNA sequence data. Numbers given at branches are Bayesian posterior probability (PP) support values. Bars and acronyms indicate the main groups discussed in the text.

and *E. lepidocarpum*, with a support of 0.68 PP, and forms part of the basal polytomy along with subclade 2D. Unlike MP, subclade 3D in the BI analysis is recovered between subclades 1D and 4D. In addition, within the H1 group, clade E is sister to clade D (0.78 PP) and clade P is sister to both clades E and D (0.99 PP).

The H1 group in both reconstructions is supported by four molecular synapomorphies, three of which correspond to substitutions and one to an indel. Clade D is defined by a single combination of seven synapomorphies, six of which are substitutions (*matK*, *trnQ-rps16*, *rpl16* and *ycf1*) and one is an indel (*rpl16*). Moreover,

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Table 4. Molecular characters that support clades.

	No. of characters	Character type, marker and position in alignment
Group *Epiphyllinae*	4	substitutions: *trnQ-rps16* (1066), *rpl16* (3795), *ycf1* (5259); 1 indel: *trnL-F* (2104–2116)
Clade *Disocactus* s.str.	7	substitutions: *matK* (706), *trnQ-rps16* (934), *trnL-F* (2438), *rpl16* (3562, 3861), *ycf1* (4434); 1 indel: *rpl16* (3866–3870)
Clade *Epiphyllum*	12	substitutions: *trnQ-rps16* (941), *psbA-trnH* (1602, 1735, 1798), *trnL-F* (2987), *ycf1* (4600, 5424, 5470, 5526); 3 indels: *psbA-trnH* (1760–1789), *trnL-F* (3115–3122), *rpl16* (3809–4162)
Clade *Pseudorhipsalis*	23	substitutions: *matK* (214), *trnQ-rps16* (930, 1431), *psbA-trnH* (1867), *trnL-F* (2471, 2767), *rpl16* (3212, 3359, 3554, 3881, 3911), *ycf1* (4790, 4923, 5272, 5339, 5430, 5460, 5587, 5643, 5645); 3 indels: *rpl16* (3880–3920), *ycf1* (4456–4458, 5049–5054)
Disocactus eichlamii *Disocactus quezaltecus*	6	substitutions: *matK* (241), *trnQ-rps16* (1053), *psbA-trnH* (1947), *rpl16* (4064); 2 indels: *psbA-trnH* (1916–1925, 1926–1946)
Disocactus biformis *Disocactus nelsonii*	4	substitutions: *ycf1* (4884); 3 indels: *psbA-trnH* (1654–1827) *trnL-F* (2128–2132, 3057–3068)
Disocactus speciosus subsp. *aurantiacus*	3	1 substitution: *ycf1* (4757); 2 indel: *psbA-trnH* (1749–1958), *rpl16* (3386–3386)
Disocactus heterodoxus	2	indel: *psbA-trnH* (1774–1838), *ycf1* (4658–4678)

12 synapomorphies support clade E (*Epiphyllum*), nine of which are substitutions and three are indels; the largest number of synapomorphies occurs in clade P (*Pseudorhipsalis*), 20 of which are substitutions and three are indels (Table 4).

Discussion

Our phylogenetic analyses did not recover *Disocactus* or the subgenera recognized by Hunt & al. (2006) as monophyletic. One group of 11 species of *Disocactus* belongs to the H1 group, which includes *D. biformis* (type species) and is defined here as *Disocactus* s.str., whereas the two analysed species of *D.* subg. *Aporocactus* are nested in the H2 group. Therefore, the genus *Aporocactus* should be re-established, and *Disocactus* must be re-circumscribed (see below). Based on its placement in group H1, *Disocactus* is more closely related to *Epiphyllum* and *Pseudorhipsalis* than to the remaining genera of *Hylocereeae*, as discussed below.

The contribution of the the markers and coded indels

The region *matK* provided the lowest number of informative sites. This region was also less variable in other groups of *Cactaceae*, such as in the genus *Pfeiffera* (Korotkova & al. 2010), the tribe *Rhipsalideae* (Korotkova & al. 2011) and the tribe *Cacteae* (Vázquez-Sánchez & al. 2013). Two sites supported the relationships at the genus level (214 for the genus *Pseudorhipsalis*, 706 for the genus *Disocactus*; Table 4), which shows that *matK* may, to a lesser degree, contribute information to resolve relationships at the genus level and not only at the family level. Conversely, markers with high evolutionary rates, such as the introns *rpl16* and *trnL-F* and the spacer regions *psbA-trnH* and *trnQ-rps16* (Borsch & Quandt 2009; Korotkova & al. 2011), provided 31 % of informative sites and allowed the establishment of relationships at primarily the genus and species levels in the genera *Epiphyllum* and *Pseudorhipsalis*. The region *ycf1* contributed 49 % of informative sites. This region is also highly variable in other groups of cacti, such as *Opuntia* (L.) Mill. (Majure & al. 2012), *Harrisia* Britton (Franck & al. 2013) and *Copiapoa* Britton & Rose (Larridon & al. 2015). The region *ycf1* produced the tree with the highest resolution, supporting the relationships at the genus and species levels; however, it did not individually resolve the relationships between close species. Moreover, the coded indels provided 13 % of the PI sites. The incorporation of coded indels in phylogenetic reconstructions is a frequent practice in *Cactaceae* (e.g. Nyffeler 2002; Butterworth & Wallace 2004; Korotkova & al. 2010, 2011; Calvente & al. 2011a, b; Franck & al. 2013; Vázquez-Sánchez & al. 2013; Bonatelli & al. 2014). Furthermore, their inclusion improved the resolution and support values for the tree compared with the tree that did not include the coded indels (data not shown), as previously suggested (Simmons & al. 2001).

Genus *Disocactus* s.str.

Based on our results, *Disocactus* consists of 13 known species, including *Epiphyllum anguliger*, *E. crenatum* and *E. lepidocarpum* (Fig. 2, 3). None of the three subgenera recognized by Hunt & al. (2006) (Table 2) was recovered; however, three subclades were recognized with some support and three sister species relationships were observed. Subclade 1D includes the relationship between *D. eichlamii* (Weing.) Britton & Rose and *D.*

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

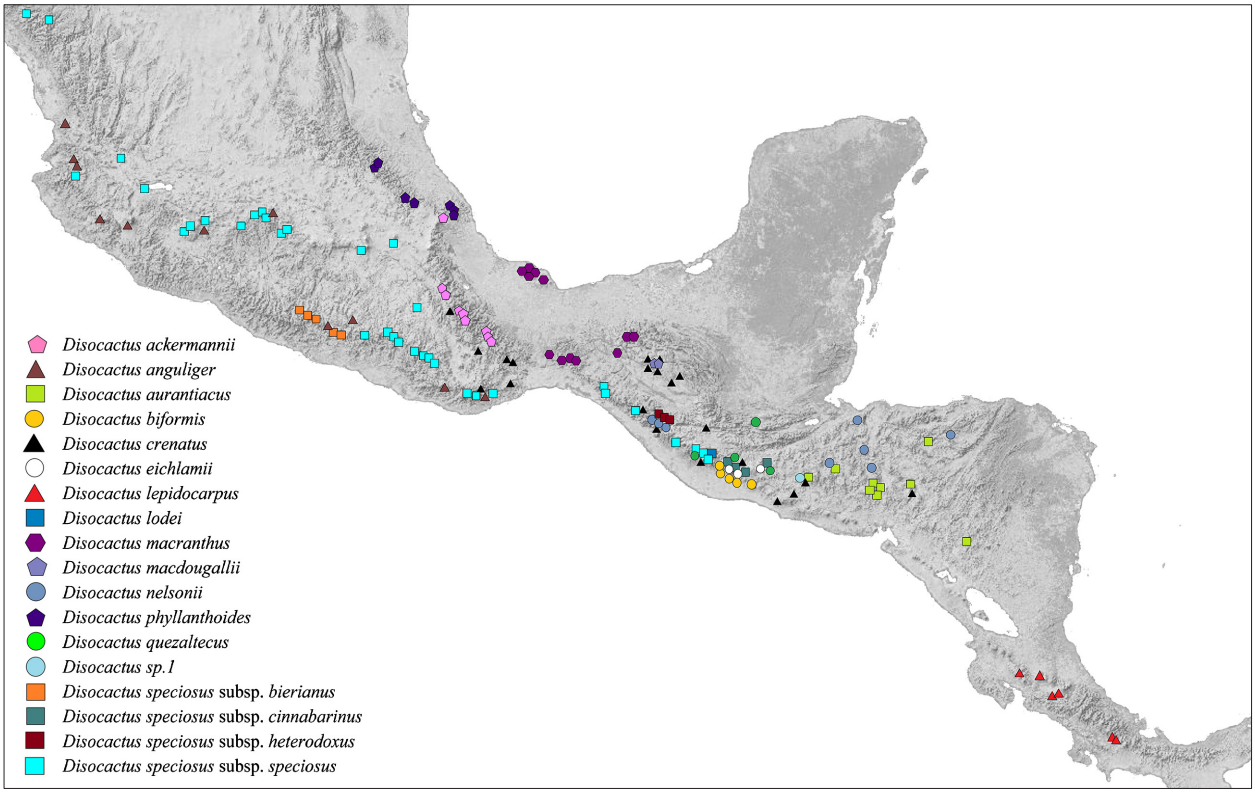


Fig. 4. Geographical distribution of *Disocactus* taxa based on georeferenced specimen records.

