

Phylogenetic Relationships of *Pilosocereus* (Cactaceae) and Taxonomic Implications

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Abstract

The aim of this paper was to investigate the phylogenetic relationships within *Pilosocereus* (Cactaceae, Cactoideae), one of the most emblematic genera of cacti of the Neotropical dry woodlands. The genus includes 42 species and eight subspecies placed in two subgenera: *Pilosocereus* subg. *Pilosocereus* and *Pilosocereus* subg. *Gounellea*. We used an expanded sampling and a broad coverage of genomic regions (*psbD-trnT*, *trnL-trnT*, *petL-psbE*, *trnS-trnG*, *ycf1* and *PhyC*) resulting in an aligned concatenated matrix of 4563 bp length. Maximum parsimony and Bayesian analyses recovered a non-monophyletic *Pilosocereus* as well as four consistently non-monophyletic species. We propose taxonomic changes to accommodate para- or polyphyletic taxa including the resurrection of three species, three new synonyms, two new names and a new circumscription for *Pilosocereus* with 42 species and four subspecies. *Xiquexique* is proposed as a new genus characterized by molecular and morphological characters, composed of three species formerly positioned in *P.* subg. *Gounellea*.

Keywords: Cacti, dry woodland, molecular phylogenetics, xerophytes, *Xiquexique*.

1. Introduction

Monophyly, the direct relationship of an ancestor and all its descendent lineages forming a clade, has been considered a key point in delimiting groups in contemporary systematics. Although initially questioned by some authors, that considered necessary to stretch the monophyletic requirement in order to retain the natural and useful grouping of organisms in the last century (see Cronquist 1968), many current taxonomical rearrangements are proposed following such rule (e.g. Clark et al. 2000; Bartish et al. 2002; Hardy and Faden 2004; Baker et al. 2006; Metzgar et al. 2008; Su et al. 2010; Middleton and Livshultz 2012; Xue et al. 2014; Ortiz-

Rodriguez et al. 2016). As more phylogenetic hypotheses emerge at the generic level using broad species sampling, particularly in speciose taxa from high diversity tropical areas, many species traditionally accepted may appear as para- or polyphyletic. Such results may be a consequence of biological processes (e.g. hybridization, incomplete lineage sorting) or mere artifacts of the earlier interpretation and taxonomy of the species. In the present work we use the genus *Pilosocereus* Byles & Rowley (Cactaceae), with high species density across the Neotropical region, to test for species delimitation using six molecular regions.

Cactaceae is one of the most representative families in the Neotropics, with 130 genera and 1850 species (Nyffeler and

Eggl 2010). Striking features of the group include: the presence of succulent photosynthetic stems; CAM metabolism; specialized root system (with high water absorption capacity) (Edwards and Diaz 2006); reduced or absent leaves; areoles (axillary meristematic regions where distinct structures, like branches, flowers and fruit arise) commonly displaying a great amount of spines, hairs and bristles (Gibson and Nobel 1986; Nyffeler 2002), as well as a great diversity of growth forms and habits (Hernández-Hernández *et al.* 2011). The group is highly variable and with many conspicuous, dominant and relevant taxa in arid and deserted areas in the Americas (Nyffeler 2002; Hernández-Hernández *et al.* 2011; Hernández-Hernández *et al.* 2014).

Among the four subfamilies (Cactoideae, Opuntioideae, Pereskioideae and Maihuenioideae), Cactoideae is the largest with six tribes and about 1,530 species (Nyffeler and Eggl 2010), which make up about 83% of the total family species richness. Cereeae is the largest of Cactoideae tribes, with 41 genera and 589 species (subdivided into the subtribes Rebutiinae, Cereinae and Trichocereinae), presenting a great variety of growth forms (Nyffeler and Eggl 2010). Except for *Harrisia* Britton, *Melocactus* Link & Otto and *Pilosocereus*, Cereeae could be recognized as an exclusively South American tribe (Nyffeler and Eggl 2010). In phylogenetic studies encompassing the whole family or for large groups (such as subfamilies and tribes) Cereeae (*sensu* Nyffeler and Eggl 2010, corresponding to the BCT clade) is nested within the RNBCCT clade (core Rhipsalideae, core Notocactaeae, plus BCT) (Nyffeler 2002; Bárcenas *et al.* 2011).

