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

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

**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, Nopalxochia* and *Pereskiopsis*). 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. biforms* 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, *Hylocersea*, *Hylocereeae*, maximum parsimony, molecular phylogeny, *Pseudorhipsalis*, taxonomy

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**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
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) | Kimmach (1993) | Hunt & al. (2006) |
|----------------|-----------------------|---------------------|---------------------|----------------|-----------------|
| *D. biformis*  | *D. biformis*          | *D. biformis*       | *D. biformis*       | *D. biformis*  | *D. biformis*   |
| *D. eichlami* | *D. eichlami*         | *D. eichlami*       | *D. eichlami*       | *D. eichlami*  | *D. eichlami*   |
| *Aporocactus* | *A. conzattii*        | *A. nelsonii*       | *A. nelsonii*       | *A. nelsonii*  | *A. nelsonii*   |
| *A. flagelliformis* | *A. flagelliformis* | *A. flagelliformis* | *A. flagelliformis* | *A. flagelliformis* | *A. flagelliformis* |
| *A. leptophi* | *A. leptophi*         | *A. leptophi*       | *A. leptophi*       | *A. leptophi*  | *A. leptophi*   |
| *A. martianu* | *A. martianus*        | *A. martianus*      | *A. martianus*      | *A. martianus* | *A. martianus*  |
| *Chiapasia*   | *C. nelsonii*         | *A. frigifemris*    | *A. frigifemris*    | *A. frigifemris* | *A. frigifemris* |
| *Heliocereus* | *H. ameacemensis*     | *H. cinnabinus*     | *H. cinnabinus*     | *H. cinnabinus* | *H. cinnabinus* |
| *H. eglantissim* | *H. eglantissim*     | *H. eglantissim*   | *H. eglantissim*   | *H. eglantissim* | *H. eglantissim* |
| *H. schrankii* | *H. speciosus*       | *H. speciosus*      | *H. speciosus*      | *H. speciosus*  | *H. speciosus*  |
| *Nopalxochia* | *N. phyllanthoides*  | *N. phyllanthoides* | *N. phyllanthoides* | *N. phyllanthoides* | *N. phyllanthoides* |
| *Pseudorhipsalis* | *P. alata*         | *P. alata*         | *P. alata*         | *P. alata*     | *P. alata*     |
| *P. himantoclad* | *P. himantoclad*   | *P. himantoclad*   | *P. himantoclad*   | *P. himantoclad* | *P. himantoclad* |
| *Wittia*      | *W. amazonica*       | *W. panamensis*     | *W. panamensis*     | *W. panamensis* | *W. panamensis* |

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. 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 subgenaera, the pericarpel exhibits spines, scales or visible areoles. In particular, *D. subg. Aporocactus* exhibits stems with 8–11 ribs, areoles...
Table 2. Summary of infrageneric groups in *Disocactus* according to recent taxonomic studies.

| 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 | | |
| Pseudorhipsalis | *Disocactus* | |
| D. macranthus | D. biformis | |
| Disocactus | | |
| D. acuminatus | | |
| D. baumgartneri | | |
| D. eichlamii | D. macranthus | |
| D. horichii | D. nelsonii | |
| D. macranchus | | |
| D. lankesterianus | D. quezaltecus | |
| D. nelsonii | | |
| D. quezaltecus | Nopalxochia | |
| | | D. amazonicus |
| Nopalxochia | D. amazonicus | |
| | | D. phyllanthoides |
| Wittia | D. amazonicus | |

