

Unravelling phylogenetic relationships of the tribe Cereeae using target enrichment sequencing

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- **Background and Aims** Cactaceae are succulent plants, quasi-endemic to the American continent, and one of the most endangered plant groups in the world. Molecular phylogenies have been key to unravelling phylogenetic relationships among major cactus groups, previously hampered by high levels of morphological convergence. Phylogenetic studies using plastid markers have not provided adequate resolution for determining generic relationships within cactus groups. This is the case for the tribe Cereeae *s.l.*, a highly diverse group from tropical America. Here we aimed to reconstruct a well-resolved phylogenetic tree of tribe Cereeae and update the circumscription of suprageneric and generic groups in this tribe.
- **Methods** We integrated sequence data from public gene and genomic databases with new target sequences (generated using the customized Cactaceae591 probe set) across representatives of this tribe, with a denser taxon sampling of the subtribe Cereinae. We inferred concatenated and coalescent phylogenetic trees and compared the performance of both approaches.
- **Key Results** Six well-supported suprageneric clades were identified using different datasets. However, only genomic datasets, especially the Cactaceae591, were able to resolve the contentious relationships within the subtribe Cereinae.
- **Conclusions** We propose a new taxonomic classification within Cereeae based on well-resolved clades, including new subtribes (Aylosterinae subtr. nov., Uebelmanniinae subtr. nov. and Gymnocalyciinae subtr. nov.) and revised subtribes (Trichocereinae, Rebutiinae and Cereinae). We emphasize the importance of using genomic datasets allied with coalescent inference to investigate evolutionary patterns within the tribe Cereeae.

Key Words: Cactaceae, succulents, systematics, phylogenomics, Angiosperm353, Cactaceae591, target sequencing.

INTRODUCTION

Cactus species are mostly succulent, popular plants found primarily in dry and arid regions in the Neotropics. This family is one of the most diverse groups of succulent plants in the Caryophyllales (Hernández-Ledesma *et al.*, 2015), and one of the most endangered plant families in the world (Goettsch *et al.*, 2015; Amaral *et al.*, 2022; Pillet *et al.*, 2022). Over the last two decades, molecular phylogenetics have been fundamental to elucidating phylogenetic relationships among major lineages of this family (Nyffeler, 2002; Bárcenas *et al.*, 2011; Hernández-Hernández *et al.*, 2011), previously hindered by the high level of morphological convergence. Traditionally, this family has been subdivided into four subfamilies, namely ‘Pereskioideae’ (possibly non-monophyletic; Edwards *et al.*, 2005; Walker *et al.*, 2018), Maihuenioideae, Opuntioideae and Cactoideae (Guerrero *et al.*, 2019), the last exhibiting the greatest species diversity and morphological variation (Anderson, 2001; Applequist and Wallace, 2002; Hunt *et al.*, 2006; Nyffeler and Egli, 2010; Hernández-Hernández *et al.*, 2011). Regardless of these efforts, the delimitation of suprageneric lineages within the subfamily

Cactoideae has long been a subject of debate, particularly for the core Cactoideae II (Guerrero *et al.*, 2019), a group of tropical American cacti with unclear phylogenetic placement.

The main systematic controversy within the core Cactoideae II is a well-supported clade composed of globose and columnar cacti from the former tribes Browningieae, Cereeae and Trichocereae (also known as the BCT clade, Nyffeler, 2002), which exhibited a recent radiation in the late Miocene (~5 Mya; Arakaki *et al.*, 2011; Hernández-Hernández *et al.*, 2014). The relationships among these tribes remained unknown due to the non-monophyly at the tribal and generic levels (Nyffeler, 2002; Ritz *et al.*, 2007, 2016; Hernández-Hernández *et al.*, 2011; Schlumberger and Renner, 2012; Franck *et al.*, 2013; Lendel, 2013; Calvente *et al.*, 2017; Franco *et al.*, 2017; Fantinati *et al.*, 2021; Romeiro-Brito *et al.*, 2022). The most recent systematic classification proposed for the family broadened the circumscription of the tribe Cereeae, including all representatives from clade BCT (tribe Cereeae *s.l.*), and divided it into three subtribes: Cereinae, Trichocereinae and Rebutiinae (Fig. 1; Nyffeler and Egli, 2010). Despite this taxonomic

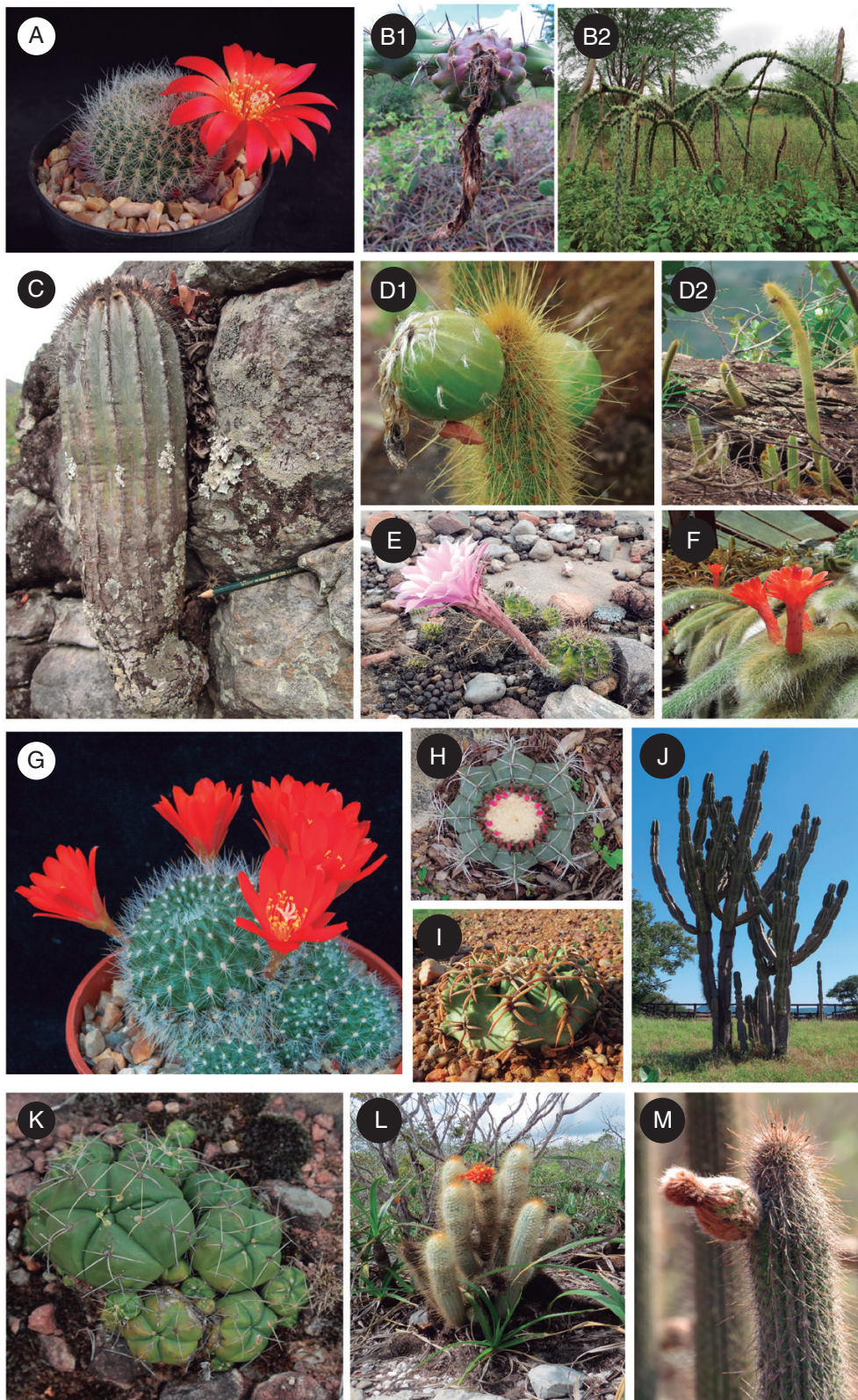


FIG. 1. Representatives of subtribe Rebutiinae (A, C, G, K), Trichocereinae (B1, B2, D1, D2, E, F) and Cereinae (H, I, J, L, M), *sensu Nyffeler and Egli (2010)*, showing different growth forms. (A) *Rebutia minuscula* K.Schum, (B1 and B2) *Harrisia adscendens* (Gürke) Britton & Rose, (C) *Uebelmannia pectinifera* Buining, (D1 and D2) *Arthrocereus rondonianus* Backeb. & Völl, (E) *Echinopsis* sp., (F) *Cleistocactus winteri* D.R.Hunt, (G) *Aylostera fiebrigii* (Gürke) Backeb., (H) *Melocactus glaucescens* Buining & Brederoo, (I) *Discocactus bahiensis* Britton & Rose, (J) *Cereus jamacaru* DC., (K) *Gymnocalycium denudatum* (Link & Otto) Pfeiff. ex Mittler, (L) *Micranthocereus auriazureus* Buining & Brederoo and (M) *Facheiroa squamosa* (Gürke) P.J.Braun & Esteves. Photo credits: A and G: M. Lowry; B, D, H, I, L and M: M. C. Telhe; C: E. M. Moraes; E and K: M. Kohler; F and J: M. Romeiro-Brito.

rearrangement, non-monophyletic groups continued to exist in the tribe Cereeae *s.l.* (Schlumberger and Renner, 2012; Romeiro-Brito et al., 2022).

Few studies have investigated phylogenetic relationships using morphological or molecular data at the suprageneric level in the tribe Cereeae *s.l.* (Taylor and Zappi, 1989; Soffiatti, 2003; Lendel, 2013; Fantinati et al., 2021). The circumscription of the subtribes within Cereeae lacks apomorphic characteristics capable of distinguishing them, particularly between the subtribes Trichocereinae and Cereinae (treated at tribal level in Taylor and Zappi, 2004). While the subtribe Trichocereinae traditionally included taxa with flowers and fruits with trichomes, bristles and scales, the subtribe Cereinae is characterized by species with naked flowers (or with minute scales; Barthlott and Hunt, 1993). Thought to be the result of convergent phenotypic evolution, these reproductive morphological characters have not been sufficient for delimiting subtribes and genera in either group (Taylor and Zappi, 1989; Schlumberger and Renner, 2012; Franck et al., 2013). Moreover, molecular systematic studies in this group have shown low phylogenetic support to delineate groups at generic levels (Lendel, 2013; Fantinati et al., 2021). The lack of phylogenetic resolution might be due to the recent and rapid diversification of this group during the late Miocene (Hernández-Hernández et al., 2014), but also insufficient phylogenetic signal when using only a few traditional molecular markers.