Pilosocereus is one of the largest genera of the tribe Cereeae and subtribe Cereinae, in number of species and range of distribution (Hunt *et al.* 2006). It currently includes 42 species and 8 subspecies distributed from the U.S.A. to the east of Brazil (Hunt *et al.* 2006; Zappi and Taylor 2011). It comprises columnar cacti with long or short areolar hairs (reproductive or non-reproductive); absent cephalium or with a pseudocephalium in some species (such as *P. densiareolatus* and *P. chrysostele*) (Zappi 1994). The flowers are usually nocturnal, and fruit are dehiscent by transversal slits, depressed-globose, with persistent floral remnants (erect or pendent) and with white or colored funicular pulp (Zappi 1994). *Pilosocereus* species may be shrubs or tree-like, densely branched above or at the base level (or unbranched), and with a wide variation in number and color of spines and number of ribs (Zappi 1994).

A single taxonomic revision published for the genus (Zappi 1994) proposed the division of *Pilosocereus* into two subgenera: *Pilosocereus* subg. *Pilosocereus* and *Pilosocereus* subg. *Gounellea*. *Pilosocereus* subg. *Gounellea* is recognized by the candelabrifform branching pattern; sinuate ribs sinuses with conspicuous podaria beneath the areoles; and erect to pendent (not immersed at the apex of the fruit) floral remnants. It includes *P. gounellei*, *P. tuberculatus* and, more recently, *P. frewenii* Zappi & Taylor (Zappi and Taylor 2011). *Pilosocereus* subg. *Pilosocereus* includes all the remaining species of the genus (39 species) and is characterized by the erect branching pattern, straight rib sinuses and pendent floral remnants (immersed in the apex of

the fruit, see Fig. 1). In addition, Zappi (1994) proposed the delimitation of five informal groups within *P.* subg. *Pilosocereus* based on floral morphology, spine and habit of the species.

Some studies have been developed for groups of species within the genus, especially for the group *P. aurisetus* (*sensu* Zappi 1994), which is composed of a complex of species with great morphological variety, overlapping diagnostic characters and taxonomic uncertainty (Jesus 2010; Moraes *et al.* 2012; Bonatelli *et al.* 2013, 2014, 2015; Menezes *et al.* 2016; Perez *et al.* 2016). However, the great majority of the studies involving *Pilosocereus* focused on isolated species and on various aspects such as morphology (e. g. Godofredo 2009; Menezes and Loiola 2015; Franck *et al.* 2019), reproductive biology (e. g. Locatelli *et al.* 1997; Rivera-Marchand and Ackerman 2006; Lucena 2007; Rocha *et al.* 2007a,b; Meiado *et al.* 2008; Munguía-Rosas *et al.* 2010; Abud *et al.* 2010; Martins *et al.* 2012; Rocha *et al.* 2019), and population genetics (e. g. Nassar *et al.* 2003; Moraes *et al.* 2005; Figueredo-Urbina *et al.* 2010; Khattab *et al.* 2014; Monteiro *et al.* 2015; Barbosa *et al.* 2020).

Calvente *et al.* (2017) published the more comprehensive molecular phylogeny focused in *Pilosocereus* to date and demonstrated the non-monophyly of the genus. Despite presenting well-resolved trees, Calvente *et al.* (2017) suggested that additional studies (encompassing more species and different markers) are still necessary for a better resolution of relationships within and outside the genus. Lavor *et al.* (2018) published the reconstructed spatio-temporal evolution for the genus, with a more complete data set, finding a very recent diversification for *Pilosocereus*. Despite the well-supported phylogeny presented by Lavor *et al.* (2018), *Pilosocereus* was recovered as polyphyletic and its relationships in the most inclusive branches remained uncertain. Lavor *et al.* (2020) also published a paper addressing the conservation, distribution, and endemism for the genus.

Hunt *et al.* (2006) mentioned that the “great battle” of delimitation in Cactaceae occurs at the generic level, since it is in this rank that the greatest uncertainties and taxonomic instabilities occur. Many cacti genera harbor many species with morphological similarity that can be only the result of convergent evolution, a process thought to be common in the family (Hernández-Hernández *et al.* 2011). Consequently, hindered by their superficial similarities, many studies of molecular phylogenetics have been recovering non-monophyletic taxa, requiring further investigation with increased taxa sampling and various genomic regions and the reevaluation of the traditional circumscriptions (Bárcenas *et al.* 2011).

Thus, to improve the knowledge of relationships within *Pilosocereus* and among lineages of the genus with other groups of the subtribe Cereinae, we added new taxa, samples and molecular data for *Pilosocereus* and outgroups to the data set used by Calvente *et al.* (2017) and present a broader and more robust phylogeny in this paper. We use this phylogenetic backbone to evaluate the monophyly of taxa and to propose taxonomic changes.