quezaltecus (Standl. & Steyerm.) Kimnach, with strong support (99 % JK, 100 % BS MP; 1 PP BI) and corroborates the hypothesis suggested by Kimnach (1959, 1993) of the closeness of these two species based on exhibiting flowers with straight tepals and exerted stamens and styles. Moreover, *D. speciosus* was not recovered as a monophyletic species. *Disocactus speciosus* subsp. *bierianus* Ralf Bauer, subsp. *cinnabarinus* (Eichlam ex Weing.) Ralf Bauer and subsp. *speciosus* form a group with moderate support (77 % JK, 89 % BS MP; 1 PP BI; Fig. 2, 3), and a poor resolution is observed within this group, which we consider to be a reflection of the morphological issues presented by the species. Our results partially support the proposal of Bauer (2003), as only three of the five subspecies were recovered in the clade mentioned above. The variability of the stems, spines and flowers shown by these three subspecies is high, and they are only distinguished by subtle morphological differences (Bauer, 2007). Their shared morphological characteristics include stems with rigid spines, large red or orange flowers (12–17 cm) and a pericarpel and receptacle tube with rigid spines. They are broadly distributed in W Mexico (Durango to Oaxaca) and C Guatemala (the Sierra de los Cuchumatanes), growing in *Pinus* forests and tropical montane cloud forest (Fig. 4). *Disocactus speciosus* subsp. *aurantiacus* (Kimnach) Ralf Bauer was not recovered as part of the group that includes the three subspecies mentioned above in either analysis (MP or BI) (Fig. 2, 3, subclade 1D). This position in the topologies is congruent with the morphological differences exhibited by *D. speciosus* subsp. *aurantiacus*, which is characterized by flexible and pendulous stems as well as longer hairs, instead of spines, on the stems and flowers. This taxon is distributed in the tropical montane cloud forests of El Salvador, Honduras and Nicaragua (Fig. 4). Bauer (2003) included it in *D. speciosus* because it also exhibits ribbed stems and large flowers (12.5–15.5 cm). Although our results do not completely clarify the relationship of this taxon within subclade 1D (Fig. 2, 3), the molecular autapomorphies revealed in this study (substitution in *ycf1*, one indel in *psbA-trnH,* and *rpl16*; Table 4) in addition to the morphological characteristics listed above indicate that it should be recognized as a separate species, as was originally proposed (Kimnach 1974; Barthlott 1991a). Hunt & al. (2006) classified *D. speciosus* and *D. ackermannii* as part of *D.* subg. *Ackermannia* because they both develop large red or orange flowers (8–20 cm). However, we indicate herein that the recognized groups of *D. speciosus* are independently related, but never to *D. ackermannii* (Fig. 2, 3, subclades 1D, 3D).

In subclade 2D, *Disocactus biformis* was recovered as a sister taxon to *D. nelsonii* (Britton & Rose) Linding. with strong support (100 % JK, 100 % BS MP; 1 PP BI). These species share morphological characteristics including expanded external tepals, a scaly pericarpel and hairs measuring less than 0.5 cm, which allowed Kimnach (1993) to suggest the closeness of the species corroborated in this study. *Disocactus biformis*, *D. eichlamii*, *D. nelsonii* and *D. quezaltecus* are native to Guatemala, Honduras and Mexico (Chiapas) and are exclusive

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

to tropical montane cloud forests (Fig. 4). Although these four species are included in the section or subgenus *Disocactus* (Table 2) because they share phylloclades, flowers, and fruits without spines, our results reveal that they do not form a clade. Five indels (two in *trnL-F* and three in *psbA-trnH*) plus five substitutions (one in *matK*, one in *psbA-trnH, trnQ-rps16*, *rpl16* and *ycf1*) maintain the two groups in independent subclades (Table 4; Fig. 2, 3).

Subclade 3D supports the relationship among *Disocactus ackermannii*, *D.* ×*kimnachii* G. D. Rowley and *D. phyllanthoides* (97 % JK, 86 % BS MP; 1 PP BI; Fig. 2, 3), in contrast to the proposal made by Hunt & al. (2006). These authors believed *D. ackermannii* to be more closely related to *D. macdougallii* (Alexander) Barthlott (not included in this analysis) and *D. speciosus*, which together comprise *D.* subg. *Ackermannia*, whereas *D. phyllanthoides* was maintained in *Nopalxochia* (Table 2). However, we identified a relationship that was more consistent with the taxonomic treatment by Bravo-Hollis (1978): with the exception of *D.* ×*kimnachii* (a taxon not recognized as a species by Hunt & al. 2006), the two remaining species and *D. macdougallii* comprise the genus *Nopalxochia*. *Disocactus ackermannii* and *D. phyllanthoides* are native to tropical montane cloud forests in E and S Mexico (Veracruz to Chiapas; Fig. 4), whereas *D.* ×*kimnachii* is found only in Costa Rica. The initial hypothesis about the origin of *D.* ×*kimnachii* proposed that it is a hybrid between a member of *Heliocereus* or *Nopalxochia* and *Epiphyllum crenatum* (Kimnach 1984), whereas a subsequent hypothesis suggested that the putative parents include *Epiphyllum crenatum* subsp. *kimnachii* (Bravo ex Kimnach) U. Guzmán and a member of the group "*Heliocereus*" (Bauer 2003). Under this proposal and according to our results, *D. phyllanthoides* may be one of the parents. The three taxa are characterized by stems that branch close to the base, a pericarpel and receptacle tube with colourful scales that increase in size towards the perianth, and areoles with trichomes and flexible spines.

Three terminals of *Disocactus speciosus* aff. subsp. *cinnabarinus* from Chiapas (Mexico) were recovered with strong support (96 % JK, 99 % BS, MP; 1 PP BI; Fig. 2, 3). Neither the MP nor BI analysis recovered this taxon as part of *D. speciosus*, where the terminal from *D. speciosus* subsp. *cinnabarinus* from Chimaltenango (Guatemala) is nested, close to the type locality (Weingart 1910). The terminals from Chiapas (*Arias 1666*, *Cruz 18*, *Cruz 22*) were initially identified as *D. speciosus* subsp. *cinnabarinus* due to their shared floral morphology (Bravo-Hollis 1978; Bauer 2003), and it was even indicated that the observed differences were due to variations between populations (Bauer 2007). However, the adult stems of the Chiapas samples exhibit a consistent difference in the presence of phylloclades. This morphological difference was observed by Standley & Steyermark (1944) and was used as a basis for describing *Heliocereus heterodoxus* Standl. & Steyerm. for the region limited by the Tacana Volcano between Mexico (Chiapas) and Guatemala (San Marcos). We identified two indels in the markers *psbA-trnH* and *ycf1* (Table 4) that are exclusive to these three terminals. Based on these molecular characteristics and the morphological difference mentioned above, we believe that the three terminals do not correspond to *D. speciosus* subsp. *cinnabarinus*; therefore, we propose that it be recombined as *D. speciosus* subsp. *heterodoxus* (Standl. & Steyerm.) M. Á. Cruz & S. Arias.

Epiphyllum anguliger, *E. crenatum* and *E. lepidocarpum* were recovered as part of *Disocactus* s.str. with strong support (Fig. 2, 3). This relationship is supported by seven shared substitutions, which we recognize as the molecular synapomorphies of the clade *Disocactus* (Table 4). The results do not allow discrimination of the closest relationships between these three species within *Disocactus* because the two topologies (MP and BI) are incongruent. However, we may recognize a possible relationship between *E. crenatum* and subclade 3D, which includes *Nopalxochia* sensu Bravo-Hollis (1978). This hypothesis is based on the shared presence of scales on the pericarpel and the receptacle tube, which are larger towards the perianth, as well as flexible spines. The generation of hybrids has also been reported for both species (*D. ackermannii* & *D. phyllanthoides*) (Anderson 2005). In addition, the distribution of *E. crenatum* complements the species of subclade 3D in México (Veracruz, Oaxaca and Chiapas), although *E. crenatum* extends towards Guatemala and Honduras. Although the relationship between *E. anguliger* and *E. lepidocarpum* is uncertain, the flexible spines observed on the stems and flowers with two sets of stamens are morphological characters shared with *Disocactus* (Bauer 2009). *Epiphyllum anguliger* is distributed in W Mexico (Guerrero, Jalisco, Nayarit and Oaxaca), whereas *E. lepidocarpum* is native to Costa Rica (Fig. 4).