The use of next-generation sequencing has been emerging as an alternative to traditional molecular markers to study phylogenetic relationships in Cactaceae (Franco et al., 2022). To date, most of the phylogenomic studies conducted in the family have focused on examining relationships at generic and specific levels using different high-throughput sequencing approaches (RAD-seq: Bombonato et al., 2020; Amaral et al., 2021b; GBS: Merklinger et al., 2021; Breslin et al., 2021; genome skimming: Majure et al., 2019, 2021, 2022). The recent availability of genome and transcriptome sequencing of Cactaceae representatives (Copetti et al., 2017; Walker et al., 2018; Wang et al., 2019; Amaral et al., 2021a; Chen et al., 2022) has enabled the development of lineage-specific target sequencing by hybridization capture in Cactaceae (Acha and Majure, 2022; Romeiro-Brito et al., 2022). The Cactaceae591, for instance, is a probe set targeting both non-coding and coding orthologue regions, mostly nuclear, which has proven to be an effective resource for unravelling relationships at shallow and deep levels in Cactaceae (Romeiro-Brito et al., 2022) as well as for resolving the contentious relationship of a recently

diverged genus in this group (N. P. Taylor et al., unpubl. res.). Therefore, the Cactaceae591 probe set is a potential source of data for establishing a phylogenetic hypothesis and proposing taxonomic adjustments within Cereeae.

In this study, our main goal was to infer suprageneric relationships within the tribe Cereeae *s.l.* and generic relationships in the subtribe Cereinae, proposing a new taxonomic classification. To this end, we integrated newly generated genome-scale data gathered with the Cactaceae591 target sequencing probe set and with sequence data from public databases. Considering the long-lasting controversies in the systematics of Cactoideae (Nyffeler and Eggli, 2010), we assume as hypotheses the non-monophyly of the current circumscription of subtribes Cereinae, Rebutiinae and Trichocereinae, as well as of the current generic delimitation within the subtribe Cereinae. Furthermore, we explored the potential of concatenated and coalescent inference approaches in resolving contentious phylogenetic relationships in the subtribe Cereinae.

MATERIALS AND METHODS

Datasets and taxon sampling

To explore distinct gene and genome-scale datasets for estimating the relationships within the tribe Cereeae, we used different datasets compiled from newly generated sequences as well as publicly available data. Our datasets were named as follows: ‘Cactaceae591’ was composed of newly generated data of more than 500 nuclear regions obtained with the Cactaceae591 probe set (Romeiro-Brito et al., 2022); ‘Angiosperm353’ was composed of data of more than 300 nuclear regions generated with the Angiosperm353 universal probe set (Johnson et al., 2016), retrieved from the Kew Tree Of Life Explorer Platform (Baker et al., 2022, <https://treeoflife.kew.org/tree-of-life>); and ‘gene-scale’ consisted of sequences of eight plastid (*rbcl*, *atpB-rbcl*, *trnK-matK*, *rpL16*, *petL-psbE*, *trnT-trnL*, *trnL-trnF*, *trnS-trnG*) and two nuclear (*phyC* and *ppc*) regions, which were recovered from the Cactaceae591 raw data and from GenBank (National Center for Biotechnology Information, NCBI). The Cactaceae591 and Angiosperm353 probe sets have 13 shared loci (Romeiro-Brito et al., 2022). However, only a limited subset of these loci was common to both datasets. Given the relatively small number of shared base pairs that could be recovered (Table 1), it was not feasible to conduct a phylogenetic analysis using both datasets simultaneously.

TABLE 1. Genetic statistics of nucleotide variation calculated for the datasets used in the present study.

Dataset	No. of loci	No. of taxa	Alignment length (bp)	Percentage missing data	S (%)	PIS (%)	Supported nodes (%)
Cactaceae591	459	146	511 090	15.75	287 596 (0.563)	171 109 (0.335)	94.1/93.5
Angiosperm353	318	84	136 589	12.63	59 975 (0.439)	27 508 (0.201)	82.3/87.1
Shared loci Cactaceae591 and Angiosperm353	5	105	2873	25.53	1222 (0.425)	601 (0.209)	–
Gene-scale	10	209	9923	34.36	3502 (0.353)	1901 (0.191)	63.4

S: variable sites, PIS (%): parsimony-informative sites (proportion of PIS). Supported nodes: percentage of supported nodes from concatenated inferences for the gene-scale (ultrafast bootstrap > 95) and from concatenated and coalescent inferences for the genome-scale datasets (ultrafast bootstrap > 95/local posterior probability > 0.8). No. of taxa includes both ingroup and outgroup sampled taxa in each dataset.

The Cactaceae591 dataset consisted of 29 of 48 recognized genera and 122 of 561 recognized species from the tribe Cereeae *s.l.* (according to Korotkova *et al.*, 2021). This sampling comprised all genera from the subtribe Cereinae (14 genera), three of seven genera from the subtribe Rebutiinae, and 12 of 27 genera from the subtribe Trichocereinae (Supplementary Data Table S1). To ascertain the phylogenetic position of monotypic genera (e.g. *Espositoopsis* Buxb. and *Leocereus* Britton & Rose) or lineages with controversial taxonomy (e.g. species from the genus *Micranthocereus* Backeb.), we sampled two specimens of each from different geographical records or different collectors.

The Angiosperm353 dataset included 43 of 48 recognized genera and 53 of 561 recognized species from the tribe Cereeae *s.l.* This sampling comprised all genera from the subtribe Cereinae, six of seven genera from the subtribe Rebutiinae, and 23 of 27 genera from the subtribe Trichocereinae (Supplementary Data Table S1).

The gene-scale sampling dataset was obtained using the SuperCRUNCH v.1.3 pipeline (Portik and Wiens, 2020) to parse, edit and generate the dataset gathered from public repositories. First, we updated the recognized species name according to synonyms from the latest checklist in Korotkova *et al.* (2021) and then selected all sequences from the tribe Cereeae *s.l.*, allowing up to 60 % of missing data for each locus. Whenever a taxon presented multiple entries for a given locus, we prioritized selecting the longest sequence or, if available, the one obtained from our Cactaceae591 dataset (details of the final dataset in Supplementary Data Table S2). This dataset included 47 of 48 recognized genera and 177 of 561 recognized species from the tribe Cereeae *s.l.* The final sampling comprised all genera from the subtribe Cereinae and Rebutiinae, and only one genus (*Weberbauerocereus* Backeb.) from the subtribe Trichocereinae is missing (Table S2).

We attempted to select the same species and/or genus for our outgroup sampling in the three datasets (Supplementary Data Tables S1 and S2). The outgroup taxa included representatives of major clades of the subfamily Cactoideae (tribe Notocactaeae, tribe Rhipsalideae, tribe Phyllocactaeae and tribe Cactaeae; *sensu* Nyffeler and Eggli, 2010), representatives of the subfamilies Opuntioideae and Pereskioideae, and one representative of Portulacaceae (*Portulaca hirsutissima* Cambess.).

DNA extraction and target capture sequencing library of the Cactaceae591 dataset

Genomic DNA from the Cactaceae591 dataset was extracted from the root and epidermis of preserved tissues or herbarium material using a high-salt CTAB protocol [modified from Martínez-González *et al.* (2017) and Inglis *et al.* (2018) and detailed in Supplementary Data Appendix S1]. We isolated and enriched 591 regions using the Cactaceae591 probe set (Romeiro-Brito *et al.*, 2022). This probe set includes 587 genic (exonic and intronic regions) and intergenic nuclear regions and also four regions commonly used in Cactaceae studies (plastidial regions: *trnK-matK* and *rbcl*; nuclear regions: *ppc* and *phyc*). Library preparation and sequencing were performed by RAPiD Genomics LLC (Gainesville, FL, USA) using their high-throughput workflow with proprietary chemistry. Samples

were pooled in equimolar concentrations and sequenced on a NovaSeq 6000 (2 × 150 and 2 × 250 bp).

Processing raw reads of the Cactaceae591 dataset

Raw reads from the Cactaceae591 dataset were trimmed using AdapterRemoval v.2 (Schubert *et al.*, 2016), removing poor quality reads (phred < 20), adapters and short reads (<60 bp). The trimmed reads were mapped and assembled using the HybPiper v.2.0.3 pipeline (Johnson *et al.*, 2016, <https://github.com/mossmatters/HybPiper>). We obtained the 591 on-target regions using the DNA sequence references available in Romeiro-Brito *et al.* (2022), setting eight reads as the minimum coverage. The putative paralogues identified in Romeiro-Brito *et al.* (2022) were removed from the final dataset. We skimmed additional plastid regions commonly used for Cereeae *s.l.* phylogenies (*atpB-rbcl*, *rpL16*, *petL-psbE*, *trnT-trnL*, *trnL-trnF*, *trnS-trnG*; Supplementary Data Appendix S1) as off-target regions by using Cactaceae reference sequences and setting the minimum coverage reads to 4.

Alignment and trimming genome-scale datasets

We used MAFFT v.7 (Katoh and Standley, 2013) for sequence alignment and trimAL v.1.3 (Capella-Gutiérrez *et al.*, 2009) for removing gaps found in at least 40 % of the sequences and poorly aligned regions.

We removed from the Cactaceae591 and Angiosperm353 datasets the nuclear regions with more than 60 % of missing data and the outlier regions using the genesortR script (Mongiardino Koch, 2021). Here the outlier loci were represented by loci with phylogenetic metrics that deviated significantly from all other loci. This script uses phylogenetic signal (e.g. average bootstrap support) and phylogenetic bias metrics (e.g. level of saturation, and root-to-tip variance) and resumes it using a principal component analysis, accounting for the increase of phylogenetic usefulness of each locus against major phylogenetic biases. This script requires concatenated alignment, a partition file, gene trees of each locus and a coalescent-based species tree. The gene trees were estimated using IQ-TREE v.2 (Minh *et al.*, 2020) with 1000 ultrafast bootstrap replicates (Hoang *et al.*, 2018) and rooted using the pxrr program available in phyx (Brown *et al.*, 2017). The species tree was summarized using ASTRAL v.5.7 (Sayyari and Mirarab, 2016). Finally, we removed 5 % of outlier genes according to the genesortR script. We estimated genetic diversity statistics from all datasets using AMAS (Borowiec, 2016).

Concatenated and coalescent phylogenetic inference

We used the genome-scale datasets Cactaceae591 and Angiosperm353 to estimate phylogenetic trees employing both the concatenated and coalescent approaches. For the gene-scale dataset, we implemented only the concatenated approach. The concatenated inferences were implemented in IQ-TREE v.2 (Minh *et al.*, 2020) with 10 000 ultrafast bootstraps (UfBoot) replicates (Hoang *et al.*, 2018) and 10 000 SH-like approximate likelihood ratio test (SH-aLRT) replicates

(Guindon *et al.*, 2010). The best substitution model for each partition was estimated by ModelFinder (Kalyaanamoorthy *et al.*, 2017), using the -m command available on IQ-TREE. We inferred coalescent species trees using the summary approach implemented in ASTRAL-hybrid (Zhang and Mirarab, 2022). Maximum-likelihood (ML) gene trees were generated in IQ-TREE v.2 (Minh *et al.*, 2020) with 10 000 ultrafast bootstrap replicates (Hoang *et al.*, 2018). We set the minimum bootstrap values of the gene tree branch support to be considered for species tree estimation as 40. Branch supports of species trees were accessed by local posterior probabilities (LPPs).