2. Material and methods

2.1 Taxon Sampling

In order to better define and confirm relationships of lineages of *Pilosocereus* in subtribal and tribal levels we used an outgroup sampling with ten taxa from two distinct subfamilies and from main clades in Cactoideae (according to the phylogenetic hypothesis presented in Hernández-Hernández *et al.* 2014): *Pereskia grandifolia* (subfamily Pereskioideae); *Copiapoa cinerea*, *Rhipsalis baccifera*, *Browningia microsperma*, *Cleistocactus* sp., *Oreocereus hempelianus*, *Arrojadoa rhodantha*, *Cereus jamacaru*, *Melocactus zehntneri* and *Stephanocereus leucosteale* (subfamily Cactoideae) (Table 1). For *Pilosocereus*, we include 43 ingroup taxa reaching nearly 85 % of the species of the group (35 species and eight subspecies out of 41 species and nine subspecies according to Hunt *et al.* (2006) classification). Appendix 1 includes voucher information and GenBank accession numbers.

2.2 DNA Extraction, Amplification and Sequencing

Genomic DNA was either extracted from silica dried stems or roots, or from herbarium specimens, using the NucleoSpin plant II kit (Macherey-Nagel, Düren, Germany) or the Qiagen DNeasy plant mini kit (Qiagen, Hilden, Germany) in accordance to manufacturers' protocols. Amplification reactions conditions and primers for six regions, four non-coding intergenic spacers of chloroplast DNA (cpDNA): *trnS-trnG*, *psbD-trnT*, *trnL-trnT*, *petL-psbE*, one nuclear low-copy gene, Phytochrome C (*PhyC*), followed protocols described in Calvente *et al.* (2017). Furthermore, we added sequences for one plastid gene, *ycf1*, in this work (Appendix 2). Amplifications for *ycf1* were conducted in a 20 µl reaction containing the following reagents (adding water to complete the final volume) and conditions: 4 µl of 5X GoTaq Buffer, 2.4 µl of 3 mM MgCl₂, 1.6 µl of each primer (120 ng), 0.2 µl of GoTaq, 0.5 µl of 250 µM dNTPs, 1 µl of template DNA; 94 °C for 3 min, followed by 40 cycles of 94 °C for 45 sec, 45 °C for 45 sec, 72 °C for 2 min and 30 sec and finishing at 72 °C for 5 min. Compared to Calvente *et al.* (2017), the present work expanded coverage of taxa and genomic regions including: the addition of 50 taxa for *ycf1*; 32 taxa for *psbD-trnT*; 29 taxa for *petL-psbE*; 27 taxa for *PhyC*; 24 taxa for *trnS-trnG* and 24 taxa for *trnL-trnT* (added taxa are listed in Appendix 1). For all cases, we coded absent data as missing characters (-). The amplification products were purified using the NucleoSpin Gel or PCR clean-up kit (Macherey-Nagel, Düren, Germany) and QIAquick PCR purification kit (QIAGEN, Crawley, UK), following the manufacturer's protocol. Automated sequencing was performed by Macrogen Inc. Korea and Netherlands.

2.3 Alignment and Phylogenetic Analyses

Complementary sequences were assembled in Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan) and aligned manually in Mesquite v. 3.04 (Maddison and Maddison 2015). Indels were coded using the simple indel coding method (Simmons and Ochoterena 2000) and included in phylogenetic analyses as presence/absence data. *Pereskia grandifolia* was used as outgroup to root the trees in all analyses based on previous knowledge of phylogenetic relationships in Cactaceae and on its position near the primary node of divergence within subfamily, Pereskioideae (Arakaki *et al.* 2011; Hernández-Hernández *et al.* 2014).

We performed maximum parsimony (MP) analyses for all regions individually, but as the analyzes for the individual regions present low resolution (see Fig. S1a-f, supplementary information: DOI 10.6084/m9.figshare.12895133), we built a concatenated matrix with two partitions (all plastid markers and *PhyC*) for a total evidence approach. All analyses were performed using heuristic searches with 1000 replicates of random taxon addition (retaining 20 trees at each replicate), tree bisection reconnection (TBR) branch swapping and equal weighting of all characters in PAUP* v4.0a147 (Swofford 2002). Support was assessed with non-parametric bootstrap analysis (BS) using 1000 replicates of random-taxon addition and TBR branch swapping. Clades with bootstrap percentages of 50–74 % are described as weakly supported, 75–89 % as moderately supported and 90–100 % as strongly supported.