Disocactus and sister genera

The topology of both phylogenies (MP and BI) is highly congruent: the tribe *Hylocereeae* consists of three groups (Fig. 2, 3). The subtribe *Epiphyllinae*, as redefined here, includes the genera *Disocactus* (Clade D), *Epiphyllum* (Clade E) and *Pseudorhipsalis* (Clade P), whereas the subtribe *Hylocereinae* consists of *Aporocactus* (Clade A), *Hylocereus*, *Selenicereus* and *Weberocereus* (HSW). *Acanthocereus* represents the third group. This result is partially consistent with the subtribes proposed by Buxbaum (1958) and the clades recovered in the phylogenetic analyses of *Peniocereus* by Arias & al. (2005). The main difference between the two subtribes is that phylloclades are more common in *Epiphyllinae*, whereas ribs are more common in *Hylocereinae*. In addition, both phylogenies indicate that *Disocactus* is more closely related to *Epiphyllum* and *Pseudorhipsalis*.

Within the subtribe *Epiphyllinae*, *Disocactus* is never recovered as a sister genus to *Pseudorhipsalis*. In

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

the MP reconstruction, *Disocactus* is a sister to *Epiphyllum* and *Pseudorhipsalis*, whereas in the BI reconstruction, *Pseudorhipsalis* is sister to *Disocactus* and *Epiphyllum*. This classification is consistent with Bauer's argument (2002), which indicates that the distribution, habitat and certain morphological characteristics differ between *Pseudorhipsalis* and *Disocactus*. *Pseudorhipsalis* is distributed from Mexico to Bolivia, but its centre of diversity is found in Costa Rica and Panama, where it grows in low-elevation zones (below 650 m) and hot, humid regions. The primary stem of *Pseudorhipsalis* is easily distinguished from the secondary stem due to its cylindrical shape; its areoles are close-set, and its flowers are very small (0.7–2.5 cm), funnel-shaped or rotate and are characterized by a short flowering period. The flowers are not oriented towards the light, and the pollen is small (45–55 µm) and polycolpate. In contrast, *Disocactus* is distributed from NC Mexico to Nicaragua, where it grows in elevated (above 1200 m) tropical montane cloud forests. The primary stem of *Disocactus* may only be distinguished from the secondary stem in certain species; the distance between the areoles is greater; and the flowers may be small (5.5–6 cm) to large (16 cm) and funnel-shaped. The flowers spend several days in anthesis and are mainly oriented towards the light, which results in a curve in the receptacle tube when they grow on pendulous stems; the pollen is large (70–100 µm) and tricolpate. These morphological differences favour the hypothesis suggested in the BI analysis, in which *Disocactus* is the sister genus to *Epiphyllum*, which is distributed from Mexico to Argentina, although its greatest diversity is found between S Mexico and Costa Rica in low zones or at high elevation (0–2300 m) in hot and temperate regions (Bauer 2003). These two genera share phylloclades with a cylindrical base, flowers larger than 2.5 cm, and a scaly pericarpel and receptacle tube. Moreover, our results allow us to exclude *Aporocactus* from *Disocactus* and recognize it as an independent genus. Nevertheless, its relationship with the subtribe *Hylocereinae* merits its own study because only the BI phylogenetic analysis showed high support.

Based on the data from cpDNA sequences, this study provided significant evidence indicating that certain floral morphological characteristics that are used to delimit *Disocactus* have arisen more than once in *Hylocereeae*. The diurnal, funnel-shaped and intensely coloured (reds and others) flowers are not exclusive to *Disocactus* (Fig. 1). Based on these data, we hypothesize a convergent origin for the nocturnal, long, funnel-shaped flowers with light colouration (white) found in *Disocactus* and *Epiphyllum*. Therefore, supplementary studies are required to clarify the relationship between *Disocactus* and its sister genera, as well as its possible origin within the subtribe *Epiphyllinae* (as it is recognized here). A comparative morphological study of vegetative (e.g. shape and succulence of cotyledons in seedlings; stem shape during the juvenile stage) and reproductive structures (e.g. number of sets of stamens; seed size and micro-ornamentation) is needed to explore the observed synapomorphies and their transformation within the subtribe. The origin of the epiphytic growth within *Hylocereeae* also needs to be explored based on climbing shape, including the change in stem growth from indeterminate to determinate. A top priority is to combine our molecular data with a set of morphological attributes in a total evidence analysis to corroborate the phylogenetic relationships obtained here and recover the currently unresolved terminal nodes. We also aim to understand the evolution of the highlighted characteristics in subtribe *Epiphyllinae.*

Taxonomy

Based on phylogenetic analysis presented here, the 14 species accepted in *Disocactus* are listed below. Five new combinations are included. In addition, one taxon of presumably hybrid origin is indicated (Kimnach 1984). An asterisk (*) marks taxa not available for molecular analysis.

1. ***Disocactus ackermannii*** (Haw.) Ralf Bauer in Cactaceae Syst. Init. 17: 15. 2003 ≡ *Epiphyllum ackermannii* Haw. in Philos. Mag. Ann. Chem. 6: 109. 1829 ≡ *Cactus ackermannii* (Haw.) Lindl. in Edwards's Bot. Reg. 16: t. 1331. 1830 ≡ *Cereus ackermannii* (Haw.) Otto ex Pfeiff., Enum. Diagn. Cact.: 123. 1837 ≡ *Phyllocactus ackermannii* (Haw.) Salm-Dyck, Cact. Hort. Dyck.: 38. 1841 ≡ *Nopalxochia ackermannii* (Haw.) F. M. Knuth in Backeb. & F. M. Knuth, Kaktus-ABC: 161. 1935. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 16. 2003): [illustration in] Lindley in Edward's Bot. Reg. 16: t. 1331. 1830.

1a. ***Disocactus ackermannii*** subsp. ***ackermannii***

= *Epiphyllum ackermannii* f. *candidum* Alexander in Cact. Succ. J. (Los Angeles) 19: 58. 1947 ≡ *Disocactus ackermannii* f. *candidus* (Alexander) Barthlott in Bradleya 9: 87. 1991. – Holotype: Mexico, Chiapas, cultivated plant in a garden in San Cristobal [de] Las Casas, 14 Apr 1945, *E. J. Alexander s.n.* (NY; isotypes: MEXU barcode 00123464!, P, US!).

1b.** **Disocactus ackermannii subsp. ***conzattianus*** (T. MacDoug.) Ralf Bauer in Cactaceae Syst. Init. 17: 17. 2003 ≡ *Nopalxochia conzattianum* T. MacDoug. in Cact. Succ. J. (Los Angeles) 19: 22. 1947 ≡ *Pseudonopalxochia conzattianum* (T. MacDoug.) Backeb., Cactaceae (Backeberg) 2: 757. 1959 ≡ *Nopalxochia ackermannii* var. *conzattianum* (T. MacDoug.) Kimnach in Cact. Succ. J. (Los Angeles) 53: 85. 1981 ≡ *Disocactus ackermannii* var. *conzattianus* (T. MacDoug.) Barthlott in Bradleya 9: 87 1991 ≡ *Heliocereus conzattianus* (T. MacDoug.) Doweld in Sukkulenty 4(1–2): 42. 2002 ≡ *Disocactus ackermannii* subsp. *conzattianus* (T. MacDoug.) U. Guzmán in

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Cactaceae Syst. Init. 16: 17. 2003. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 17. 2003): [illustration] "*Nopalxochia conzattianum* new species" in MacDougall in Cact. Succ. J. (Los Angeles) 19: 22, fig. 15. 1947.

2. ***Disocactus anguliger*** (Lem.) M. Á. Cruz & S. Arias, **comb. nov.** ≡ *Phyllocactus anguliger* Lem. in Jard. Fleur. 1: t. 92. 1851 ≡ *Epiphyllum anguliger* (Lem.) G. Don, Encycl. Pl., ed. 3: 1380. 1855. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 26. 2003): [illustration] "*Phyllocactus anguliger* Ch. L." in Lemaire in Jard. Fleur. 1: t. 92. 1851.