We assessed gene tree conflict against species trees inferred from the Cactaceae591 dataset using PhyParts (Smith *et al.*, 2015) and checked for gene tree incongruences using PhyPartsPieCharts (<https://github.com/mossmatters/phyloscripts/tree/master/phypartspiecharts>).

RESULTS

Variability of genetic and genomic datasets

The efficiency of capture of Cactaceae591 regions was higher within representatives of Cactoideae (ranging from 480 to 567 regions) compared to representatives of Opuntioideae (ranging from 383 to 404 regions), Pereskioideae (ranging from 485 to 521 regions) and Portulacaceae (137 regions) (Supplementary Data Fig. S1). After removing regions with more than 60 % of missing data, paralogues and outlier regions, the Cactaceae591 dataset included 459 nuclear regions and the Angiosperm353 dataset included 318 nuclear regions (Table 1). The Cactaceae591 dataset presented the highest proportion of variable sites, parsimony-informative sites and branch support among the three datasets delimited in this study (Table 1). The Angiosperm353 presented the smallest percentage of missing data and the gene-scale dataset displayed the most complete taxon sampling regarding genera and species sampling (Table 1).

Phylogenetic relationships among major clades of tribe Cereeae

All phylogenetic inferences recovered the tribe Cereeae as a well-supported clade and sister to the tribe Notocactaceae (Fig. 2). None of the Cereeae subtribes circumscribed by Nyffeler and Eggli (2010) was recovered as monophyletic, regardless of the dataset used in the present study. The genome-scale datasets (Cactaceae591 and Angiosperm353) provided a higher phylogenetic resolution along all nodes of the tribe Cereeae (Table 1; Fig. 2A, B, respectively). The gene-scale dataset exhibited insufficient node support for both the backbone and shallow relationships of the Cereeae phylogeny (Fig. 2C; Supplementary Data Fig. S2). The major clades within tribe Cereeae were recovered with high resolution regardless of the datasets and phylogenetic inferences used: *Uebelmannia* Buining clade, *Aylosteria* Speg clade, *Browningia* Britton & Rose clade (including *Rebutia* K.Schum. and *Weingartia* Werderm.), Trichocereinae clade (excluding *Espositoopsis dybowskii*), *Gymnocalycium* Mittler clade and Cereinae clade [including *Stetsonia coryne* (Salm-Dyck) Britton & Rose and *Espositoopsis dybowskii*].

Due to low phylogenetic resolution (Fig. 2C; UfBoot = 49) or inadequate taxon representation in each dataset (Fig. 2), we could not determine whether *Uebelmannia* or *Aylosteria* was the earliest diverging group within the Cereeae tribe. In other way, both genome-scale datasets were consistent in recovering the *Browningia* clade as an early divergent lineage sister to the remaining representatives from the tribe Cereeae *s.l.*: subtribe Trichocereinae, *Gymnocalycium* clade and subtribe Cereinae (Figs 3 and 5).

Phylogenetic relationships within major clades of tribe Cereeae *s.l.*

The Cactaceae591 dataset recovered *Arthrocerus* A.Berger and *Harrisia* as the early divergent lineages of subtribe Trichocereinae (Fig. 3), whereas the Angiosperm353 dataset recovered *Reicheocactus* Backeb. and *Harrisia* Britton as the early divergent lineages of this group (Fig. 5). The phylogenetic inferences of all datasets supported the *Cleistocactus* Lem. *s.s.* clade as sister to the *Oreocereus* (A.Berger) Riccob clade, although the genus *Cleistocactus* was recovered as a non-monophyletic group in either genomic-scale or gene-scale datasets (Figs 3 and 4; Supplementary Data Fig. S2, respectively). Both *Cleistocactus* and *Oreocereus* clades were sisters to the *Echinopsis s.l.* clade using the genome-scale datasets, considering that in the Angiosperm353 dataset the *Echinopsis* Zucc. clade included the genus *Denmoza* Britton & Rose (Fig. 4). The gene-scale dataset did not support *Echinopsis s.l.* as monophyletic, recovering some representatives closely related to the genus *Harrisia* and the remaining *Echinopsis* representatives in a clade including the genus *Denmoza* (Fig. S2). Another incongruence among datasets is related to the genus *Lasiocereus* F.Ritter, which is allied to the *Browningia* and *Rebutia* clade in the gene-scale dataset (Fig. S2), but closely related to *Espositoa* in the Angiosperm353 dataset (Fig. 5).

Regardless of the dataset used, all phylogenetic inferences consistently resolve the genus *Gymnocalycium* as a sister group to the subtribe Cereinae *s.l.*, which includes *Stetsonia coryne* and *Espositoopsis dybowskii*. The genomic dataset resolved the generic relationships within the subtribe Cereinae (especially the Cactaceae591 dataset, Fig. 4), while the gene-scale dataset did not present enough phylogenetic support in the deep and shallow nodes of subtribe Cereinae (Fig. S2).

The backbone of the subtribe Cereinae showed several short internal branches (Fig. 4). The first group to diverge in this subtribe was the genus *Stetsonia*, followed by the genus *Praecereus* Buxb., the *Cereus* clade (including *Cereus* Mill. *Cipocereus* F.Ritter, the *Facheiroa* clade (including *Facheiroa* Britton & Rose, *Brasillicereus* Backeb. and *Leocereus*), and the monotypic genus *Espositoopsis*. The next well-supported group comprised most of the diversity of the subtribe Cereinae.

Melocactus Link & Otto, *Discocactus* Pfeiff., *Coleocephalocereus* Backeb. and *Xiquexique* Lavor *et al.* represent the few monophyletic genera within subtribe Cereinae. We highlight the polyphyly of the genus *Micranthocereus*, whose representatives are scattered within the three main clades assigned as: the *Micranthocereus s.s.* clade [which includes *Micranthocereus polyanthus* (Werderm.) Backeb. and its allies, *Xiquexique* and *Pilosocereus bohlei* Hofacker],

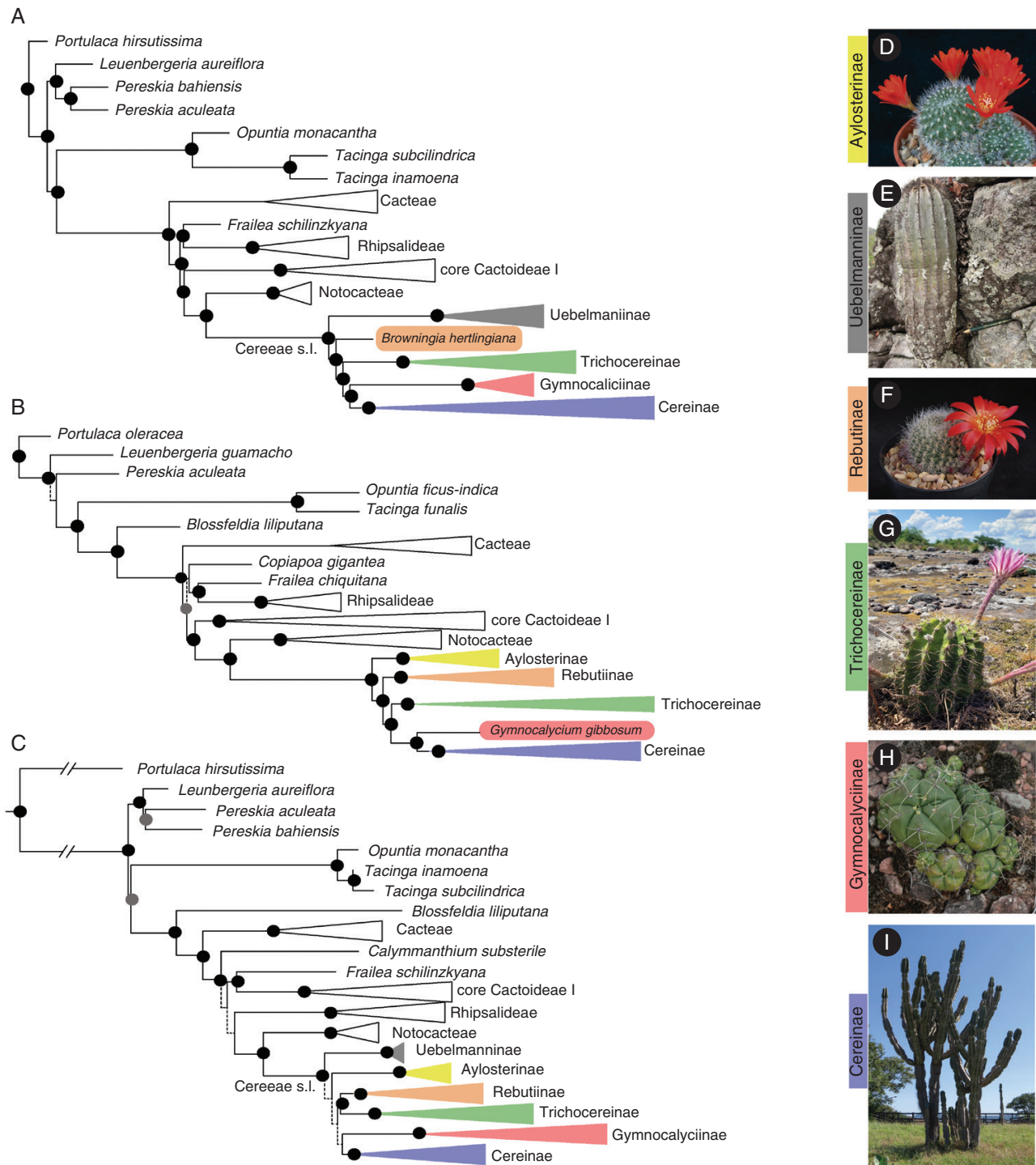


FIG. 2. Phylogenetic tree reconstructions showing major clades of subfamily Cactoideae and tribe Cereae from coalescent-based inference of the Cactaceae591 dataset (A), coalescent-based inference of the Angiosperm353 dataset (B) and maximum-likelihood inference of the gene-scale dataset (C). Highly supported branches (LPP > 0.9 for coalescent-based inference and BS/SH-aLRT > 95/80 for maximum-likelihood inference) are depicted with black circles; moderately supported branches ($0.9 > \text{LPP} > 0.7$ for coalescent-based inference and $95 > \text{BS}$ and $80 < \text{SH-aLRT}$ for maximum-likelihood) are depicted with grey circles. Low supported nodes (LPP < 0.7 in coalescent-based inference and BS/SH-aLRT < 95/80 in maximum-likelihood inference) are represented by dashed branch lines. Representatives of Cereae clades: (D) *Aylosteria fiebrigii*, (E) *Uebelmannia pectinifera* Buining, (F) *Rebutia minuscula* K.Schum, (G) *Echinopsis* sp., (H) *Gymnocalycium denudatum* (Link & Otto) Pfeiff. ex Mittler, (I) *Cereus jamacaru* DC. Photo credits: D and F: M. Lowry; E: M. Moraes; G and H: M. Kohler; and J: M. Romeiro-Brito.