We also inferred phylogenetic relationships using Bayesian inference (BI) in MrBayes 3.2.2 (Ronquist *et al.* 2011), hosted on the CIPRES Science Gateway (Miller *et al.* 2010). Choice of substitution models used the Akaike information criterion implemented in MrModelTest 2.2 (Nylander 2004) and run in PAUP* v4.0a147 (Swofford 2002). Bayesian analyses performed on individual genes used molecular substitution models selected for each marker: GTR for *psbD-trnT*, GTR + I for *petL-psbE*, GTR + G for *ycf1*, GTR + I + G for *trnS-trnG*, F81 + I for *trnL-trnT* and HKY + G for *PhyC*. As the analyzes for the individual regions present low resolution (see Fig. S2a-f, supplementary information), we built a concatenated matrix with two partitions (all plastid/*PhyC*), using GTR + I + G and HKY + G as the best-fit model for each partition, respectively, and the overall substitution rate unlinked between partitions. MrBayes was set to build two independent runs of four chains each, for 10 million generations, sampling every 1000th generation. We assessed convergence between runs by monitoring the standard deviation of split frequencies (< 0.01) and using the potential scale reduction factor (PSRF). After discarding the first 25% samples as burn-in, we pooled the remaining trees to construct a 50% majority rule consensus tree. We describe clades as weakly supported when the posterior probability (PP) < 0.8; as moderately supported when PP ranged from 0.8 to 0.95, and as strongly supported when PP > 0.95.

TABLE 1. Species sampled in this study and their classification following Nyffeler and Eggli (2010) (outgroups are printed in bold). Type species of *Pilosocereus* subgenera are underlined (Zappi 1994).

Subfamily	Tribe	Subtribe	Species and subspecies
Pereskioideae	Rhipsalideae	Rebutiinae Trichocereinae	1. <i>Pereskia grandifolia</i> Haw.
			2. <i>Copiapoa cinerea</i> (Phil.) Britton & Rose
			3. <i>Rhipsalis baccifera</i> (J. S. Mueller) Stearn.
			4. <i>Browningia microsperma</i> (Werderm. & Backeb.) W. T. Marshall.
			5. <i>Cleistocactus</i> sp. Lem.
			6. <i>Oreocereus hempelianus</i> (Gurke) D. R. Hunt
			7. <i>Arrojadoa rhodantha</i> Britton & Rose
			8. <i>Cereus jamacaru</i> D. C.
			9. <i>Melocactus zehntneri</i> (Britton & Rose) Luetzelb.
			10. <i>Stephanocereus leucostele</i> (Gurke) A.
	<i>Pilosocereus</i> subg. <i>Pilosocereus</i> :		
	11. <i>Pilosocereus albisummus</i> P. J. Braun & Esteves		
	12. <i>Pilosocereus alensis</i> (F. A. C. Weber) Byles & G. D. Rowley		
	13. <u><i>Pilosocereus arrabidae</i></u> (Lem.) Byles & G. D. Rowley		
	14. <i>Pilosocereus aureispinus</i> (Buining & Brederoo) F. Ritter		
	15. <i>Pilosocereus aurisetus</i> (Werderm.) Byles & G. D. Rowley subsp. <i>aurisetus</i>		
	16. <i>Pilosocereus aurisetus</i> subsp. <i>aurilanatus</i> (F. Ritter) Zappi		
	17. <i>Pilosocereus azulensis</i> (F. A. C. Weber ex Rol. Goss) Byles & G. D. Rowley		
	18. <i>Pilosocereus bohlei</i> Hofacker		
	19. <i>Pilosocereus brasiliensis</i> (Britton & Rose) Backeb subsp. <i>brasiliensis</i>		
	20. <i>Pilosocereus brasiliensis</i> subsp. <i>ruschianus</i> (Buining & Brederoo) Zappi		
	21. <i>Pilosocereus catingicola</i> (Gurke) Byles & G. D. Rowley subsp. <i>catingicola</i>		
22. <i>Pilosocereus catingicola</i> subsp. <i>salvadorensis</i> (Werderm.) Zappi			
Cactoideae	Cereeae	Cereinae	23. <i>Pilosocereus chrysacanthus</i> (F. A. C. Weber) Byles & G. D. Rowley
			24. <i>Pilosocereus chrysostele</i> subsp. <i>cearensis</i> P. J. Braun & Esteves
			25. <i>Pilosocereus flavipulvinatus</i> (Buining & Brederoo) F. Ritter
			26. <i>Pilosocereus floccosus</i> subsp. <i>quadricostatus</i> (F. Ritter) Zappi
			27. <i>Pilosocereus fulvilanatus</i> (Buining & Brederoo) F. Ritter subsp. <i>fulvilanatus</i>