= *Phyllocactus darrahii* K. Schum., Gesamtb. Kakt. Nachtr.: 69. 1903 ≡ *Epiphyllum darrahii* (K. Schum.) Britton & Rose in Contr. U.S. Natl. Herb. 16: 256. 1913. – **Neotype (designated here):** [illustration] "*Phyllocactus darrahi* K. Schum." in Gürke, Bluh. Kakteen: t. 91. 1910.

3. ***Disocactus aurantiacus*** (Kimnach) Barthlott in Bradleya 9: 87. 1991 ≡ *Heliocereus aurantiacus* Kimnach in Cact. Succ. J. (Los Angeles) 46: 67. 1974 ≡ *Disocactus speciosus* subsp. *aurantiacus* (Kimnach) Ralf Bauer in Cactaceae Syst. Init. 17: 16. 2003. – Holotype: Nicaragua, Jinotega, Potter's Folly, between Santa Maria Ostumes and Jinotega, 4500 feet, 1959, *A. H. Heller s.n.* (UC barcode 1229424!; isotypes: HNT barcode 0000028!, US barcode 00115677!).

4. ***Disocactus biformis*** (Lindl.) Lindl. in Bot. Reg. 31: t. 9. 1845 ≡ *Cereus biformis* Lindl. in Bot. Reg. 29: misc. 51. 1843 ≡ *Disisocactus biformis* (Lindl.) Kunze in Bot. Zeitung (Berlin) 3: 533. 1845 ≡ *Phyllocactus biformis* (Lindl.) Labour., Monogr. Cact.: 418. 1853 ≡ *Epiphyllum biforme* (Lindl.) G. Don, Encycl. Pl., ed. 3: 1378. 1855. – Neotype (designated by Kimnach in Haseltonia 1: 111. 1993): Guatemala, Sacatepéquez, 6 km N of Alotenango, gorge of Río Guacalate, 1300 m, 9 Mar 1957, *C. K. Horich s.n.* (UC; isoneotypes: HNT!, MO!).

5. ***Disocactus crenatus*** (Lindl.) M. Á. Cruz & S. Arias, **comb. nov.** ≡ *Cereus crenatus* Lindl. in Edwards´s Bot. Reg. 30: t. 31. 1844 ≡ *Phyllocactus crenatus* (Lindl.) Lem. in Hort. Universel 6: 87. 1845 ≡ *Phyllocactus crenatus* (Lindl.) Walp. in Repert. Bot. Syst. 5: 820. 1846 ≡ *Epiphyllum crenatum* (Lindl.) G. Don, Encycl. Pl., ed. 3: 1378. 1855. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 27. 2003): [illustration in] Lindley in Edwards's Bot. Reg. 30: t. 31. 1844.

5a. ***Disocactus crenatus*** subsp. ***crenatus***

= *Phyllocactus caulorrhizus* Lem. in Jard. Fleur. 1: misc. 6. 1851 ≡ *Epiphyllum caulorrhizum* (Lem.) G. Don, Encycl. Pl., ed. 3: 1380. 1855.

5b. ***Disocactus crenatus*** subsp. ***kimnachii*** (Bravo ex Kimnach) M. Á. Cruz & S. Arias, **comb. nov.** ≡ *Epiphyllum crenatum* var. *kimnachii* Bravo ex Kimnach in Cact. Succ. J. (Los Angeles) 39: 207. 1967 ≡ *Epiphyllum crenatum* subsp. *kimnachii* (Bravo ex Kimnach) U. Guzmán in Cactaceae Syst. Init. 16: 17. 2003. – **Lectotype (designated here):** [illustration] "fig. 1 *Epiphyllum crenatum* var. *kimnachii*" in Bravo in Anales Inst. Biol. Univ. Nac. México 35: 78. 1964.

6. ***Disocactus eichlamii*** (Weing.) Britton & Rose in Contr. U.S. Natl. Herb. 16: 259. 1913 ≡ *Phyllocactus eichlamii* Weing. in Monatsschr. Kakteenk. 21: 5. 1911 ≡ *Trochilocactus eichlamii* (Weing.) Linding. in Beih. Bot. Centralbl. 61: 383. 1942 ≡ *Epiphyllum eichlamii* (Weing.) L. O. Williams in Fieldiana, Bot. 29: 378. 1962. – Lectotype (designated by Kimnach & Hutchison in Cact. Succ. J. (Los Angeles) 29: 78. 1957): Guatemala, locality unknown, 13 Nov 1910, *F. Eichlam s.n.* (US!)

7. ***Disocactus lepidocarpus*** (F. A. C. Weber) M. Á. Cruz & S. Arias, **comb. nov.** ≡ *Phyllocactus lepidocarpus* F. A. C. Weber in Bull. Mus. Hist. Nat. (Paris) 8: 462. 1902 ≡ *Epiphyllum lepidocarpum* (F. A. C. Weber) Britton & Rose in Contr. U.S. Natl. Herb. 16. 257. 1913. – Neotype (designated by Bauer in Cactaceae Syst. Init. 17: 27. 2003): Costa Rica, Heredia, S of Volcán Barva and N of Heredia, Paso El Llano, 2100 m, 13 Mar 1983, *M. Kimnach 2440* (ZSS).

8.** ***Disocactus lodei Veliz & al. in Cact.-Avent. Int. 104: 2. 2014. – Holotype: Guatemala, Quetzaltenango, Zunil, on volcanic rocks with *Tillandsia tecpanensis* and *Epiphyllum crenatum*, 2060 m, 10 Apr 2008, *M. Véliz 20100* (BIGU 42261; isotype: MEXU).

9.** ***Disocactus macdougallii (Alexander) Barthlott in Bradleya 9: 88. 1991 ≡ *Lobeira macdougallii* Alexander in Cact. Succ. J. (Los Angeles) 16: 178. 1944 ≡ *Nopalxochia macdougallii* (Alexander) W. T. Marshall in Cactus 4: 6. 1946 ≡ *Heliocereus macdougallii* (Alexander) Doweld in Sukkulenty 4(1–2): 42. 2002. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 17. 2003): [illustration] "fig. 162. *Lobeira macdougallii* sp. nov., natural size" in Alexander in Cact. Succ. J. (Los Angeles) 16: 176. 1944.

10. ***Disocactus macranthus*** (Alexander) Kimnach & Hutchinson in Cact. Succ. J. (Los Angeles) 29: 78. 1957 ≡ *Pseudorhipsalis macrantha* Alexander in Cact. Succ. J. (Los Angeles) 14: 20. 1942 ≡ *Disisorhipsalis macrantha* (Alexander) Doweld in Sukkulenty 4(1–2): 40. 2002. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 18. 2003): Mexico, Oaxaca, N of Niltepec, on the N side of the main Sierra near La Gloria, 830 m, 1939/1940, *T. MacDougall A42* (US!; isotypes: M, MO!, P).

11. ***Disocactus nelsonii*** (Britton & Rose) Linding. in Beih. Bot. Centralbl. 61: 383. 1942 ≡ *Epiphyllum nelsonii* Britton & Rose in Contr. U.S. Natl. Herb. 16: 257. 1913 ≡ *Phyllocactus nelsonii* (Britton & Rose) Vaupel in Monatsschr. Kakteenk. 23: 116. 1913 ≡ *Chiapasia nelsonii* (Britton & Rose) Britton & Rose, Cact. 4: 203. 1923. – Holotype: Mexico, Chiapas, near Chicharras, c. 1000 m, 12–15 Feb 1869, *E. W. Nelson 3761* (US!; isotype: NY!).

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

11a.** ***Disocactus nelsonii subsp. ***hondurensis*** (Kimnach) Doweld in Sukkulenty 4 (1–2): 41. 2002 ≡ *Disocactus nelsonii* var. *hondurensis* Kimnach in Cact. Succ. J. (Los Angeles) 37: 33. 1965. – Holotype: Honduras, Comayagua, 4 miles beyond El Rincon, on way from Siguatepeque, in canyon along road, 11 Aug 1962, *M. Kimnach 394* (UC; isotype: HNT barcode 0000082!).

11b. ***Disocactus nelsonii*** subsp. ***nelsonii***

= *Phyllocactus chiapensis* J. A. Purpus in Monatsschr. Kakteenk. 28: 118. 1918. – **Lectotype (designated here):** [illustration] "*Phyllocactus chiapensis* J. A. Purp. Von dem Autor photographisch aufgenommen" in Purpus in Monatsschr. Kakteenk. 28: 119. 1918.