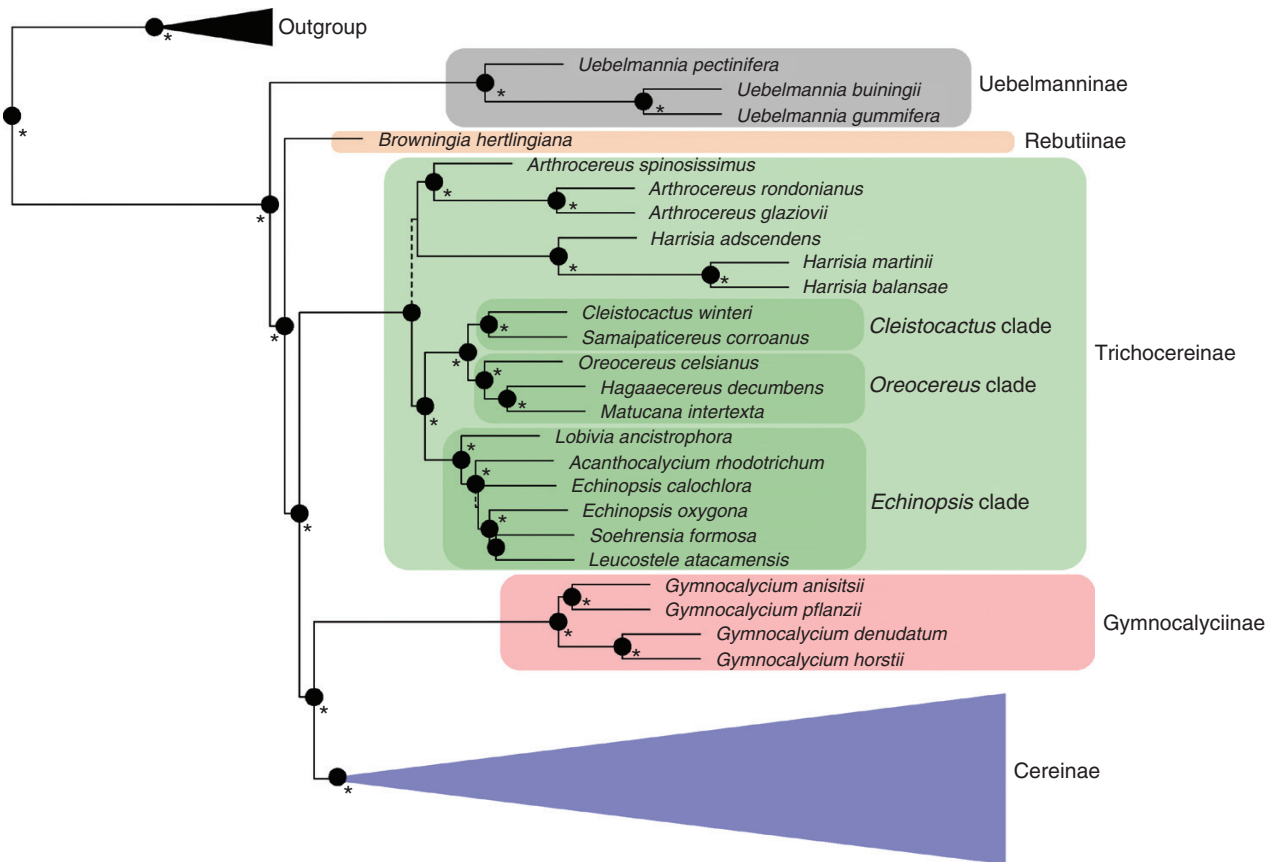


FIG. 3. Phylogenetic tree reconstruction of coalescent-based inference using the Angiosperm353 dataset, showing the relationships within major clades of tribe Cereeae. Highly supported branches (LPP > 0.95) are depicted by black circles at nodes. Nodes with LPP = 1 are highlighted with an asterisk. Moderately supported branches are depicted with dark grey (0.95 > LPP > 0.8) and light grey (0.8 > LPP > 0.75) circles. Low supported nodes (LPP < 0.7) are shown with dashed lines in respective branches.

the *Melocactus* clade [which includes *Coleocephalocereus*, *Micranthocereus* subgen. *Siccobaccatus* (P.J.Braun & Esteves) N.P.Taylor, *Discocactus* and *Melocactus*], and the *Pilosocereus* clade [including *Pilosocereus* Byles & G.D.Rowley, *Arrojadoa* Britton & Rose, *Stephanocereus* A.Berger, *Cipocereus pusilliflorus* (F.Ritter) Zappi & N.P.Taylor, *Micranthocereus violaciflorus* Buining., *Micranthocereus albicephalus* (Buining & Brederoo) F.Ritter and *Micranthocereus auriazureus* Buining & Brederoo]. The position of one representative from the genus *Micranthocereus* [*Micranthocereus purpureus* (Gürke) F.Ritter] was nested within the outgroup taxa in the Angiosperm353 phylogenetic tree (Fig. 4), although this same species is recovered as closely related to the *Micranthocereus s.s.* clade in the Cactaceae591 dataset (Fig. 5).

Incongruences among concatenated and coalescent inferences using the genomic dataset

The backbone of the phylogenies obtained from the genome-scale datasets was congruent across different phylogenetic approaches (Supplementary Data Fig. S3 and S4), differing only in the presence of the lineages *Uebelmannia* and *Aylosteria* in each genomic dataset. At shallower levels, incongruences among

phylogenetic approaches occurred within the genus *Melocactus*, and within the *Cereus*, *Micranthocereus* and *Pilosocereus* clade in the Cactaceae591 dataset (Fig. S3), and within the *Brasilicereus* and *Cleistocactus* clade in the Angiosperm353 dataset (Fig. S4). Most incongruences presented high phylogenetic support in both phylogenetic approaches (represented by red lines in Figs S3 and S4). Incongruences involving the generic level are related to the placement of *Cipocereus pusilliflorus* (whether close to the *Arrojadoa s.l.* clade or to *Micranthocereus albicephalus*) and the relationship between *Cereus* and *Cipocereus* in the Cactaceae591 dataset (Fig. S3).

Gene tree discordances have been shown to be widespread throughout the evolutionary history of the tribe Cereeae, with no dominant alternative topology among the gene trees in the Cactaceae591 dataset (Supplementary Data Fig. S5). The genera that display a higher proportion of gene tree–species tree concordant topology are *Uebelmannia*, *Harrisia*, *Gymnocalycium*, *Praecereus* and *Xiquexique* (Fig. S5).

DISCUSSION

Target sequencing strategies have demonstrated their effectiveness in unravelling contentious phylogenetic relationships in plant groups that experienced recent radiation, despite extensive

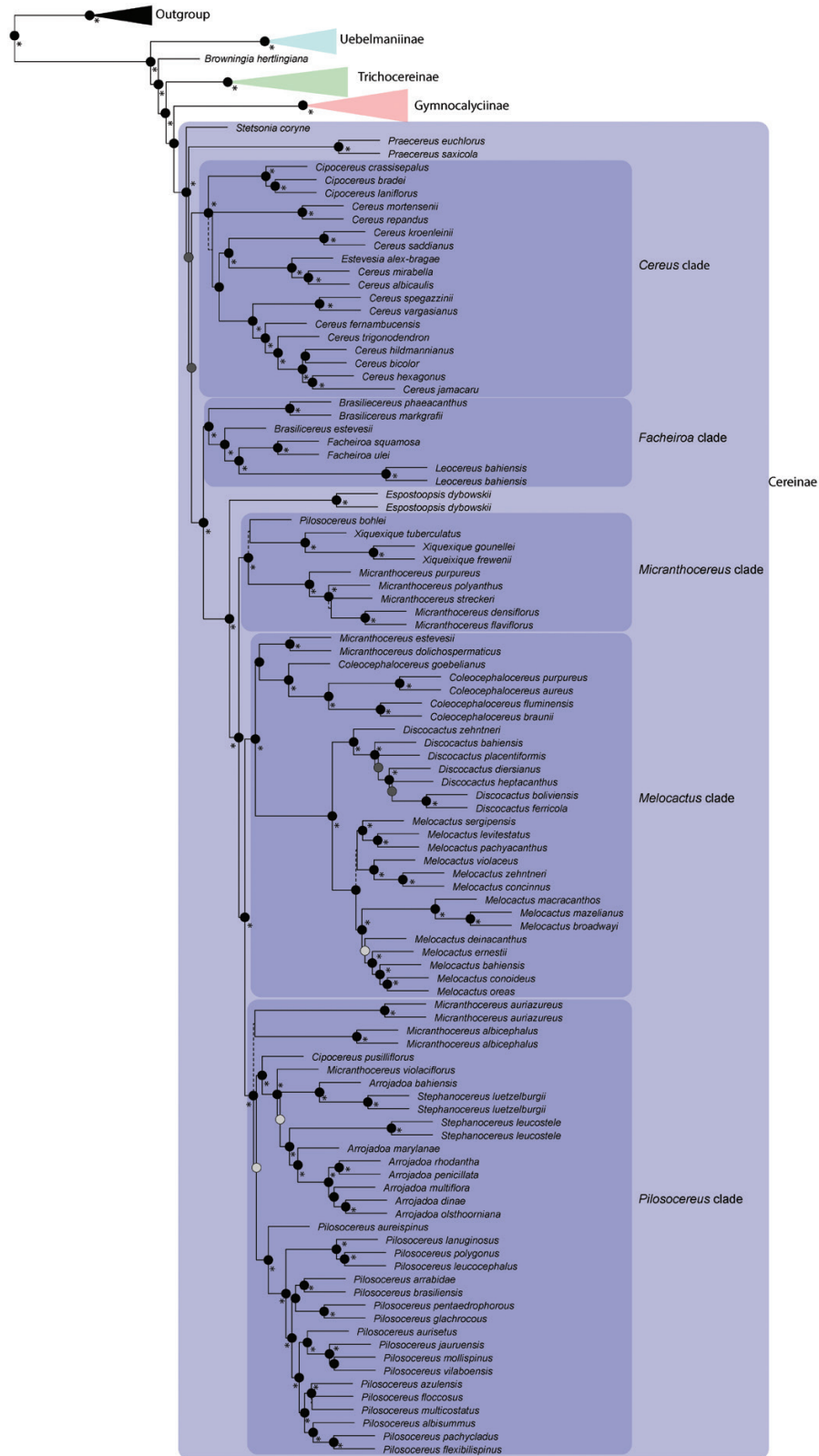


Fig. 4. Phylogenetic tree reconstruction of coalescent-based inference using the Cactaceae591 dataset, highlighting the relationships of early-diverging lineages of tribe Cereae and within subtribe Trichocereinae. Highly supported branches (LPP > 0.95) are depicted by black circles at nodes. Nodes with LPP = 1 are highlighted with an asterisk. Moderately supported branches (0.95 > LPP > 0.8) are depicted by dark grey circles. Low supported nodes (LPP < 0.7) are shown with dashed lines in respective branches.