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 *Epiphylllum* Haw., along with seven other genera (*Chiapasia*, *Eccremocactus* Britton & Rose, *Epiphylliatus* A. Berger, *Nopalxochia*, *Schlumbergera* Lem., *Wittia* and *Zygocactus* K. Schum.), comprise the subtribe *Epiphyllineae*, 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 *Hylocereae*, which also includes *Epiphyl- lumn*, *Hylocereus* (A. Berger) Britton & Rose, *Pseudo- rhipsalis*, *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 *Hylocereae*, 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. (Schlumberger & 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 *Hylocereae* 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 *Hylocereae*. However, in the analyses performed by Hernández-Hernández & al. (2011), *Epiphylllum* diverged early within *Hylocereae*, whereas
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. bifrons*, 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 24324 (INB); L: *Aporocactus flagelliformis*, Mexico, Veracruz, M. Á. Cruz 1 (MEXU).
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 to: (1) carry out a phylogenetic reconstruction of the genus Disocactus using six chloroplast molecular markers (matK, psbA-trnH, rpl16, trnL-F, trnQ-rrps16 and ycf1) to evaluate whether Disocactus and the subgenus recognized by Hunt et 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 Hylocereaeae; 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 et 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. biflorus (Lindl.) Lindl., D. macranthus (Alexander) Kimmich & 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. Lophostocereus (A. Berger) Britton & Rose (Hartmann & al. 2001), Mammillaria (Harpe & 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-rrps16) 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, but the incubation time in tissue lysis buffer was extended to 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 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 trnL intron was amplified using the primers trnL1F and trnL1R (Shaw & al. 2007), with initial denaturation of the DNA for 2 min at 94 °C, followed by 26 cycles of 1 min at 94 °C, 50 s at 55 °C and 2 min at 72 °C, 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 °C, 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-rrps16 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...
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). Using the MP criteria, 57 equally parsimonious trees were recovered; 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 anguilliger (Lem.) H. P. Kelsey & Dayton was recovered as sister species of sub-
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*...
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,
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.
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) Lind. 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 Kimmach (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
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. xkimnachii* 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. xkimnachii* (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. xkimnachii* is found only in Costa Rica. The initial hypothesis about the origin of *D. xkimnachii* 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 (Bauer-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 *Hylocereeinae* 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 *Hylocereeinae*. 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
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 polycoplate. 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 (16 cm) and funnel-shaped. The flowers are not oriented towards the light, and 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 *Hylocereeae* 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 Edward’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

≡ *Nopalxochia conzattianum* T. MacDoug. Ralf Bauer in Cactaceae Syst. Init. 17: 17. 2003 ≡ *Disocactus conzattianus* T. MacDoug. in Cact. Succ. J. (Los Angeles) 19: 22. 1947 = *Psudonopalxochia 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 = *Helilocereus conzattianus* (T. MacDoug.) Doweld in Sukkulenty 4(1–2): 42. 2002 ≡ *Disocactus ackermannii* subsp. conzattianus (T. MacDoug.) U. Guzmán in
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 darrahi* K. Schum., Gesamtb. Kakt. Nachr.: 69. 1903 = *Epiphyllum darrahi* (K. Schum.) Britton & Rose in Contr. U.S. Natl. Herb. 16: 256. 1913. – **Neotype (designated here):** [illustration] “*Phyllocactus darrahi* K. Schum.” in Gürke, Bluh. Katkeen. 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, type: 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 = *Disocactus 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 Atozaco, gorge of Rio Guacalote, 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 eichliamii* (Weing.) Britton & Rose in Contr. U.S. Natl. Herb. 16: 259. 1913 = *Phyllocactus eichliamii* Weing. in Monatschr. Katkeen. 21: 5. 1911 = *Trochilocactus eichliamii* (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 lepido- carpus* 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* Véliz & al. in Cact.-Avent. Int. 104: 2. 2014. – Holotype: Guatemala, Quetzaltenango, Zunil, on volcanic rocks with *Tillandsia tectanensis* 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) Dowell in Sukkulenty 42(1–2): 42. 2002. – Lectotype (designated by Bauer in Cactaceae Syst. Init. 17: 27. 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 = *Disorhispalis macrantha* (Alexander) Dowell 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 Monatschr. Katkeen. 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!).
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: 119. 1918.

12. *Disocactus phyllanthoides* (DC.) Doweld in Sukkulenty 4 (1): 205. 1923 (DC.) Link, Handbuch 2: 11. 1829 = *Epiphyllum phyllanthoides* (Phyllocactus 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, Launert L02/60I (ZSS).

13. *Disocactus quezaltecus* (Standl. & Steyerm.) Kimnach in Cact. Succ. J. (Los Angeles) 31: 137. 1959 = *Bonini 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 Mujliá, 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. Pflanzenfam. 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, Guerre-ro, zwischen Fito de Caballos und Puerto del Gallo, c. 2000 m, 23 Feb 1992, Launert 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, Chiafas, Cerro Sabandillo, near rio Monoblanco and the border of Oaxaca, Mar 1951, T. MacDougall A202 (HNT!; isotype: CAS barcode 0027330!).

14c. *Disocactus speciosus* subsp. *cinnaBARNARius* (Eichl. ex Wéing.) Ralf Bauer in Cactaceae Syst. Init. 17: 15. 2003 = *Cereus cinnaBARNARius* (Eichl. ex Wéing.) Britton & Rose, Cact. 2: 129. 1920 = *Disocactus cinnaBARNARius* (Eichl. ex Wéing.) Barthlott in Bradleya 9: 87. 1991. – Neotype (designated by Doweld in Sukkulenty 4(1–2); 42. 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 rio Vega between San Rafael and the NE portion of Volcán de Tacaná, 2500–3000 m, 21 Feb 1940, J. A. Steyermark 32691 (F barcode 0052879!).