12. ***Disocactus phyllanthoides*** (DC.) Barthlott in Bradleya 9: 88. 1991 ≡ *Cactus phyllanthoides* DC., Cat. Pl. Horti Monsp.: 84. 1813 ≡ *Epiphyllum phyllanthoides* (DC.) Sweet, Hort. Brit. 1: 172. 1826 ≡ *Cereus phyllanthoides* (DC.) DC., Prodr. 3: 469. 1828 ≡ *Phyllocactus phyllanthoides* (DC.) Link, Handbuch 2: 11. 1829 ≡ *Nopalxochia phyllanthoides* (DC.) Britton & Rose, Cact. 4: 205. 1923 ≡ *Heliocereus phyllanthoides* (DC.) Doweld in Sukkulenty 4(1–2): 42. 2002. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 17. 2003): [illustration] "Tab. CCXLVII" in Plukenet, Phytographia: t. 247, fig. 5. 1692. – Epitype (designated by Bauer in Cactaceae Syst. Init. 17: 17. 2003): Mexico, Puebla, near rio San Pedro in steep rocky cliffs, 1500 m, 8 Mar 2002, *Lautner L02/60I* (ZSS).

13. ***Disocactus quezaltecus*** (Standl. & Steyerm.) Kimnach in Cact. Succ. J. (Los Angeles) 31: 137. 1959 ≡ *Bonifazia quezalteca* Standl. & Steyerm. in Publ. Field Mus. Nat. Hist., Bot. Ser. 23: 66. 1944 ≡ *Epiphyllum quezaltecum* (Standl. & Steyerm.) L. O. Williams in Fieldiana, Bot. 29: 378. 1962. – Holotype: Guatemala, Quezaltenango, above Mujuliá, between San Martín Chile Verde and Colomba, 1800 m, 1 Feb 1941, *P. C. Standley 85603* (F!).

14. ***Disocactus speciosus*** (Cav.) Barthlott in Bradleya 9: 87. 1991 ≡ *Cactus speciosus* Cav. in Anales Ci. Univ. Madrid 6: 339. 1803 ≡ *Cereus speciosus* (Cav.) K. Schum. ex Engl. & Prantl, Nat. Pflazenfam. 3(6a): 179. 1894 ≡ *Heliocereus speciosus* (Cav.) Britton & Rose in Contr. U.S. Natl. Herb. 12: 434. 1909. – Neotype (designated by Bauer in Cactaceae Syst. Init. 17: 13. 2003): Mexico, Distrito Federal, native on the pedregal of the botanical garden of the University of Mexico, 6 Aug 1962, *M. Kimnach 383* (HNT!).

14a. ***Disocactus speciosus*** subsp. ***bierianus*** Ralf Bauer in EPIG 60: 31. 2008. – Holotype: Mexico, Guerrero, zwischen Filo de Caballos und Puerto del Gallo, c. 2000 m, 23 Feb 1992, *Lautner L92/46* (ZSS).

14b.** ***Disocactus speciosus subsp. ***blomianus*** (Kimnach) Ralf Bauer in Cactaceae Syst. Init. 17: 15. 2003 ≡ *Heliocereus aurantiacus* var. *blomianus* Kimnach in Cact. Succ. J. (Los Angeles) 62: 270. 1990 ≡ *Disocactus aurantiacus* var. *blomianus* (Kimnach) E. Meier in Kakteen And. Sukk. 48: 7: 1997. – Holotype: Mexico, Chiapas, Cerro Sabandillo, near río Monoblanco and the border of Oaxaca, Mar 1951, *T. MacDougall A202* (HNT!; isotype: CAS barcode 0027330!).

14c. ***Disocactus speciosus*** subsp. ***cinnabarinus*** (Eichlam ex Weing.) Ralf Bauer in Cactaceae Syst. Init. 17: 15. 2003 ≡ *Cereus cinnabarinus* Eichlam ex Weing. in Monatsschr. Kakteenk. 20: 161. 1910 ≡ *Heliocereus cinnabarinus* (Eichlam ex Weing.) Britton & Rose, Cact. 2: 129. 1920 ≡ *Disocactus cinnabarinus* (Eichlam ex Weing.) Barthlott in Bradleya 9: 87. 1991. – Neotype (designated by Doweld in Sukkulenty 4(1–2): 45. 2002): Guatemala, Quezaltenango, Las Nubes, 12 Mar 1957, *C. K. Horich s.n.* (MO barcode 148794!).

14d. ***Disocactus speciosus*** subsp. ***heterodoxus*** (Standl. & Steyerm.) M. Á. Cruz & S. Arias, **comb. & stat. nov.** ≡ *Heliocereus heterodoxus* Standl. & Steyerm. in Publ. Field Mus. Nat. Hist., Bot. Ser. 23: 67. 1944. – Holotype: Guatemala, San Marcos, along río Vega between San Rafael and the NE portion of Volcán de Tacaná, 2500–3000 m, 21 Feb 1940, *J. A. Steyermark 36291* (F barcode 0052879F!).

14e. ***Disocactus speciosus*** subsp. ***speciosus***

= *Cactus speciosissimus* Desf. in Mém. Mus. Hist. Nat. 3: 193. 1817 ≡ *Cactus speciosissimus* (Desf.) DC., Prodr. 3: 468. 1828 ≡ *Heliocereus speciosissimus* (Desf.) Y. Itô, Cacti: 146. 1952. – **Lectotype (designated here):** [illustration] "*Cactus speciosissimus*" in Desfontaines in Mém. Mus. Hist. Nat. 3: [t. ix] pre 193. 1817.

= *Cereus coccineus* Salm-Dyck ex Pfeiff., Enum. Diagn. Cact.: 122. 1837 [non Salm-Dyck ex DC., Prodr. 3: 469. 1828] ≡ *Heliocereus coccineus* Britton & Rose in Contr. U.S. Nat. Herb. 12: 433. 1909 ≡ *Heliocereus elegantissimus* Britton & Rose, Cact. 2: 127. 1920, nom. illeg. ≡ *Cereus elegantissimus* A. Berger, Kakteen: 131. 1929, nom. illeg. – **Neotype (designated here):** [illustration] "Taf. XV *Cereus coccineus*" in Pfeiffer & Otto, Abbild. Beschr. Cact. 1: t. 15. 1839.

= *Cereus schrankii* Zucc. ex Seitz in Allg. Gartenzeitung 2: 244. 1834 ≡ *Heliocereus schrankii* (Zucc. ex Seitz) Britton & Rose in Contr. U.S. Natl. Herb. 12: 434. 1909 ≡ *Disocactus schrankii* (Zucc. ex Seitz) Barthlott in Bradleya 9 : 87. 1991. – **Neotype (designated here)**: [illustration] "Taf. XXVII *Cereus schrankii*" in Pfeiffer & Otto, Abbild. Beschr. Cact. 1: t. 27. 1843.

= *Cereus superbus* Ehrenb. in Bot. Zeitung (Berlin) 4: 324. 1846 ≡ *Heliocereus superbus* (Ehrenb.) A. Berger, Kakteen: 131, 341. 1929 ≡ *Heliocereus speciosus* var. *superbus* (Ehrenb.) Backeb., Cactaceae (Backeberg) 4: 2120. 1960.

= *Cereus amecamensis* Heese in Rother, Prakt. Ratgeber Obst-Gartenbau 11: 442. 1896 ≡ *Heliocereus amecamensis* (Heese) Britton & Rose in Contr. U.S. Natl. Herb. 12: 433. 1909 ≡ *Cereus speciosus* var. *ameca-*

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

mensis (Heese) Weing. in Berger, Kakteen: 131. 1929 ≡ *Heliocereus speciosus* var. *amecamensis* (Hesse) Bravo in Cact. Suc. Mex. 19: 47. 1974 ≡ *Disocactus speciosus* f. *amecamensis* (Hesse) Barthlott in Bradleya 9: 87. 1991 ≡ *Heliocereus speciosus* subsp. *amecamensis* (Hesse) Doweld in Sukkulenty 4(1–2): 41. 2002.

= *Cereus serratus* Weing. in Monatsschr. Kakteenk. 22: 185. 1912 ≡ *Heliocereus serratus* (Weing.) Borg, Cacti: 176. 1951 ≡ *Heliocereus speciosus* var. *serratus* (Weing.) Backeb., Cactaceae 4: 2121. 1960.