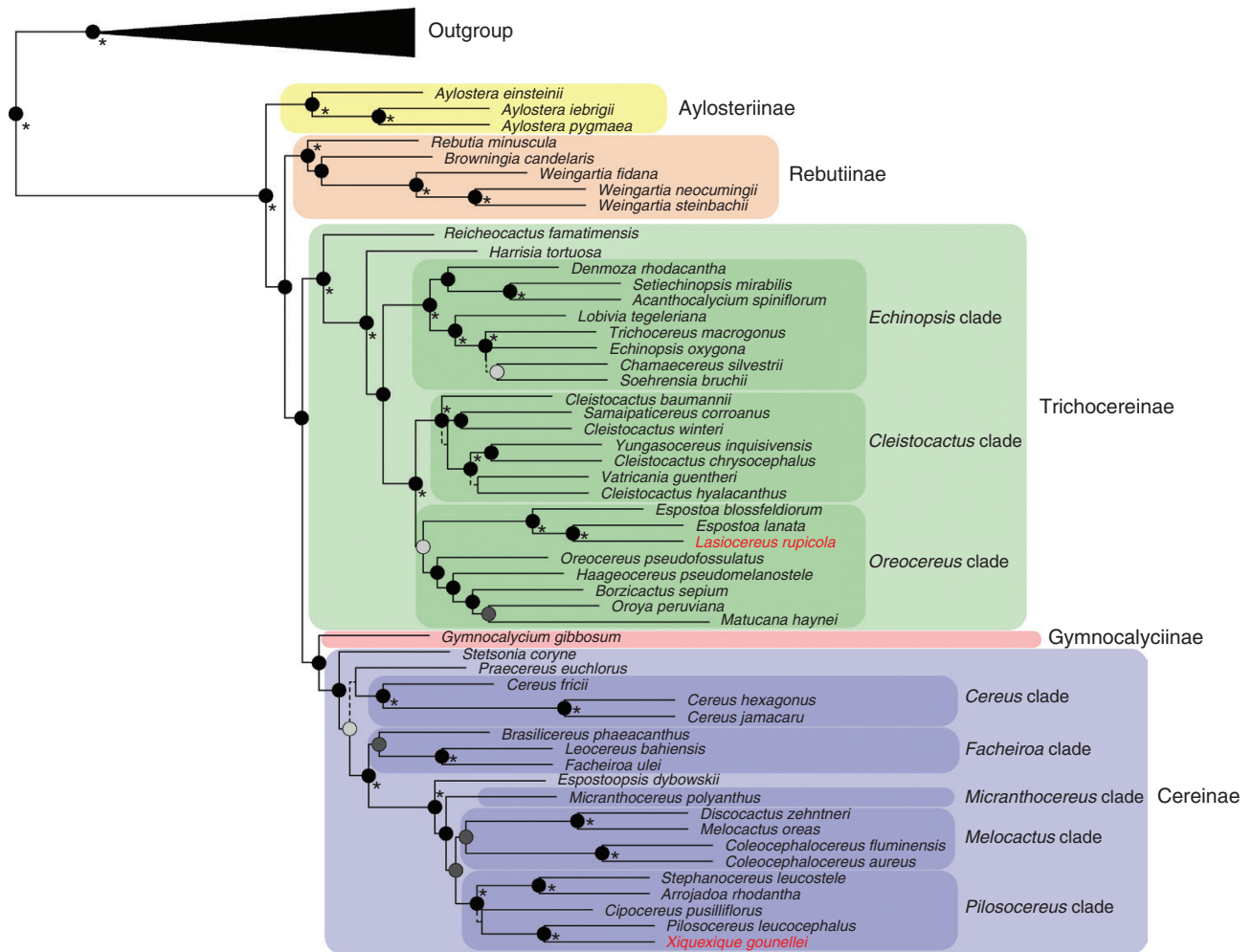


FIG. 5. Phylogenetic tree reconstruction of coalescent-based inference using the Cactaceae591 dataset, highlighting the relationships within subtribe Cereinae. Highly supported branches (LPP > 0.95) are depicted by black circles at nodes. Nodes with LPP = 1 are highlighted with an asterisk. Moderately supported branches (0.95 > LPP > 0.8) are depicted by dark grey circles. Low supported nodes (LPP < 0.7) are shown with dashed lines in respective branches.

patterns of morphological convergence and gene tree–species tree discordance (Lagomorsino et al., 2022). These strategies may contribute to the progress toward a robust taxonomic classification at multiple levels in Cactaceae, particularly using the customized Cactaceae591 probe set (Romeiro-Brito et al., 2022). Although both genomic datasets used in this study presented similar topologies, the Cactaceae591 dataset recovered higher genetic variability and higher node support than the Angiosperm353 dataset. For instance, only the Cactaceae591 dataset successfully elucidated the relationship at the backbone of subtribe Cereinae. Hence, we agree with previous studies indicating that lineage-specific panels may outperform universal panels when conducting phylogenetic studies at the interspecific level or within challenging plant groups (Christe et al., 2021; Eserman et al., 2021; Yardeni et al., 2021).

On the other hand, the combination of lineage-specific and universal probe sets has been a useful strategy for recovering well-supported topologies in large-scale plant groups and resolving major clades at lineage-specific groups (e.g. Ogutcen et al., 2021; Siniscalchi et al., 2021; Ufimov et al., 2021). For

instance, both genomic datasets revealed a surprisingly close and well-supported relationship between *Frailia* Britton & Rose (an orphan genus of tiny globose cacti) and tribe Rhipsalideae (a group of epiphytic cacti) (Fig. 2A, B). This example shows that robust relationships provided by the genomic datasets can also shed light on relationships of orphan lineages within the subfamily Cactoideae and may help to understand the evolutionary trend of convergent growth forms across Cactaceae.

This study explored comprehensive genetic dataset information, from a few molecular markers to hundreds of orthologous loci, and across contrasting phylogenetic inferences. In the meantime, this is the first phylogenomic analysis comprising most representatives of the tribe Cereeae, resulting in a higher-resolution topology. The presence of the same major clades in our findings as well as in early phylogenetic studies (Ritz et al., 2007; Schlumpberger and Renner, 2012; Lendel, 2013) provides further support for the need to delimit these subtribes and genera in Cereeae. Moreover, the well-supported topology obtained here will provide a solid basis for exploring the evolutionary and biogeographical patterns within this group.

Major groups of the tribe Cereeae *s.l.*

The monophyly of the tribe Cereeae *s.l.* and the close relationship with the tribe Notocactaceae, as evidenced by the present study, agree with previous taxonomic (Nyffeler and Egli, 2010) and molecular phylogenetic studies in this group (Nyffeler, 2002; Hernández-Hernández *et al.*, 2011). Likewise, the non-monophyly of the Cereeae subtribes was previously observed in molecular phylogenetic studies within this group (Ritz *et al.*, 2007; Bárcenas *et al.*, 2011; Schlumpberger and Renner, 2012; Bombonato *et al.*, 2020; Romeiro-Brito *et al.*, 2022). While previous studies (Lendel, 2013; Fantinati *et al.*, 2021) have proposed the monophyly of the subtribes Cereinae and Trichocereinae, the genera *Espositoopsis* and *Stetsonia* (representatives of the subtribes Trichocereinae and Rebutiinae, respectively) were not previously included for testing. Here, both genera were recovered with high support within the subtribe Cereinae (Figs 4 and 5), suggesting the inclusion of these taxa in an expanded delimitation of this subtribe. Therefore, the circumscription of the subtribe Trichocereinae should exclude the genus *Espositoopsis*, while the circumscription of the subtribe Rebutiinae should exclude the genus *Stetsonia*.

We identified six well-supported major clades across all phylogenetic inferences, indicating the existence of six subtribes within Cereeae. These subtribes consist of three monogeneric lineages and three clades with high support: *Uebelmannia*, *Aylostera*, *Gymnocalycium*, Rebutiinae *s.s.* (excluding *Uebelmannia*, *Aylostera*, *Gymnocalycium* and *Stetsonia*), Trichocereinae *s.s.* (excluding *Espositoopsis*) and Cereinae *s.l.* (including *Espositoopsis* and *Stetsonia*). As observed in previous phylogenetic studies (Ritz *et al.*, 2007; Demaio *et al.*, 2011; Mosti *et al.*, 2011), the monogeneric clades were recovered as well-supported monophyletic groups in all inferences of the present study. Considering that the relationship among the major groups of the tribe Cereeae is now clarified using genome-scale datasets, these subgroups should be finally recognized as independent lineages within Cereeae *s.l.*

The earliest divergent lineage within Cereeae *s.l.* remains unknown. Previous phylogenetic studies have pointed to *Uebelmannia* as the first divergent lineage (Ritz *et al.*, 2007; Hernández-Hernández *et al.*, 2011; Lendel, 2013; Romeiro-Brito *et al.*, 2022), although these studies did not include representatives from *Aylostera*, or they lacked phylogenetic support. Both issues were also the main concern in the present study. To properly address this matter within the tribe Cereeae, it is crucial to incorporate both lineages into future phylogenomic studies.

Generic relationships of subtribe Cereinae

To date, the Cactaceae591 dataset includes the most comprehensive sampling of the subtribe Cereinae in phylogenetic studies, including all genera and nearly half of its species diversity. To identify generic relationships and revise taxonomic classifications within this subtribe, it is essential to sample this subtribe broadly in light of its diversity and taxonomic complexity.

The present study corroborates the inclusion of two genera first placed in the subtribes Trichocereinae (*Espositoopsis*

dybowskii) and Rebutiinae (*Stetsonia coryne*) in the subtribe Cereinae. All phylogenetic inferences in this study are in line with previous phylogenetic studies of the tribe Cereeae *s.l.*, which grouped all taxa of the subtribe Cereinae in the same clade (Ritz *et al.*, 2007; Schlumpberger and Renner, 2012; Lendel, 2013; Bombonato *et al.*, 2020; Romeiro-Brito *et al.*, 2022).

The early divergent lineages of the subtribe Cereinae consist of genera with few species, such as *Stetsonia* and *Praecereus*, followed by a clade that contains most of the subtribe's diversity. The relationships of its major clade are partially congruent with the findings of Fantinati *et al.* (2021). Both studies confirm the non-monophyly of *Arrojadoa*, *Micranthocereus*, *Pilosocereus* and *Cipocereus*, and the close relationship between the following genera: (1) *Cereus* and *Cipocereus s.s.*, (2) *Arrojadoa* and *Stephanocereus*, and (3) *Micranthocereus s.s.* and *Xiquexique*. On the other hand, our phylogenomic inferences disagreed with the close relationship between *Facheiroa* and *Arrojadoa*, and the non-monophyly of *Cereus*, *Discocactus* and *Coleocephalocereus*, as recovered by Fantinati *et al.* (2021).