Table 1 (continue)

Subfamily	Tribe	Subtribe	Species and subspecies
Cactoideae	Cereeae	Cereinae	28. <i>Pilosocereus fulvilanatus</i> subsp. <i>rosae</i> (P. J. Braun) Zappi
			29. <i>Pilosocereus glaucochrous</i> (Werderm.) Byles & G. D. Rowley
			30. <i>Pilosocereus jauruensis</i> (Buining & Brederoo) P. J. Braun
			31. <i>Pilosocereus leucocephalus</i> (Poselg.) Byles & G. D. Rowley
			32. <i>Pilosocereus machrisii</i> (E. Y. Dawson) Backeb
			33. <i>Pilosocereus magnificus</i> (Buining & Brederoo) F. Ritter
			34. <i>Pilosocereus multicostatus</i> F. Ritter
			35. <i>Pilosocereus oligolepis</i> (Vaupel) Byles & G. D. Rowley
			36. <i>Pilosocereus pachycladus</i> F. Ritter subsp. <i>pachycladus</i>
			37. <i>Pilosocereus pachycladus</i> subsp. <i>pernambucoensis</i> (F. Ritter) Zappi
			38. <i>Pilosocereus parvus</i> (Diers & Esteves) P. J. Braun
			39. <i>Pilosocereus pentaedrophorus</i> (Labour.) Byles & G. D. Rowley subsp. <i>pentaedrophorus</i>
			40. <i>Pilosocereus pentaedrophorus</i> subsp. <i>robustus</i> Zappi
			41. <i>Pilosocereus piauihyensis</i> (Gurke) Byles & G. D. Rowley
			42. <i>Pilosocereus polygonus</i> (Lam.) Byles & G. D. Rowley
			43. <i>Pilosocereus purpusii</i> (Britton & Rose) Byles & G. D. Rowley
			44. <i>Pilosocereus pusillibaccatus</i> P. J. Braun & Esteves
			45. <i>Pilosocereus quadricentralis</i> (E. Y. Dawson) Backeb
			46. <i>Pilosocereus royenii</i> (L.) Byles & G. D. Rowley
			47. <i>Pilosocereus splendidus</i> F. Ritter
			48. <i>Pilosocereus ulei</i> (K. Schum.) Byles & G. D. Rowley
			49. <i>Pilosocereus vilaboensis</i> (Diers & Esteves) P. J. Braun
			<i>Pilosocereus</i> subg. <i>Gounellea</i> :
			50. <i>Pilosocereus gounellei</i> (F. A. C. Weber ex K. Schum.) Byles & G. D. Rowley
			51. <i>Pilosocereus tuberculatus</i> (Werderm.) Byles & G. D. Rowley

3. Results

In this work, we assess phylogenetic relationships for *Pilosocereus* based on 220 new sequences produced here and added to the data set used by Calvente et al. (2017). In total, we used 447 sequences for 46 species and eight subspecies (total 75 specimens) from six markers (Appendix 1). Statistics for each genomic region studied are summarized in Table 2. There were no significant differences in the topological analysis with and without indels. The results of the MP and BI analyzes for the individual regions present low resolution but confirm the paraphyly of *Pilosocereus* sensu lato (s.l.). Already for concatenated matrix, the phylogenetic relationships recovered with MP (Fig. 2) and BI (Fig. 3) analyses were generally similar with majority of nodes receiving moderate (BS of 75-89 % and PP > 0.8) to strong support values (BS of 90-100 % and PP > 0.95) (described below).

The tribe Cereeae emerges strongly supported as monophyletic (BS = 100, PP = 1) sister of *Rhipsalis baccifera* (BS = 98, PP = 1). The position of *Browningia microsperma* (subtribe Rebutiinae) and the clade representing subtribe Trichocereinae (*Cleistocactus* sp., and *Oreocereus hempelianus*) is controversial; *Browningia microsperma* appears more closely related to Cereinae in the MP topology (BS = 80), whereas the clade (*Cleistocactus* sp., *Oreocereus hempelianus*) is more closely related to Cereinae in the BI topology (PP = 0.81). Subtribe Cereinae is monophyletic in a strongly supported clade in both analyses (BS = 100, PP = 1).