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. Itó, Cacti: 146. 1952. – Lectotype (designated here): [illustration] “Cactus speciosissimum” in Desfontaines in Mém. Mus. Hist. Nat. 3: [t. ix] p. 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. Natl. 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. Gartenzei-tung 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. 1929 = *Heliocereus speciosus* var. *superbus* (Ehrenb.) Backeb., Cactaceae (Backeb.) 4: 2120. 1960.

= *Cereus amecamecensis* Heese in Rother, Prakt. Ratgeber Obst-Gartenbau 11: 442. 1896 = *Heliocereus amecamecensis* (Heese) Britton & Rose in Contr. U.S. Natl. Herb. 12: 433. 1909 = *Cereus speciosus* var. *ameca-
mensis (Heese) Weing. in Berger, Kakteen: 131. 1929 = Helianthus speciosus var. amecamensis (Heesse) Bravo in Cact. Succ. Mex. 19: 47. 1974 = Disocactus speciosus f. amecamensis (Heesse) Barthlott in Bradleyn 9: 87. 1991 = Helianthus speciosus subsp. amecamensis (Heesse) Doweld in Sukkulenty 4(1–2): 41. 2002.

Cereus serratius Weing. in Monatsschr. Kakteenk. 22: 185. 1912 = Helianthus serratius (Weing.) Borg, Cacti: 176. 1951 = Helianthus speciosus var. serratia (Weing.) Backeb., Cactaceae 4: 2121. 1960.

Heliocereus elegantissimus var. stenopetalus Bravo ex S. Arias & al. in Cact. Succ. Mex. 37: 72. 1992 = Helianthus schrankii var. stenopetalus (Bravo ex S. Arias & al.) Kimnach in Cact. Succ. J. (Los Angeles) 56: 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. helena Scheinvar in Phytologia 49: 317. 1981 = Heliocereus schrankii var. helena (Scheinvar) Kimmich in Cact. Succ. J. (Los Angeles) 68: 217. 1996 = Heliocereus elegantissimus subsp. helena (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 skimmnachii G. D. Rowley in Brit. Cact. Succ. J. 5: 84. 1987 (pro sp.) = Nopalxochia horichii Kimmich in Cact. Succ. J. (Los Angeles) 56: 6. 1984 [non Disocactus horichii Kimmich 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 Rio Durazno, c. 1400 m, [7 Jan] 1971, C. K. Horich s.n. (HNT barcode 0000023!; isotypes: CR, F, US barcode 00115768!)

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**Appendix**

Taxa included in plastid *rpl16*, *trnL-F*, *psbA-trnH*, *trnQ-psi16*, *matK* and *ycf1* phylogenetic analyses. Two-letter country codes follow ISO 3166-1 alpha-2.