The controversial relationship between *Cereus* and *Cipocereus* was first documented by phylogenetic inferences with few molecular markers (Franco *et al.*, 2017). Later, the use of genome-scale datasets allied with coalescent phylogenetic inferences was decisive in untangling the relationship between these genera (Fig. 2; Bombonato *et al.*, 2020; N. P. Taylor *et al.*, unpubl. res.). Our phylogenetic analysis based on the Cactaceae591 dataset supported the monophyly of *Cereus* and *Cipocereus*. Moreover, we observed a significant proportion of incongruences among gene trees and species trees associated with these genera, particularly at the ancestral node of *Cereus* (Supplementary Data Fig. S5). This supports the hypothesis of Bombonato *et al.* (2020) that *Cipocereus* and *Cereus* are sister taxa that diverged rapidly during a radiation event, resulting in extensive incomplete lineage sorting and creating an 'anomaly zone' (Degnan and Rosenberg, 2006) near their ancestral node.

The *Micranthocereus s.s.* clade comprises *Xiquexique* and *Pilosocereus bohlei*. Although *P. bohlei* shares many characteristics with *Pilosocereus* species, it has long been recovered outside the *Pilosocereus s.s.* group (Calvente *et al.*, 2017; LAVOR *et al.*, 2018; Fantinati *et al.*, 2021; Romeiro-Brito *et al.*, 2023). The close relationship of these taxa within the *Micranthocereus s.s.* clade gives insight into common characteristics shared among them, such as the branching pattern occurring only at the base, hairy areoles and curved hypanthium (Hunt *et al.*, 2006). *Pilosocereus bohlei* is similar to *Xiquexique gounellei* with regard to the broader and hairy areoles, but its branching pattern is similar to that of *Micranthocereus* species. The interesting discovery of the proximity of *Micranthocereus s.s.*, *Xiquexique* and *P. bohlei*, with the possibility of the last of these being a hybrid taxon within this clade, highlights the need for additional scrutiny in future phylogenomic studies.

Three closely related genera (*Facheiroa*, *Brasilicereus* and *Leocereus*) were previously placed within the tribes Trichocereae and Browningieae (Taylor and Zappi, 2004) due to the presence of scales and hair-spines on the pericarpel of the flower. Early molecular phylogeny inferences including these genera (Soffiatti, 2003) suggested a position within the tribe Cereeae (Soffiatti, 2003). Here, we find these genera compose a monophyletic group regardless of the dataset and phylogenetic

inference. These results reinforce that the similarity of floral traits previously used to delineate major groups in Cactoideae may at least partially result from convergent evolution, particularly within the BCT clade (Guerrero *et al.*, 2019).

The *Melocactus* clade clustered genera into two distinct groups, one including *Melocactus* and *Discocactus*, and the other comprising *Coleocephalocereus* and *Micranthocereus* subgen. *Siccobaccatus*. The former group consists of globose plants with apical cephalia, while the latter includes columnar plants with unilateral cephalia. The close relationship between the genera *Melocactus* and *Discocactus* has been consistently established by molecular phylogenetic studies (Hernández-Hernández *et al.*, 2011; Santos, 2013; Silva *et al.*, 2017). However, the present study is the first to mention the close relationship between *M.* subgen. *Siccobaccatus* and *Coleocephalocereus*. Indeed, the species from *M.* subgen. *Siccobaccatus* share characteristics with most *Coleocephalocereus* species, including a columnar, erect, growth habit, sunken lateral cephalium and association with rock outcrops. Based on these findings, we suggest the inclusion of the former to be recognized as a subgenus of *Coleocephalocereus*.

The *Pilosocereus* clade was composed of the genera *Arrojadoa*, *Stephanocereus* and *Pilosocereus s.s.*, and two *incertae sedis*: *Micranthocereus albicephalus* and *Micranthocereus auriazureus*. This clade comprises columnar and shrubby species inhabiting the Caatinga, rock outcrops in the Cerrado, and *campos rupestres* from Minas Gerais to Bahia (Brazil). The inclusion of two representatives of *M. albicephalus* and *M. auriazureus* corroborates its position in the *Pilosocereus* clade, even though the low support could not delineate its placement within the *Pilosocereus* or *Arrojadoa* clades. Further studies investigating the source of low phylogenetic resolution among these taxa should be carried out.

The *Pilosocereus* clade was divided into two distinct groups with strong support in our study: one consisting of the *Pilosocereus* species, and the other comprising the genus *Arrojadoa* and its allies. Our findings support the monophyly of *Pilosocereus*, excluding *Pilosocereus bohlei*, a taxon previously positioned outside the genus *Pilosocereus* (Calvente *et al.*, 2017; LAVOR *et al.*, 2018; Romeiro-Brito *et al.*, 2023). Although our analysis detected inconsistencies in the species relationships across various phylogenetic inference methods in the genus *Pilosocereus*, we obtained a topology comparable to those of previous phylogenetic studies (LAVOR *et al.*, 2018; Romeiro-Brito *et al.*, 2023), but with stronger node support (Fig. 5). These findings underline the significance of using a genome-wide dataset to gain a thorough understanding of the intricate evolutionary past of *Pilosocereus*.

The well-supported *Arrojadoa* clade is composed of *Arrojadoa*, *Stephanocereus*, *Cipocereus pusilliflorus* and *Micranthocereus violaciflorus*. The early divergent lineages of *Arrojadoa* consist of *Cipocereus pusilliflorus* and *Micranthocereus violaciflorus*, both species lacking a true cephalia. The following clade comprises *Arrojadoa* and *Stephanocereus* species, which split into two groups: one composed of species bearing terminal/ring-cephalia (most *Arrojadoa* species and *Stephanocereus leucostele*) and the

other lacking or with a primitive chlorophyllous cephalium (*Arrojadoa bahiensis* and *Stephanocereus luetzelburgii*). Previous phylogenetic studies on this group (Soffiatti, 2003; Fantinati *et al.*, 2021) have suggested a close relationship between *Cipocereus pusilliflorus* and *Stephanocereus* to *Arrojadoa* species, but the positioning of *Micranthocereus violaciflorus* near *Arrojadoa* is a novelty. These results highlight the need to recognize an expanded *Arrojadoa* that includes *Cipocereus pusilliflorus*, *Micranthocereus violaciflorus* and *Stephanocereus* species.

Generic relationships of subtribe Trichocereinae

Though the non-monophyly of subtribes is widespread in Cereeae *s.l.* (Guerrero *et al.*, 2019), many phylogenetic studies using few molecular markers have recovered Trichocereinae as a monophyletic subtribe (Hernández-Hernández *et al.*, 2011; Schlumberger and Renner, 2012; Lendel, 2013). Here we highlight an updated circumscription of the subtribe Trichocereinae, excluding *Espositoopsis dyboswkii*. Although this taxon resembles *Espositoa*, suggesting a close relationship with the subtribe Trichocereinae, its naked flower tube corroborates its inclusion in the subtribe Cereinae (Taylor and Zappi, 2004). Considering this aspect, flower tube characteristics are also variable within the subtribes of Cereeae and should be treated with caution in taxonomic classifications of these groups.

According to gene-scale dataset phylogenetic inferences, the genus *Reicheocactus* was the earliest lineage to diverge within the subtribe Trichocereinae, followed by the genus *Arthrocerus*. We were unable to confirm this relationship using the genome-scale datasets because one of those genera was missing in each dataset. Nonetheless, the phylogenetic inferences with the Angiosperm353 and Cactaceae591 datasets recovered *Reicheocactus* and *Arthrocerus* as early divergent lineages in subtribe Trichocereinae, respectively, somehow agreeing with the gene-scale results. The next divergent clade is commonly recovered by the genomic dataset, placing the genus *Harrisia* as a sister group to all remaining representatives of Trichocereinae. The genus *Harrisia* was consistently identified as closely related to either the *Cleistocactus* clade (Franck *et al.*, 2013) or the *Echinopsis s.s.* clade (Schlumberger and Renner, 2012; Lendel, 2013). However, the present study suggests that *Harrisia* may not be closely related to either of these clades. The inclusion of *Reicheocactus*, *Arthrocerus* and *Harrisia* in future phylogenomic studies may shed light on *Reicheocactus* as the first divergent group of Trichocereinae and a closer relationship between *Arthrocerus* and *Harrisia*. The latter two genera are the only members of the subtribe Trichocereinae occurring outside the Andean region, spanning across Central-western and Eastern Brazil (*Arthrocerus*) to the Caribbean region (*Harrisia*).

Echinopsis was recovered as a polyphyletic group in our gene-scale phylogenetic tree, corroborating previous phylogenetic studies (Schlumberger and Renner, 2012; Lendel, 2013). Indeed, the recent taxonomic checklist from Korotkova *et al.* (2021) segregated *Echinopsis* into multiple genera. However, all trees inferred from the Cactaceae591 and Angiosperm353 datasets recovered the monophyly of this genus, including *Denmoza* and excluding *Reicheocactus*. Thus, to properly

address whether *Echinopsis* is or is not monophyletic, as once defined by Hunt *et al.* (2006), we recommend the use of genome-scale datasets with comprehensive taxonomic sampling in future phylogenetic studies.

The remaining complex groups of Trichocereinae mostly agree with previous plastid phylogenetic inferences, consisting of the *Cleistocactus* clade and *Oreocereus* clade (Schlumberger and Renner, 2012; Lendel, 2013). The *Cleistocactus* clade includes species pollinated by bats (*Samaipaticereus*, *Yungasocereus* and *Vatricania guentheri*) or hummingbirds (*Cleistocactus* and *Cephalocleistocactus*). The *Oreocereus* clade, as recovered from genomic datasets, comprises the genera *Borzicactus*, *Espostoa*, *Mila*, *Oreocereus*, *Oroya*, *Haageocereus*, *Matucana* and *Lasiocereus*. The position of *Lasiocereus* among Trichocereinae in the Angiosperm353 tree should be taken with caution, considering that previous phylogenetic studies placed *Lasiocereus* species within the subtribe Rebutiinae (Supplementary Data Fig. S3; Ritz *et al.*, 2007; Schlumberger and Renner, 2012; Lendel, 2013).

Potential causes of extensive gene tree and species tree discordance

Resolving the phylogenetic relationships among representatives of the subtribe Cereinae has been a persistent challenge for many decades (Taylor and Zappi, 1989), due primarily to the absence of apomorphic characteristics (Taylor and Zappi, 2004) and low phylogenetic resolution (e.g. Calvente *et al.*, 2017; Franco *et al.*, 2017; Fantinati *et al.*, 2021). The use of genome-scale datasets to resolve relationships at multiple levels is not only a trending practice for studying Cactaceae groups that underwent rapid and recent divergence (Franco *et al.*, 2022), but it is an essential approach for investigating the evolutionary and diversification histories of its tribes. Moreover, the extensive level of gene-tree/species-tree discordance in Cactaceae also highlighted the usefulness of coalescent inference approaches in this group, which may outperform concatenated inference approaches at contentious nodes (Bombonato *et al.*, 2020; Romeiro-Brito *et al.*, 2022, 2023).