= *Heliocereus elegantissimus* var. *stenopetalus* Bravo ex S. Arias & al. in Cact. Suc Mex. 37: 72. 1992 ≡ *Heliocereus schrankii* var. *stenopetalus* (Bravo ex S. Arias & al.) Kimnach in Cact. Succ. J. (Los Angeles) 68: 217. 1996 ≡ *Heliocereus elegantissimus* subsp. *stenopetalus* (Bravo ex S. Arias & al.) Doweld in Sukkulenty 4(1–2): 41. 2002. – Holotype: Mexico, Durango, km 1120 carretera Durango a Mazatlán, 8 May 1965, *H. Bravo Hollis 84* (MEXU barcode 00155748!).

= *Heliocereus elegantissimus* var. *helenae* Scheinvar in Phytologia 49: 317. 1981 ≡ *Heliocereus schrankii* var. *helenae* (Scheinvar) Kimnach in Cact. Succ. J. (Los Angeles) 68: 217. 1996 ≡ *Heliocereus elegantissimus* subsp. *helenae* (Scheinvar) Doweld in Sukkulenty 4(1–2): 41. 2002. – Holotype: Mexico, Estado de México, Amecameca, Tocino, Agua Viva, 13 Jul 1977 [24 Apr], *L. Scheinvar 2240* (MEXU barcode 00273025!; isotypes: MEXU barcode 00241071; US barcode 01049960!).

= *Heliocereus luzmariae* Scheinvar in Cact. Succ. J. (Los Angeles) 57: 268. 1985 ≡ *Heliocereus schrankii* subsp. *luzmariae* (Scheinvar) U. Guzmán in Cactaceae Syst. Init. 16: 17. 2003. – Holotype: Mexico, Jalisco, 3 km al noreste de la Joya, Sierra de Manantlán, 1980 m, 29 May 1984, *H. J. Arreola Nava 33* (MEXU; isotypes: IBUG barcode 0040851!, 0040851_a!).

15. ***Disocactus* ×*kimnachii*** G. D. Rowley in Brit. Cact. Succ. J. 5: 84. 1987 (pro sp.) ≡ *Nopalxochia horichii* Kimnach in Cact. Succ. J. (Los Angeles) 56: 6. 1984 [non *Disocactus horichii* Kimnach in Cact. Succ. J. (Los Angeles) 51: 169. 1979]. – Holotype: Costa Rica, San José, NE outskirts of San Isidro de Coronado along trail W of Río Durazno, c. 1400 m, [7 Jan] 1971, *C. K. Horich s.n.* (HNT barcode 0000023!; isotypes: CR, F, US barcode 00115768!).

Acknowledgements

This study is part of the requirements for Miguel Ángel Cruz to obtain a Doctor of Science degree from the Posgrado en Ciencias Biológicas (PCB), UNAM. M.A.C. thanks PCB, UNAM, for providing the facilities for this project and the Consejo Nacional de Ciencia y Tecnología for the Ph.D. scholarship (215188). We are grateful to Mario Veliz (Universidad San Carlos de Guatemala), Gabriel Cerén, Jennie Menjivar (Museo de Historia Natural El Salvador), and Barry Hammel (Missouri Botanical Garden, Instituto Nacional de Biodiversidad, Museo Nacional de Costa Rica) for their kind help. We also thank the curators of the herbaria BIGU, CR, ENCB, HNT, INB, MEXU, MHES, MO, UAMIZ, US, USJ and XAL for loaning specimens critical to this study, as well as the program UNAM-DGAPA-PAPIIT <IN208315> to S.A., which supported this work. We thank three anonymous reviewers and Nicholas Turland for their useful comments to improve the manuscript.

References

Anderson E. F. 2005: Das große Kakteen-Lexikon. – Stuttgart: Eugen Ulmer.

Arias S., Terrazas T., Arreola-Nava H. J., Vázquez-Sánchez M. & Cameron K. M. 2005: Phylogenetic relationships in *Peniocereus* (*Cactaceae*) inferred from plastid DNA sequence data. – J. Pl. Res. **118:** 317–328.

Arias S., Terrazas T. & Cameron K. 2003: Phylogenetic analysis of *Pachycereus* (*Cactaceae*, *Pachycereeae*) based on chloroplast and nuclear DNA sequences. – Syst. Bot. **28:** 547–557.

Bárcenas R. T., Yesson C. & Hawkins J. A. 2011: Molecular systematics of the *Cactaceae*. – Cladistics **27:** 1–20.

Barthlott W. 1991a: *Disocactus*. – Pp. 86–88 in: Hunt D. & Taylor N. P. (ed.), Notes on miscellaneous genera of *Cactaceae*. – Bradleya **9:** 81–92.

Barthlott W. 1991b: *Pseudorhipsalis*. – Pp. 90 in: Hunt D. & Taylor N. P. (ed.), Notes on miscellaneous genera of *Cactaceae*. Bradleya **9:** 81–92.

Barthlott W., Burstedde K., Geffert J. L., Ibisch P. L., Korotkova N., Miebach A., Rafiqpoor M. D., Stein A. & Mutke J. 2015: Biogeography and biodiversity of cacti. – Shumannia **7.**

Bauer R. 2002: The genus *Pseudorhipsalis* Britton & Rose. – Haseltonia **9:** 94–120.

Bauer R. 2003: A synopsis of the tribe *Hylocereeae* F. Buxb. – Cactaceae Syst. Init. **17:** 3–63.

Bauer R. 2007: *Disocactus speciosus* (Cavanilles) Barthlott und seine Unterarten-mit Beschreibung der neuen Unterart *D. speciosus* ssp. *bierianus* Ralf Bauer ssp. nov. – EPIG **60:** 4–61.

Bauer R. 2009: *Epiphyllum anguliger* (Lemaire) Don ex Loudon, eine botanisch interessante Art aus dem westlichen Mexiko. – EPIG **63:** 5–15.

Bonatelli I. A. S., Perez M. F., Peterson A. T., Taylor N. P., Zappi D. C., Machado M. C., Koch I., Pires A. C. H. & Morales E. M. 2014: Interglacial microrefugia and diversification of a cactus species complex: phylogeography and paleodistributional reconstructions for *Pilosocereus arisetus* and allies. – Molec. Ecol. **23:** 3044–3063.

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Borsch T. & Quandt D. 2009: Mutational dynamics and phylogenetic utility of noncoding chloroplast DNA. – Pl. Syst. Evol. **282:** 169–199.

Bravo-Hollis H. 1978: *Las cactáceas de México*, vol. I. – México D.F.: Universidad Nacional Autónoma de México.

Britton N. L. & Rose J. N. 1923: The Cactaceae **4.** – Washington D.C.: The Carnegie Institution.

Butterworth C. A. & Wallace R. S. 2004: Phylogenetic studies of *Mammillaria* (*Cactaceae*) insights from chloroplast sequence variation and hypothesis testing using the parametric bootstrap. – Amer. J. Bot. **91:** 1086–1098.

Buxbaum F. 1958: The phylogenetic division of the subfamily *Cereoideae*, *Cactaceae*. – Madroño **14:** 177–216.

Calvente A., Zappi D. C., Forest F. & Lohmann L. G. 2011a: Molecular phylogeny of tribe *Rhipsalideae* (*Cactaceae*) and taxonomic implications for *Schlumbergera* and *Hatiora*. – Molec. Phylogen. Evol. **58:** 456–468.

Calvente A., Zappi D. C., Forest F. & Lohmann L. G. 2011b: Molecular phylogeny, evolution, and biogeography of South American epiphytic cacti. – Int. J. Pl. Sci. **172:** 902–914.

Darriba D., Taboada G. L., Doallo R. & Posada D. 2012: jModelTest 2: more models, new heuristics and parallel computing. – Nature, Meth. **9:** 772.

Edwards E. J., Nyffeler R. & Donoghue M. J. 2005: Basal cactus phylogeny: implications of *Pereskia* (*Cactaceae*) paraphyly for the transition to the cactus life form. – Amer. J. Bot. **92:** 1177–1188.

Franck A. R., Cochrane B. J. & Garey J. R. 2012: Low-copy nuclear primers and *ycf1* primers in *Cactaceae*. – Amer. J. Bot. **99:** 405–407.

Franck A. R., Cochrane B. J. & Garey J. R. 2013: Relationships and dispersal of the Caribbean species of *Harrisia* (sect. *Harrisia*: *Cactaceae*) using AFLPs and seven DNA regions. – Taxon **62:** 486–497.

Griffith M. P. 2002: *Grusonia pulchella* classification and its impacts on the genus *Grusonia*: morphological and molecular evidence. – Haseltonia **9:** 86–93.

Harpke D. & Peterson A. 2006: Non-concerted ITS evolution in *Mammillaria* (*Cactaceae*) – Molec. Phylogen. Evol. **41:** 579–593.