| Taxon                        | Source, voucher | GenBank accession number |
|------------------------------|-----------------|--------------------------|
| *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.) Hummelínck | 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 |
| *Aporocactus flagelliformis* | MX: Veracruz, Cruz 02, MEXU | KU597983 KU598035 KU597930 KU598088 KU597877 KU598141 |
| *Aporocactus martianus* (Zucc.) Britton & Rose | MX: Oaxaca, Cruz 09, MEXU | KU597986 KU598033 KU597933 KU598091 KU597880 KU598144 |
| *Aporocactus martianus* | MX: Veracruz, Cruz 02, MEXU | KU597989 KU598041 KU597936 KU598094 KU597883 KU598147 |
| *Disocactus ackermannii* (Haw.) Ralf Bauer | MX: Oaxaca, Cruz 13, MEXU | KU597985 KU598037 KU597932 KU598090 KU597879 KU598143 |
| *Disocactus ackermannii* | MX: Oaxaca, Cruz 10, MEXU | KU597988 KU598040 KU597935 KU598093 KU597882 KU598146 |
| *Disocactus anguliger* (Lem.) M. Á. Cruz & S. Arias | MX: Jalisco, Garcia 897, MEXU | KU598026 KU598074 KU597974 KU598135 KU597921 KU598182 |
| *Disocactus aurantiacus* (Kimnach) Barthlott | SV: Santa Ana, Ceréén 2580, MHES | KU598010 KU598062 KU597957 KU598115 KU597904 KU598167 |
| *Disocactus aurantiacus* | SV: Santa Ana, Menjivar 2429, MHES | KU598013 KU598066 KU597961 KU598119 KU597908 KU598171 |
| *Disocactus biforis* (Lindl.) Lindl. | GT: Sacatepéquez, Veliz 19901, BIGU | KU598016 KU598069 KU597964 KU598122 KU597911 KU598174 |
| *Disocactus biforis* | 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 xkimnachii* 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)
| 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.) Kimmich | GT: Quezaltenango, Veliz 20054, BIGU | KU597995 | KU598047 | KU597942 | KU598100 | KU597889 | KU598152 |
| Disocactus speciosus subsp. bierianus Ralf Bauer | MX: Chiapas, Cruz 22, MEXU | KU597996 | KU598048 | KU597943 | KU598101 | KU597890 | KU598153 |
| Disocactus speciosus subsp. heterodoxus (Standl. & Steyerm.) M. A. Cruz & S. Arias | MX: Chiapas, Cruz 18, MEXU | KU598006 | KU598058 | KU597953 | KU598111 | KU597900 | KU598163 |
| 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, MHS | KU598007 | KU598059 | KU597954 | KU598112 | KU597901 | KU598164 |
| Disocactus sp. 1 | SV: Santa Ana, Menjivar 2428, MHS | 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)
| Taxon                                    | Source, voucher                  | GenBank accession number |
|-----------------------------------------|----------------------------------|-------------------------|
| *Epiphyllum phyllanthus* (L.) Haw. subsp. *phyllanthus* | SR: Hammel 22377, INB             | rpl16 KU598015, trnL-F KU598068, psbA-trnH KU597963, trnQ-rps16 KU598121, matK KU597910, ycf1 KU598173 |
| *Epiphyllum pumilum* Britton & Rose    | MX: Veracruz, Cruz 06, MEXU      | rpl16 KU597993, trnL-F KU598045, psbA-trnH KU597940, trnQ-rps16 KU598098, matK KU597887, ycf1 KU598173 |
| *Epiphyllum thomasianum* (K. Schum.) Britton & Rose | PA: Coce, Hammel 22020, INB     | rpl16 KU598018, trnL-F KU598071, psbA-trnH KU597966, trnQ-rps16 KU598124, matK KU597913, ycf1 KU598173 |
| *Hylocereus ocamponis* (Salm-Dyck) Britton & Rose | MX: Guerrero, Gama 104, MEXU    | rpl16 KU598027, trnL-F KU598080, psbA-trnH KU597975, trnQ-rps16 KU598133, matK KU597922, ycf1 KU598183 |
| *Hylocereus purpurii* (Weing.) Britton & Rose | MX: Oaxaca, Guzmán 1095, MEXU  | rpl16 KU598028, trnL-F KU598081, psbA-trnH KU597976, trnQ-rps16 KU598134, matK KU597923, ycf1 KU598184 |
| *Lemaireocereus hollianus* (F. A. C. Weber) Britton & Rose | MX: Puebla, Arias 1373, MEXU  | rpl16 KU598012, trnL-F KU598064, psbA-trnH KU597959, trnQ-rps16 KU598117, matK KU597906, ycf1 KU598169 |
| *Pereskia lychnidiflora* DC.            | MX: Oaxaca, Arias 1153, MEXU    | rpl16 KU598024, trnL-F KU598077, psbA-trnH KU597972, trnQ-rps16 KU598130, matK KU597919, ycf1 KU598188 |
| *Pseudorhipsalis amazonica* (K. Schum.) Ralf Bauer | PA: Colon, Hammel 24524, INB | rpl16 KU597994, trnL-F KU598046, psbA-trnH KU597941, trnQ-rps16 KU598099, matK KU597888, ycf1 KU598151 |
| *Pseudorhipsalis himantoclada* (Rol.-Goss.) Britton & Rose | CR: San José, Hammel 22076, INB | rpl16 KU597998, trnL-F KU598050, psbA-trnH KU597945, trnQ-rps16 KU598103, matK KU597892, ycf1 KU598155 |
| *Selenicereus atropilosus* Kimmich     | MX: Jalisco, Arreola 1473, MEXU | rpl16 KU598029, trnL-F KU598082, psbA-trnH KU597977, trnQ-rps16 KU598135, matK KU597924, ycf1 KU598185 |
| *Selenicereus donkelarrii* Britton & Rose | MX: Yucatán, Arias 1089, MEXU | rpl16 KU598022, trnL-F KU598075, psbA-trnH KU597970, trnQ-rps16 KU598128, matK KU597917, ycf1 KU598179 |
| *Selenicereus grandiflorus* (L.) Britton & Rose | MX: Veracruz, Guzmán 1365, MEXU | rpl16 KU598023, trnL-F KU598076, psbA-trnH KU597971, trnQ-rps16 KU598129, matK KU597918, ycf1 KU598180 |
| *Weberocereus glaber* (Eichlam) G. D. Rowley | MX: Chiapas, Bravo 5614, MEXU | rpl16 KU598031, trnL-F KU598084, psbA-trnH KU597979, trnQ-rps16 KU598137, matK KU597926, ycf1 KU598187 |