Despite the extensive gene tree discordance, the Cereeae topology is stable using different genomic resources and phylogenetic approaches. Some minor discordances at terminal branches were found among concatenated and coalescent inferences, including the *Cereus*, *Pilosocereus* and *Melocactus* clades in subtribe Cereinae, and *Cleistocactus* clade in subtribe Trichocereinae. Incomplete lineage sorting has been discussed as the main source of gene-tree and species-tree discordance in cacti (Copetti *et al.*, 2017; Walker *et al.*, 2018; Wang *et al.*, 2019; Romeiro-Brito *et al.*, 2022, 2023), especially if we consider that the rapid diversification experienced by cactus lineages (Arakaki *et al.*, 2011) may also lead to short internal branches generating an ‘anomaly zone’ in the tree (Bombonato *et al.*, 2020). However, other sources of phylogenetic conflict have been poorly investigated in these groups, such as hybridization/introgression events.

Hybridization may be an important source of phylogenetic discordance in the complex diversification of tribe Cereeae, given the large number of natural intergeneric and congeneric hybrids described within this group in the past years

(Rowley, 1994; Machado, 2008; Khan *et al.*, 2020; Taylor and Albuquerque-Lima, 2020; Arakaki *et al.*, 2021). However, the effects of recent and ancient introgression on diversification within this group remain unknown. Previous studies with *Melocactus* have demonstrated that despite the widespread occurrence of hybridization events within these genera, there are low levels of introgression within different species (Khan *et al.*, 2020). Hence, this pattern results in the maintenance of species boundaries due to the prevalence of genetic integrity of the parental lineages.

Considering the substantial evidence of hybridization observed in many rapid plant radiations (Schley *et al.*, 2022), we hypothesize that the intricate diversification of tribe Cereeae may be attributed to deep hybridization events. These events, when combined with incomplete lineage sorting, probably contribute to the significant levels of gene tree incongruence observed. The expansion of arid conditions during the Miocene and Pliocene (Arakaki *et al.*, 2011) may have enabled the rapid diversification within this group and promoted the contact and subsequent hybridization among related lineages. So far, the extensive phylogenomic discordance of North American cacti has primarily been linked to incomplete lineage sorting rather than introgression (Copetti *et al.*, 2017). In addition to different methods available for detecting introgression events (e.g. Hibbins and Hahn, 2022), we now have the capability to discern the contributions of different sources of phylogenetic discordance (e.g. Cai *et al.*, 2021; Morales-Briones *et al.*, 2021). Investigating the roles played by different sources of phylogenetic conflict would provide valuable insights into the diversification of Cereeae and shed light on the persistently contentious and poorly resolved relationships within this group.

Taxonomic synopsis of Cereeae

Species are listed in cases where the circumscription of the genus or subgenus is being changed significantly from that in the standard works by Hunt *et al.* (2006, 2013). Newly published names are indicated in **bold** type.

Tribe Cereeae Salm-Dyck (as ‘Cereastreae’)

Superficially like Echinocereae (Pachycereae), but pericarpel, hypanthial tube and pericarp of unripe fruit usually lacking areoles and stiff spines (if spiny, then plant very slender, not pachycaul). *Type*: *Cereus* Mill. Comprising the following six subtribes:

1. Subtribe **Uebelmanniinae** N.P.Taylor, **subtr. nov.**

Globular to shortly columnar, many ribbed/tuberculate-ribbed cacti. Stems unbranched, with internal mucilage ducts; epidermis roughened, bearing waxy scales (cf. *Copiapoa*), grey-green to reddish; areoles on mature stems with long hairs and short straight spines. Cephalium lacking. Flowers small, apical, diurnal, pericarpel and very short hypanthial tube with bract-scales woolly in their axils, perianth-segments expanding, green to yellow, stigma-lobes few. Fruit scarcely fleshy, more or less naked but with the hairy perianth remains attached at apex, reddish. Seeds few, medium-sized, testa smooth.

Type and only genus: *Uebelmannia* Buining (3 spp.).

Distribution: Narrowly endemic to the central-northern part of Minas Gerais state, Brazil.

2. Subtribe **Aylosterinae** N.P.Taylor, **subtr. nov.**

Dwarf globular, simple or caespitose, sometimes semi-geophytic; stems with \pm spiralled tubercles or indistinct ribs; spines short, but often dense; cephalium absent. Flowers diurnal, from the sides or base of the stem, shortly funnellform, pericarpel and tube with bract-scales, the style and tube \pm fused in the lower half or more. Fruits and seeds small.

Type and only genus: *Aylostera* Spegazzini (incl. *Mediolobivia* Backeb., *Digitorebutia* Donald) (~11 spp.).

Distribution: Eastern Andes of Bolivia and Argentina.

3. Subtribe **Rebutiinae** Donald [incl. *Browningieae* F.Buxb., Krainz, *Die Kakteen*, Lfg 33: CIV/1 (1966)].

Tall columnar, ribbed pachycaul cacti, branched and treelike, rarely single-stemmed, or dwarf globular-stemmed and simple or clustering (caespitose). Stems 7–many-ribbed or with spiralled tubercles, when ribbed these mostly low and rounded. Areoles spiny on juvenile plants, later sometimes spineless on fertile stems. Cephalium lacking (but cf. *Browningia columnaris* F.Ritter). Flowers borne laterally to basally, nocturnal and whitish or diurnal and brightly coloured, shortly tubular to infundibuliform, pericarpel and hypanthial tube bearing small to large and often overlapping bract-scales, sometimes these shortly hairy, persisting on the fleshy indehiscent fruit. Seeds small, very numerous, testa variously ornamented.

Type: *Rebutia* K.Schum. (3 spp.)

Distribution: Andes.

Browningia Britt. & Rose (8 spp.)

Weingartia Werderm. (incl. *Sulcorebutia* Backeb., *Cintia* Knize & Riha) (~16 spp.).

4. Subtribe **Gymnocalycinae** N.P.Taylor, **subtr. nov.**

Dwarf to medium-sized globular to discoid cacti; ribs few to many, mostly low, often tuberculate. Areoles spiny or sometimes almost spineless. Cephalium lacking. Flowers diurnal, from near the stem apex or from the ‘shoulder’ of the stem, pericarpel and short-to-long hypanthial tube scaly but otherwise naked, perianth variously coloured. Fruit dehiscent, revealing the funicular pulp in which the seeds of diverse testa morphology are embedded.

Type and only genus: *Gymnocalycium* Mittler (~65 spp.)

Distribution: southeastern South America from the eastern Andes to southern Brazil and central Argentina.

5. Subtribe **Trichocereinae** F.Buxb.

Stems of diverse size and form, from depressed globose to tall columnar, unbranched to treelike, many-ribbed. Areoles usually spiny. Cephalium lacking or occasionally present, then lateral. Flowers of diverse size and shape, from small to very large, nocturnal or diurnal, pericarpel and hypanthial tube clothed in discrete scales bearing abundant hairy spines (woolly hairs) in their axils, perianth expanded or segments remaining erect to incurved; stamens often inserted in the tube in two series; stigma-lobes many. Fruits scaly-hairy, mostly dehiscent to reveal the funicular pulp. Seeds medium-sized, testa smooth to tuberculate.

Type: *Trichocereus* Britt. & Rose (= *Echinopsis* Zucc.)

Distribution: Caribbean and Andes to southeastern South America.

Genera:

Mila Britt. & Rose

Pygmaeocereus Johns. & Backeb.

Haageocereus Backeb.

Espostoa Britt. & Rose (incl. *Thrixanthocereus* Backeb.)

Rauhocereus Backeb.

Weberbauerocereus Backeb.

Cleistocactus Lem. (incl. *Samaipaticereus* Cárđ., *Yungasocereus* F.Ritter, *Vatricania* Backeb.)

The following new name combinations are required: *Cleistocactus corroanus* (Cárđ.) N.P.Taylor, **comb. nov.** Basionym: *Samaipaticereus corroanus* Cárđ., *Cact. Succ. J. (US)* 24: 141 (1952). *Cleistocactus inquisivensis* (Cárđ.) N.P.Taylor, **comb. nov.** Basionym: *Samaipaticereus inquisivensis* Cárđ., *Cactus (Paris)* 12(57): 246–247 (1957). *Cleistocactus guentheri* (Kupper) N.P.Taylor, **comb. nov.** Basionym: *Cephalocereus guentheri* Kupper, *Monatss. Deuts. Kakteen-Gesels.* 3: 159 (1931).

Borzicactus Riccob.

Oreocereus (A.Berger) Riccob.

Matucana Britt. & Rose

Oroya Britt. & Rose

Harrisia Britt.

Arthroocereus A.Berger

Echinopsis Zucc. (incl. *Denmoza* Britt. & Rose)

Reicheocactus Backeb.

Incertae sedis: *Lasiocereus* F.Ritter (cf. *Espostoa*)

6. Subtribe **Cereinae** Britt. & Rose

Habit diverse as in *Trichocereinae*. Lateral or terminal cephalia developed in many taxa. Flowers of diverse size and shape, from small to very large, but sometimes very small (e.g. *Melocactus*), mostly lacking areoles and hairs, bract-scales often inconspicuous or widely spaced. Fruits scaly or more often naked, dehiscent or indehiscent; seeds mostly small.

Type: *Cereus* Mill.

Distribution: Mexico and the Caribbean to eastern Andes and southeastern South America.

Genera:

Stetsonia Britt. & Rose (1 sp.)

Praecereus F.Buxb. (2 spp.)

Cipocereus F.Ritter (5 spp.)

Type:

1. *C. pleurocarpus* F.Ritter

2. *C. minensis* (Werderm.) F.Ritter

3. *C. bradei* (Backeb. & Voll) Zappi & N.P.Taylor

4. *C. laniflorus* N.P.Taylor & Zappi

5. *C. crassisepalus* (Buin. & Brederoo) Zappi & N.P.Taylor

Cereus Mill. *Type:* *C. hexagonus* (L.) Mill. (~33 spp.)

C. subg. Oblongicarpus (Croizat) D.R.Hunt & N.P.Taylor. (5 spp.). *Type:*

1. *C. repandus* (L.) Mill.;

2. *C. fricii* Backeb.

3. *C. horrispinus* Backeb.

4. *C. mortensenii* Croizat.

5. *Cereus serruliflorus* Haw. (*C. haitiensis* A.R.Franck, nom. illeg.).

C. subg. *Mirabella* (F.Ritter) N.P.Taylor (incl. *Mirabella* F.Ritter, *Estevesia* P.J.Braun).

Type:

6. *C. mirabella* N.P.Taylor (*Mirabella minensis* F.Ritter)
7. *C. albicaulis* (Britt. & Rose) Luetzelb.

Incertae sedis:

8. *C. saddianus* (Rizzini & Matos-F.) P.J.Braun
9. *C. phatnospermus* K.Schum. (incl. *C. kroenleinii* N.P.Taylor)
10. *C. aethiops* Haw.
11. *C. adelmarii* (Rizzini & Mattos-F.) P.J.Braun
12. *C. estevesii* P.J.Braun (cf. *C. albicaulis*).