The genus *Pilosocereus* as circumscribed in Hunt et al. (2006) (*Pilosocereus* sensu lato, Fig. 1) was recovered as paraphyletic in both MP and BI analyses as the clade (*M. zehntneri* (*A. rhodantha*, *C. jamacaru*)) is nested inside the genus. *Pilosocereus* species are distributed in three strongly supported main clades: (I) containing *P. gounellei* and *P. tuberculatus* (*P.* subg. *Gounellea* clade; BS = 100, PP = 1); (II) *P. bohlei* isolated (BS = 100, PP = 1); and (III) the remaining of *Pilosocereus* species (*Pilosocereus* sensu stricto

(s.s.); BS = 100, PP = 1). A closer relationship of the (*M. zehntneri* (*A. rhodantha*, *C. jamacaru*)) clade with the *Pilosocereus* s.s. clade is recovered in both MP and BI results although not strongly supported (BS = 57, PP = 0.86).

In *Pilosocereus* s.s. species are clustered in three main clades: (1) *Pilosocereus aureispinus* isolated; (2) a clade (BS = 80, PP = 1) including all non-Brazilian species clustered (BS = 100, PP = 1) (the relationships are better resolved in the BI topology, although poorly supported) and a clade of the Brazilian species *P. glaucochrous*, *P. pachycladus* subsp. *pernambucoensis*, *P. pentaedrophorus* subsp. *pentaedrophorus* and *P. piauhensis* (BS = 99, PP = 1); (3) a major strongly supported clade (BS = 94, PP = 1) clustering all remaining Brazilian species, but with poor resolution in more inclusive nodes. In the MP topology (Fig. 2) *P. aureispinus* is the first diverging lineage inside *Pilosocereus* s.s. (BS = 100), however in the BI topology the relationships between clades 1, 2 and 3 are uncertain (Fig. 3).

The relationships within clade 3 are not fully resolved, however six weakly to strongly supported multispecies clades are recovered both in MP and BI analyses: (A) *Pilosocereus ulei*, *P. arrabidae*, *P. catingicola* subsp. *catingicola* and *P. catingicola* subsp. *salvadorensis* (BS = 98, PP = 1); (B) *Pilosocereus oligolepis*, *P. chrysostele* subsp. *cearensis* and *P. flavipulvinatus* (BS = 95, PP = 1); (C) *Pilosocereus brasiliensis* subsp. *brasiliensis*, *P. brasiliensis* subsp. *ruschianus*, *P. magnificus* and *P. multicostatus* (BS = 85, PP = 0.80); (D) *Pilosocereus fulvilanatus* subsp. *fulvilanatus* and *P. pentaedrophorus* subsp. *robustus* (BS = 79; PP = 0.99); (E) *Pilosocereus azulensis* and *P. floccosus* subsp. *quadricostatus* (BS = 100; PP = 1); (F) *Pilosocereus fulvilanatus* subsp. *rosae* and *P. aurisetus* subsp. *aurilanatus* (BS = 96, PP = 1); (G) *Pilosocereus densiareolatus*, *P. parvus*, *P. pusillibaccatus*, *P. splendidus*, *P. pachycladus* subsp. *pachycladus* e *P. albisummus* (BS = 96, PP = 1); and (H) *Pilosocereus pachycladus* subsp. *pachycladus* and *P. albisummus* (BS = 92;

TABLE 2. Summary statistics from the maximum parsimony analyses of *Pilosocereus* (ingroup and outgroup) for plastid and nuclear regions used in this study (CI = consistency index; RI = retention index).

	<i>trnL-trnT</i>	<i>psbD-trnT</i> ^{GGU}	<i>petL-psbE</i>	<i>trnS-trnG</i>	<i>ycf1</i>	<i>PhyC</i>	Combined dataset
Size of aligned matrix	308	616	555	1327	751	1006	4563
Informative sites (bp)	22	41	42	139	163	48	454
Length of best tree	48	76	103	473	534	136	1418
Number of most parsimonious trees	3974	386	2	120	8732	210	19,338
CI	0.79	0.94	0.87	0.78	0.71	0.88	0.75
RI	0.85	0.96	0.93	0.79	0.83	0.90	0.82