Hartmann S., Nason J. D. & Bhattacharya D. 2001: Extensive ribosomal DNA genetic variation in the columnar cactus *Lophocereus*. – J. Molec. Evol. **53:** 124–134.

Hernández-Hernández T., Hernández H. M., De-Nova J. A., Eguiarte L. E. & Magallón S. 2011: Phylogenetic relationship and evolution of growth form in *Cactaceae* (*Caryophyllales*, *Eudicotyledoneae*). – Amer. J. Bot. **98:** 44–61.

Huelsenbeck J. P. & Ronquist F. 2001: MRBAYES: Bayesian inference of phylogenetic trees. – Bioinformatics **17:** 754–755.

Hunt D., Taylor N. P. & Charles G. 2006: The new cactus lexicon. – Milborne Port: Dh Books.

Katoh K., Misawa K., Kuma K. & Miyata T. 2002: MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. – Nucl. Acids Res. **14:** 3059–3066.

Kimnach M. 1959: *Disocactus quezaltecus*. – Cact. Succ. J. (Los Angeles) **31:** 137–141.

Kimnach M. 1974: *Heliocereus aurantiacus*. – Cact. Succ. J. (Los Angeles) **46:** 67–69.

Kimnach M. 1984: *Nopalxochia horichii*, a new cactus from Costa Rica. – Cact. Succ. J. (Los Angeles) **56:** 4–8.

Kimnach M. 1993: The genus *Disocactus*. – Haseltonia **1:** 95–139.

Korotkova N., Borsch T., Quandt D., Taylor N. P., Müller K. F. & Barthlott W. 2011: What does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic *Rhipsalideae* (*Cactaceae*). – Amer. J. Bot. **98:** 1549–1572.

Korotkova N., Zabel L., Quandt D. & Barthlott W. 2010: A phylogenetic analysis of *Pfeiffera* and the reinstatement of *Lymanbensonia* as an independently evolved lineage of epiphytic *Cactaceae* within a new tribe *Lymanbensonieae*. – Willdenowia **40:** 151–172.

Larridon I., Walter H. E., Guerrero P. C., Duarte M., Cisternas M. A., Peña-Hernández C., Bauters K., Asselman P., Goetghebeur G. & Samain M. S. 2015: An integrative approach to understanding the evolution and diversity of *Copiapoa* (*Cactaceae*), a threatened endemic Chilean genus from the Atacama Desert. – Amer. J. Bot. **102:** 1506–1520.

Lindley J. 1845: *Disocactus*. – Edwards's Bot. Reg. **31:** t. 9.

Lodé J. 2013: *Acanthocereus*. – Cact.-Avent. Int. **97** (Suppl.): 2–11.

Majure L. C., Puente R., Griffith M. P., Judd W. S., Soltis P. S. & Soltis D. E. 2012: Phylogeny of *Opuntia* s.s. (*Cactaceae*): clade delineation, geographic origins, and reticulate evolution. – Amer. J. Bot. **99:** 847–864.

Müller K. F. 2005: SeqState – primer design and sequence statistics for phylogenetic DNA datasets. – Appl. Bioinformatics **4:** 65–69.

Nyffeler R. 2002: Phylogenetic relationships in the cactus family (*Cactaceae*) based on evidence from *trnK*/*matK* and *trnL*- *trnF* sequences. – Amer. J. Bot. **9:** 312–326.

Plume O., Straub S. C. K., Tel-Zur N., Cisneros A., Schneider B. & Doyle J. J. 2013: Testing a hypothesis of intergeneric allopolyploidy in vine cacti (*Cactaceae*: *Hylocereeae*). – Syst. Bot. **38:** 737–751.

Rambaut A. & Drummond A. J. 2009: TRACER: MCMC Trace Analysis Tool Version v1. 5.0. – University of Oxford.

Ritz C. M., Martins L., Mecklenburg R., Goremykin V. & Hellwig F. H. 2007: The molecular phylogeny of *Rebutia* (*Cactaceae*) and its allies demonstrates

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

the influence of paleogeography on the evolution of South American mountain cacti. – Amer. J. Bot. **94:** 1321–1332.

Sang T., Crawford D. & Stuessy T. F. 1997: Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (*Paeoniaceae*). – Amer. J. Bot. **84:** 1120–1136.

Schlumpberger B. O. & Renner S. S. 2012: Molecular phylogenetics of *Echinopsis* (*Cactaceae*): polyphyly at all levels and convergent evolution of pollination modes and growth forms. – Amer. J. Bot. **99:** 1335–1349.

Shaw J., Lickey E. B., Schilling E. E. & Small R. L. 2007: Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. – Amer. J. Bot. **94:** 275–288.

Simmons M. P. & Ochoterena H. 2000: Gaps as characters in sequence-based phylogenetic analyses. – Syst. Biol. **49:** 369–381.

Simmons M. P., Ochoterena H. & Carr T. G. 2001: Incorporation, relative homoplasy and effect of gap characters in sequence-based phylogenetic analyses. – Syst. Biol. **50:** 454–462.

Standley P. C. & Steyermark J. A. 1944: *Heliocereus heterodoxus*. – Publ. Field Mus. Nat. Hist., Bot. Ser. **23:** 67–68.

Swofford D. L. 2002: PAUP*: Phylogenetic analysis using parsimony (and other methods) 4.0. – Sunderland: Sinauer Associates.

Taberlet P., Gielly L., Pautou G. & Bouvet J. 1991: Universal primers for amplification of the tree non-coding regions of chloroplast DNA. – Pl. Molec. Biol. **17:** 1105–1109.

Tate J. A. & Simpson B. B. 2003: Paraphyly of *Tarasa* (*Malvaceae*) and diverse origins of the polyploid species. – Syst. Bot. **28:** 723–737.

Vázquez-Sánchez M., Terrazas T., Arias S. & Ochoterena H. 2013: Molecular phylogeny, origin and taxonomic implications of the tribe *Cacteae* (*Cactaceae*). – Syst. Biodivers. **11:** 103–116.

Weingart W. 1910: *Cereus cinnabarinus* Eichlam spec. nov. – Monatsschr. Kakteenk. **20:** 161–162.

Willdenowia

Open-access online edition www.bioone.org/loi/will BioOne

Online ISSN 1868-6397 · Print ISSN 0511-9618 · Impact factor 0.721

Published by the Botanic Garden and Botanical Museum Berlin, Freie Universität Berlin

© 2016 The Authors · This open-access article is distributed under the CC BY 4.0 licence

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Appendix

Taxa included in plastid *rpl16*, *trnL-F*, *psbA-trnH*, *trnQ-rps16*, *matK* and *ycf1* phylogenetic analyses. Two-letter country codes follow ISO 3166-1 alpha-2.

Taxon	Source, voucher	GenBank accession number					
		rpl16	***trnL-F***	***psbA-trnH***	***trnQ-rps16***	***matK***	***ycf1***
Acanthocereus chiapensis Bravo	MX: Chiapas, *Guzmán 999*, MEXU	KU598005	KU598057	KU597952	KU598110	KU597899	KU598162
Acanthocereus oaxacensis (Britton & Rose) Lodé	MX: Oaxaca, *Arias 2185*, MEXU	KU598008	KU598060	KU597955	KU598113	KU597902	KU598165
Acanthocereus tetragonus (L.) Hummelinck	MX: Chiapas, *Guzmán 1002*, MEXU	KU598021	KU598074	KU597969	KU598127	KU597916	KU598178
Aporocactus flagelliformis (L.) Lem.	MX: Veracruz, *Cruz 01*, MEXU	KU597980	KU598032	KU597927	KU598085	KU597874	KU598138
Aporocatus flagelliformis	MX: Veracruz, *Cruz 02*, MEXU	KU597983	KU598035	KU597930	KU598088	KU597877	KU598141
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *Cruz 09*, MEXU	KU597986	KU598038	KU597933	KU598091	KU597880	KU598144
Aporocactus martianus	MX: Oaxaca, *Cruz 13*, MEXU	KU597989	KU598041	KU597936	KU598094	KU597883	KU598147
Disocactus ackermannii (Haw.) Ralf Bauer	MX: Oaxaca, *Cruz 10*, MEXU	KU597985	KU598037	KU597932	KU598090	KU597879	KU598143
Disocactus ackermannii	MX: Oaxaca, *Cruz 14*, MEXU	KU597988	KU598040	KU597935	KU598093	KU597882	KU598146
Disocactus anguliger (Lem.) M. Á. Cruz & S. Arias	MX: Jalisco, *Garcia 897*, MEXU	KU598026	KU598079	KU597974	KU598132	KU597921	KU598182
Disocactus aurantiacus (Kimnach) Barthlott	SV: Santa Ana, *Cerén 2580*, MHES	KU598010	KU598062	KU597957	KU598115	KU597904	KU598167
Disocactus aurantiacus	SV: Santa Ana, *Menjivar 2429*, MHES	KU598013	KU598066	KU597961	KU598119	KU597908	KU598171
Disocactus biformis (Lindl.) Lindl.	GT: Sacatepéquez, *Veliz 19901*, BIGU	KU598016	KU598069	KU597964	KU598122	KU597911	KU598174
Disocactus biformis	GT: Sacatepéquez, *Veliz 23600*, BIGU	KU598019	KU598072	KU597967	KU598125	KU597914	KU598176
Disocactus crenatus (Lindl.) M. Á. Cruz & S. Arias	MX: Oaxaca, *Cruz 11*, MEXU	KU598014	KU598067	KU597962	KU598120	KU597909	KU598172
Disocactus crenatus	MX: Chiapas, *Cruz 23*, MEXU	KU598017	KU598070	KU597965	KU598123	KU597912	KU598175
Disocactus eichlamii (Weing.) Britton & Rose	GT: Chimaltenango, *Veliz 19887*, BIGU	KU598011	KU598063	KU597958	KU598116	KU597905	KU598168
Disocactus ×*kimnachii* G. D. Rowley	CR: Heredia, *Hammel 26706*, INB	KU597984	KU598036	KU597931	KU598089	KU597878	KU598142
Disocactus lepidocarpus (F. A. C. Weber) M. Á. Cruz & S. Arias	CR: Limón, *Hammel 25624*, INB		KU598065	KU597960	KU598118	KU597907	KU598170
Disocactus macranthus (Alexander) Kimnach & Hutchison	MX: Veracruz, *Cruz 07*, MEXU	KU597982	KU598034	KU597929	KU598087	KU597876	KU598140
Disocactus macranthus	MX: Veracruz, *Cruz 08*, MEXU	KU597990	KU598042	KU597937	KU598095	KU597884	KU598148