C. subg. *Cereus* [incl. *C.* subg. *Ebneria* (Backeb.) D.Hunt]

13. *C. spegazzinii* F.A.C.Weber
14. *C. vargasianus* Cárđ.
15. *C. trigonodendron* Ule
16. *C. pierre-braunianus* E.Esteves-Pereira
17. *C. calcirupicola* F.Ritter
18. *C. lepidotus* Salm-Dyck
19. *C. gerardi* N.P.Taylor
20. *C. jamacaru* DC.
21. *C. ingens* N.P.Taylor & M.C.Machado
22. *C. sericifer* (F.Ritter) P.J.Braun
23. *C. fernambucensis* Lem.
24. *C. insularis* Hemsl.
25. *C. bicolor* Rizz. & Mattos-F.
26. *C. hildmannianus* K.Schum.
27. *C. stenogonus* K.Schum.

Incertae sedis:

28. *C. cochabambensis* Cárđ.
29. *C. huilunchu* Cárđ.
30. *C. hankeanus* K.Schum.
31. *C. lamprospermus* K.Schum.
32. *C. lanosus* (F.Ritter) P.J.Braun
33. *C. braunii* Cárđ.

Micranthocereus Backeb. (6 spp.). *Type:*

1. *M. polyanthus* (Werderm.) Backeb.
2. *M. flaviflorus* Buin. & Brederoo
3. *M. alvinii* (Hofacker & M.C.Machado) N.P.Taylor & M.Lowry
4. *M. streckeri* Van Heek. & Van Criek.
5. *M. hofackerianus* (P.J.Braun & E.Esteves-Pereira) M.C.Machado
6. *M. purpureus* (Gürke) F.Ritter.

Xiquexique Lavor & Calvente (*Pilosocereus* subg. *Gounellea* Zappi; incl. *Caerulocereus* Guiggi). (4 spp.). *Type:*

1. *X. gounellei* (F.A.C.Weber) Lavor & Calvente
2. *Xiquexique bohlei* (Hofacker) N.P.Taylor, **comb. nov.**
Basionym: *Pilosocereus bohlei* Hofacker, Kakt. and. Sukk. 52: 253–257 (2001).
3. *X. tuberculatus* (Werderm.) Lavor & Calvente
4. *X. frewenii* (Zappi & N.P.Taylor) Lavor & Calvente.

Arrojadoa Britt. & Rose (incl. *Stephanocereus* A.Berger, *Floribunda* F.Ritter, *Pierrebraunia* E.Esteves-Pereira, *Arrojadoopsis* Guiggi) (13 spp.).

Revised description: Dwarf to medium-tall (to 4 m) cylindrical-stemmed cacti, erect to decumbent, shrub-like or sometimes solitary columnar; subterranean stem base and rootstock often tuberous, vascular cylinder woody. Stems 7–many ribbed, ribs low, rounded, never acute, axes sometimes segmented, and then interrupted by ring cephalia. Areoles usually bearing long hairs, at least when young, always spiny. Flowers mostly from or from near the apex of stem-segments, often from bristly terminal or encircling ring cephalia, small to medium-sized (2–10 cm), shortly tubular, pericarpel very small, it and hypanthial tube almost naked or with few inconspicuous bract-scales, with relatively small, mostly scarcely expanded perianth-segments, diurnal or nocturnal, hummingbird or bat syndrome, reddish pink to magenta, or bicoloured with paler to whitish inner segments, or greenish white. Fruits mostly indehiscent or opening by a basal pore (*A. leucosteles*), fleshy, never dry when ripe, variously coloured, perianth remains persistent, blackish. Seeds mostly small, 1–2 mm. Seedlings, where known, globular at first, only later becoming elongate-cylindrical. *Type:*

1. *A. rhodantha* (Gürke) Britt. & Rose
2. *Arrojadoa pusilliflora* (F.Ritter) N.P.Taylor, **comb. nov.**
Basionym: *Floribunda pusilliflora* F.Ritter, Kakt. Südamer. 1: 58–60 (1979).
3. *Arrojadoa violaciflora* (Buining & Brederoo) N.P.Taylor, **comb. nov.** Basionym: *Micranthocereus violaciflorus* Buin., Kakt. and. Sukk. 20: 129–130 (1969).
4. *A. bahiensis* (P.J.Braun & E.Esteves-Pereira) N.P.Taylor & Egli
5. *Arrojadoa luetzelburgii* (Vaupel) N.P.Taylor, **comb. nov.** Basionym: *Cereus luetzelburgii* Vaupel, Zeitschr. Sukkulente. 1: 57 (1923).
6. *A. marylandiae* Soares-F. & M.C.Machado
7. *Arrojadoa leucosteles* (Gürke) N.P.Taylor, **comb. nov.**
Basionym: *Cereus leucosteles* Gürke, Monatsschr. Kakt.-Kunde 18: 53 (1908).
8. *A. dinae* Buin. & Brederoo
9. *A. albiflora* Buin. & Brederoo
10. *A. eriocaulis* Buin. & Brederoo
11. *A. multiflora* F.Ritter
12. *A. olsthoorniana* Hofacker & M.C.Machado
13. *A. penicillata* (Gürke) Britt. & Rose.

Facheiroa Britt. & Rose (incl. *Zehntnerella* Britt. & Rose, *Leocereus* Britt. & Rose, *Brasilicereus* Backeb., *Bragiaia* P.J.Braun) (7 spp.).

Revised description: Medium to tall cylindrical cacti, erect and self-supporting or slender and sometimes leaning on surrounding vegetation, shrubby to treelike, sparsely to many-branched, vascular cylinder woody. Stems unsegmented, 7–many-ribbed, ribs low, rounded, never acute. Areoles always spiny, mostly lacking long-hairs. Flowers lateral, never terminal, sometimes borne from a bristly/woolly unilateral cephalium, shortly tubular, to 7.5 × 7.5 cm, pericarpel and hypanthial tube scaly, woolly or spiny, never naked, perianth small and hardly expanded or as broad as the flower is long, diurnal to

nocturnal, bird, bat or moth syndrome, reddish, green or white. Fruit indehiscent or disintegrating when mature, scaly or with deciduous spiny areoles, fleshy. Seeds small to medium-sized, 1–2.5 mm. Seedlings, where known, globular at first, later elongating. *Type*:

1. *F. ulei* (Gürke) Werderm.
2. *F. cephaliomelana* Buining & Brederoo
3. *F. squamosa* (Gürke) P.J.Braun & E.Esteves-Pereira
4. *Facheiroa phaeacantha* (Gürke) N.P.Taylor, **comb. nov.** Basionym: *Cereus phaeacanthus* Gürke, Monatsschr. Kakt.-Kunde 18: 57 (1908).
5. *Facheiroa markgrafii* (Backeb. & Voll) N.P.Taylor, **comb. nov.** Basionym: *Brasilicereus markgrafii* Backeb. & Voll, Arch. Jard. Bot. Rio de Janeiro 9: 155 (1949, publ. 1950).
6. *Facheiroa bragaia* N.P.Taylor, **nom. nov.** Replaced synonym: *Bragaia estevesii* Hofacker & P.J.Braun, Kakt. and. Sukk. 60(12): 328 (2009), non *Facheiroa estevesii* P.J.Braun (= *F. cephaliomelana* subsp. *estevesii* (P.J.Braun) N.P.Taylor & Zappi).
7. *Facheiroa bahiensis* (Britt. & Rose) N.P.Taylor, **comb. nov.** Basionym: *Leocereus bahiensis* Britt. & Rose, Cact. 2: 108 (1920).

Melocactus Link & Otto, nom. cons. (~40 spp.). *Type*: *Cactus melocactus* L., typ. cons.

Discocactus Pfeiff. (13 spp.). *Type*: *D. insignis* Pfeiff. [= *D. placentiformis* (Lehm.) K.Schum.].

Coleocephalocereus Backeb. (incl. *Buiningia* F.Buxb., *Siccobaccatus* P.J.Braun & E.Esteves-Pereira, *Mariottia* Guiggi) (9 spp.). *Type*: *C. fluminensis* (Miq.) Backeb.

C. subg. Coleocephalocereus

1. *C. fluminensis* (Miq.) Backeb.
2. *C. decumbens* F.Ritter
3. *C. pluricostatus* Buin. & Brederoo
4. *C. buxbaumianus* Buin.

C. subg. Simplex N.P.Taylor. *Type and only species*:

5. *C. goebelianus* (Vaupel) Buin.

C. subg. Buiningia (F.Buxb.) P.J.Braun. *Type*: *Buiningia brevicylindrica* Buin. (= *C. aureus* F.Ritter)

6. *C. aureus* F.Ritter
7. *C. purpureus* (Buin. & Brederoo) F.Ritter.

Coleocephalocereus subg. *Siccobaccatus* (P.J.Braun & E.Esteves-Pereira) N.P.Taylor, **comb. nov.** Basionym: *Siccobaccatus* P.J.Braun & E.Esteves-Pereira, Succulenta (NL) 69: 7 (1990) (2 spp.). *Type*: *S. dolichospermaticus* (Buin. & Brederoo) P.J.Braun & E.Esteves-Pereira.

Differs from other subgenera of *Coleocephalocereus* in fruits which are dry and disintegrate at maturity; seeds elongate, testa ±smooth (wind dispersed).

8. *Coleocephalocereus dolichospermaticus* (Buin. & Brederoo) N.P.Taylor, **comb. nov.** Basionym: *Austrocephalocereus dolichospermaticus* Buin. & Brederoo, Kakt. and. Sukk. 25: 76–79 (1974).
9. *Coleocephalocereus neoestevesii* N.P.Taylor, **nom. nov.** Replaced synonym: *Austrocephalocereus estevesii* Buin.

& Brederoo, Cact. Succ. J. (US) 47: 267 (1975), non *Coleocephalocereus estevesii* L.Diers (= *C. buxbaumianus* subsp. *flavisetus* (F.Ritter) N.P.Taylor & Zappi).

Pilosocereus Byles & G.Rowley (excl. *P. subg. Gounellea* Zappi and *Caerulocereus* Guiggi) (~60 spp.). *Type*: *P. leucocephalus* (Poselger) Byles & G.D.Rowley.

Incertae sedis within Cereinae:

1. *Micranthocereus albicephalus* (Buin. & Brederoo) F.Ritter
2. *M. auriazureus* Buin. & Brederoo.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following.

Table S1. Sampling information of Cactaceae and Portulacaceae species used for the genomic dataset. Table S2. Sequence matrix of species and regions included in the gene-scale dataset. Figure S1. Heatmap indicating 591 orthologue recovery success per sample. Figure S2. Maximum-likelihood phylogenetic inference estimated by IQ-TREE2 using the gene-scale dataset. Figure S3. Phylogenetic tree reconstructions of coalescent-based inference (A) and maximum-likelihood (B) using the Cactaceae591 dataset. Figure S4. Phylogenetic tree reconstructions of coalescent-based inference (A) and maximum-likelihood (B) using the Angiosperm353 dataset. Figure S5. Gene-tree and species-tree conflicts estimated in PhyParts using coalescent-based phylogeny inferred with the Cactaceae591 dataset.

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