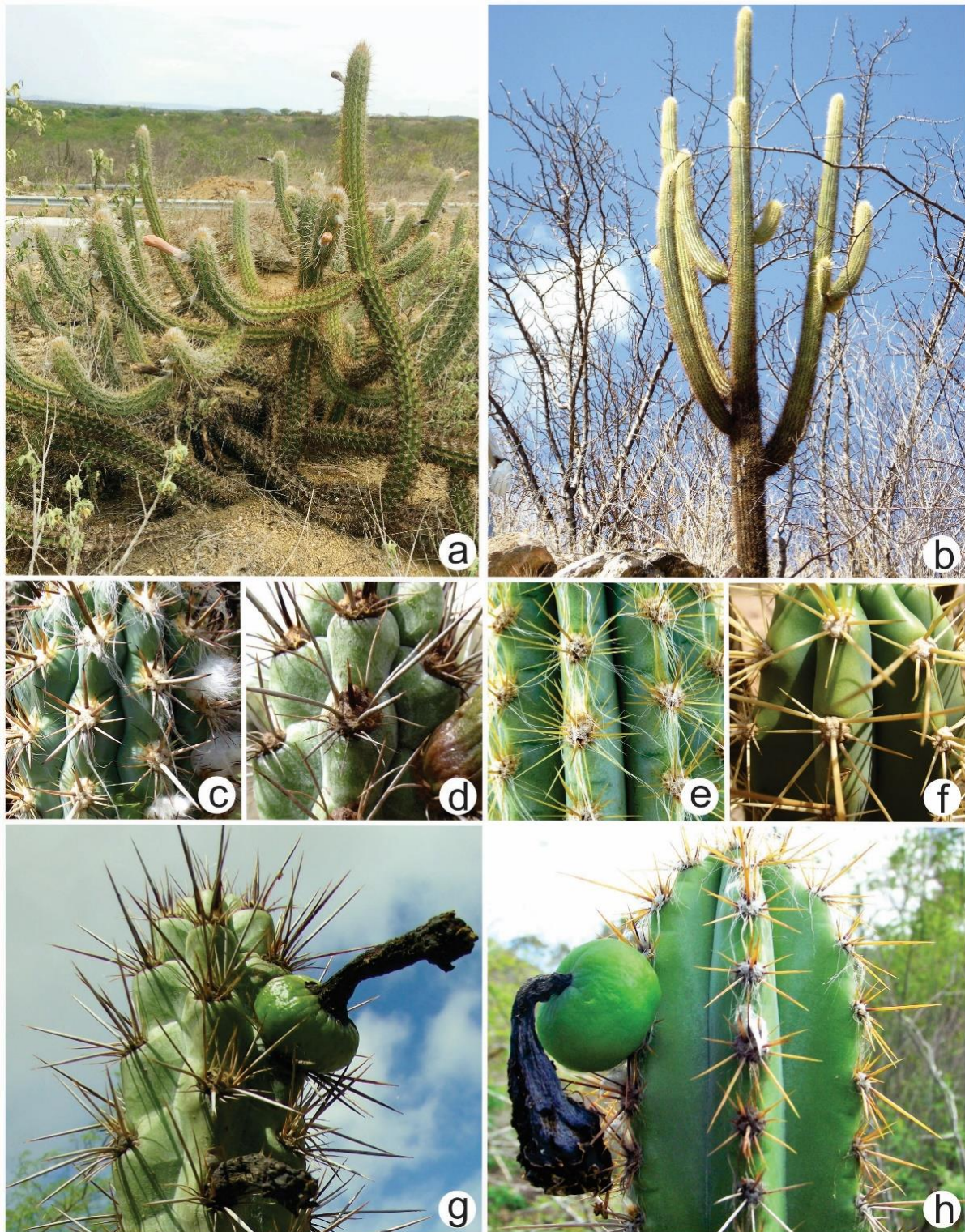


FIGURE 1. Main differences between *Pilosocereus* subgenus *Gounellea* (photos on the left) and *Pilosocereus* subgenus *Pilosocereus* (photos on the right), relative to the branching (**a** – candelabriform pattern; **b** – erect pattern); ribs (**c**, **d** – sinuses sinuate with conspicuous podaria beneath the areoles; **e**, **f** – sinuses straight) and fruit (**g** – floral remnant erect or pendent, not sunken into the apex of the pericarp, circular at point of attachment; **h** – floral remnant pendent, sunken into the apex of the pericarp, forming a linear insertion point) (Species: **a**, **c** – *P. gounellei* subsp. *gounellei*; **b**, **e** – *P. pachycladus* subsp. *pernambuconensis*; **d**, **g** – *P. tuberculatus*; **f** – *P. flavipulvinatus*; **h** – *P. oligolepis*).

PP = 1). In the BI topology (Fig. 2) clade A is the first diverging lineage inside *Pilosocereus* s.s. (BS = 100) while B and C are sister clades, however in the MP topology the relationships between clades A, B and C are uncertain (Fig. 3).