(continued on next page)

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

(Appendix continued from previous page)

Taxon	Source, voucher	GenBank accession number					
		rpl16	***trnL-F***	***psbA-trnH***	***trnQ-rps16***	***matK***	***ycf1***
Disocactus nelsonii (Britton & Rose) Linding.	MX: Chiapas, *Cruz 17*, MEXU	KU597999	KU598051	KU597946	KU598104	KU597893	KU598156
Disocactus phyllanthoides (DC.) Barthlott	MX: Puebla, *Arias 1432*, MEXU	KU597981	KU598033	KU597928	KU598086	KU597875	KU598139
Disocactus phyllanthoides	MX: Veracruz, *Arias 2201*, MEXU	KU598025	KU598078	KU597973	KU598131	KU597920	KU598181
Disocactus quezaltecus (Standl. & Steyerm.) Kimnach	GT: Quezaltenango, *Veliz 20054*, BIGU	KU597995	KU598047	KU597942	KU598100	KU597889	KU598152
Disocactus speciosus subsp. *bierianus* Ralf Bauer	MX: Guerrero, *Salazar 6564B*, MEXU	KU597987	KU598039	KU597934	KU598092	KU597881	KU598145
Disocactus speciosus subsp. *cinnabarinus* (Eichlam ex Weing.) Ralf Bauer	GT: Chimaltenango, *Veliz 19579*, BIGU	KU598000	KU598052	KU597947	KU598105	KU597894	KU598157
Disocactus speciosus subsp. *heterodoxus* (Standl. & Steyerm.) M. Á. Cruz & S. Arias	MX: Chiapas, *Cruz 18*, MEXU	KU598006	KU598058	KU597953	KU598111	KU597900	KU598163
Disocactus speciosus subsp. *heterodoxus*	MX: Chiapas, *Cruz 22*, MEXU	KU597996	KU598048	KU597943	KU598101	KU597890	KU598153
Disocactus speciosus subsp. *heterodoxus*	MX: Chiapas, *Arias 1666*, MEXU	KU598020	KU598073	KU597968	KU598126	KU597915	KU598177
Disocactus speciosus (Cav.) Barthlott subsp. *speciosus*	MX: Jalisco, *Morales 01*, MEXU	KU597992	KU598044	KU597939	KU598097	KU597886	KU598150
Disocactus speciosus subsp. *speciosus*	GT: Quezaltenango, *Veliz 20104*, BIGU	KU598004	KU598056	KU597951	KU598109	KU597898	KU598161
Disocactus speciosus subsp. *speciosus*	MX: Oaxaca, *Cruz 16*, MEXU	KU597991	KU598043	KU597938	KU598096	KU597885	KU598149
Disocactus sp. 1	SV: Santa Ana, *Cerén 2578*, MHES	KU598007	KU598059	KU597954	KU598112	KU597901	KU598164
Disocactus sp. 1	SV: Santa Ana, *Menjivar 2428*, MHES	KU598003	KU598055	KU597950	KU598108	KU597897	KU598160
Epiphyllum cartagense Britton & Rose	CR: Alajuela, *Hammel 19793*, INB	KU598002	KU598054	KU597949	KU598107	KU597896	KU598159
Epiphyllum chrysocardium Alexander	MX: Chiapas, *MacDougall 198*, MEXU	KU598030	KU598083	KU597978	KU598136	KU597925	KU598186
Epiphyllum chrysocardium	MX: Chiapas, *Arias 1708*, MEXU	KU598001	KU598053	KU597948	KU598106	KU597895	KU598158
Epiphyllum hookeri Haw. subsp. *hookeri*	MX: Veracruz, *Cruz 03*, MEXU	KU597997	KU598049	KU597944	KU598102	KU597891	KU598154
Epiphyllum hookeri subsp. *pittieri* (F. A. C. Weber) Ralf Bauer	CR: Puntarenas, *Hammel 22194*, INB	KU598009	KU598061	KU597956	KU598114	KU597903	KU598166

(continued on next page)

Downloaded From: https://complete.bioone.org/journals/Willdenowia on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

(Appendix continued from previous page)

Taxon	Source, voucher	GenBank accession number					
		rpl16	***trnL-F***	***psbA-trnH***	***trnQ-rps16***	***matK***	***ycf1***
Epiphyllum phyllanthus (L.) Haw. subsp. *phyllanthus*	SR: *Hammel 22377*, INB	KU598015	KU598068	KU597963	KU598121	KU597910	KU598173
Epiphyllum pumilum Britton & Rose	MX: Veracruz, *Cruz 06*, MEXU	KU597993	KU598045	KU597940	KU598098	KU597887	
Epiphyllum thomasianum (K. Schum.) Britton & Rose	PA: Cocle, *Hammel 22020*, INB	KU598018	KU598071	KU597966	KU598124	KU597913	
Hylocereus ocamponis (Salm-Dyck) Britton & Rose	MX: Guerrero, *Gama 104*, MEXU	KU598027	KU598080	KU597975	KU598133	KU597922	KU598183
Hylocereus purpusii (Weing.) Britton & Rose	MX: Oaxaca, *Guzmán 1095*, MEXU	KU598028	KU598081	KU597976	KU598134	KU597923	KU598184
Lemaireocereus hollianus (F. A. C. Weber) Britton & Rose	MX: Puebla, *Arias 1373*, MEXU	KU598012	KU598064	KU597959	KU598117	KU597906	KU598169
Pereskia lychnidiflora DC.	MX: Oaxaca, *Arias 1153*, MEXU	KU598024	KU598077	KU597972	KU598130	KU597919	KU598188
Pseudorhipsalis amazonica (K. Schum.) Ralf Bauer	PA: Colon, *Hammel 24524*, INB	KU597994	KU598046	KU597941	KU598099	KU597888	KU598151
Pseudorhipsalis himantoclada (Rol.-Goss.) Britton & Rose	CR: San José, *Hammel 22076*, INB	KU597998	KU598050	KU597945	KU598103	KU597892	KU598155
Selenicereus atropilosus Kimnach	MX: Jalisco, *Arreola 1473*, MEXU	KU598029	KU598082	KU597977	KU598135	KU597924	KU598185
Selenicereus donkelaarii Britton & Rose	MX: Yucatán, *Arias 1089*, MEXU	KU598022	KU598075	KU597970	KU598128	KU597917	KU598179
Selenicereus grandiflorus (L.) Britton & Rose	MX: Veracruz, *Guzmán 1365*, MEXU	KU598023	KU598076	KU597971	KU598129	KU597918	KU598180
Weberocereus glaber (Eichlam) G. D. Rowley	MX: Chiapas, *Bravo 5614*, MEXU	KU598031	KU598084	KU597979	KU598137	KU597926	KU598187