Inside *Pilosocereus* s.s., four species are consistently non-monophyletic in their current delimitation in both MP and BI

topologies since their respective subspecies are clustered separately in distinct strongly supported clades: *Pilosocereus pachycladus*, *P. pentaedrophorus*, *P. fulvilanatus* and *P. aurisetus*. *Pilosocereus aurisetus* subsp. *aurilanatus* is closer related to *P. fulvilanatus* subsp. *rosae* than to *P. aurisetus* subsp. *aurisetus*. *Pilosocereus fulvilanatus* subsp. *fulvilanatus* is closer to *P. pentaedrophorus* subsp. *robustus* than to

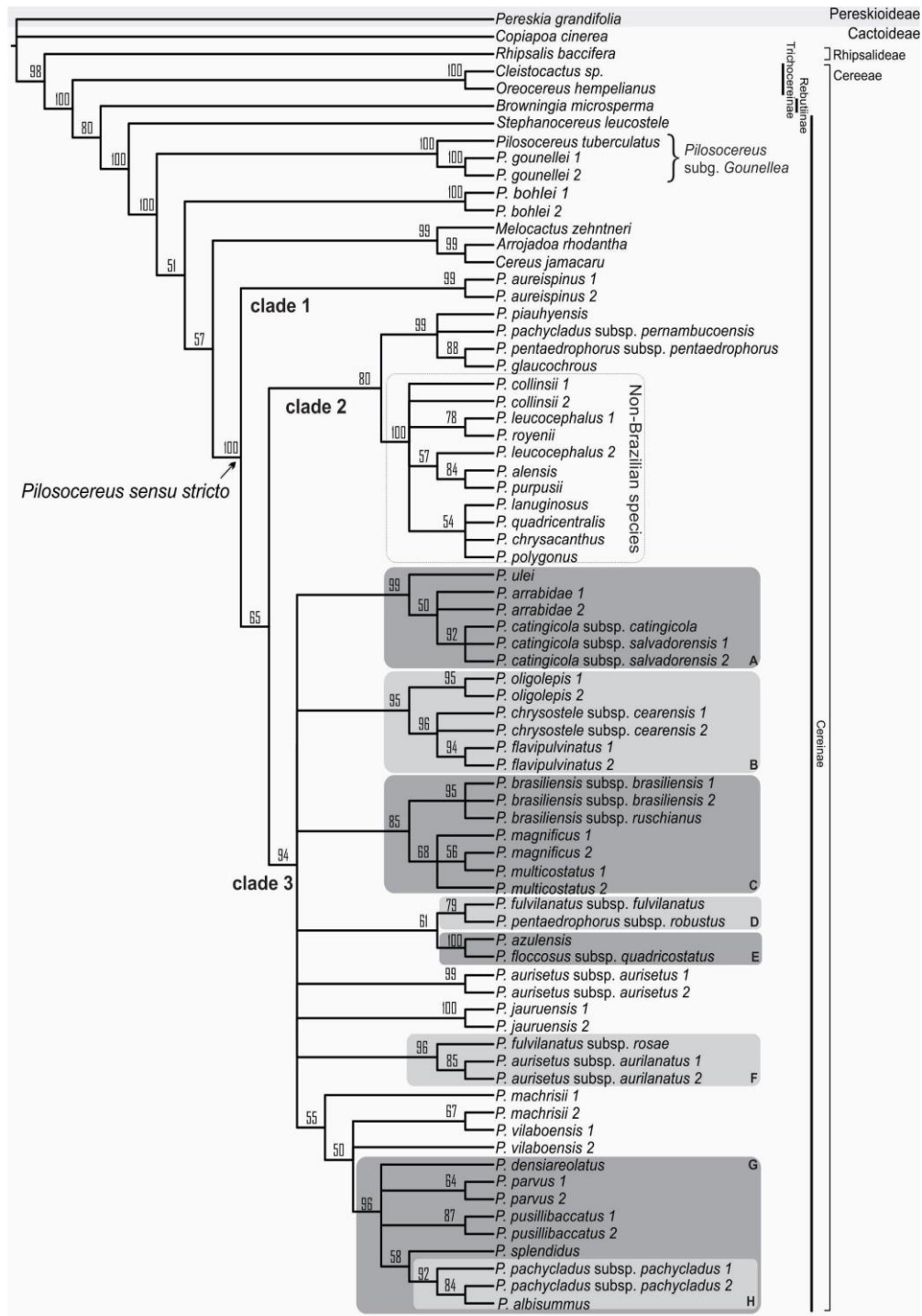


FIGURE 2. Strict consensus tree from maximum parsimony analyses of the combined dataset of (*trnS-trnG*, *psbD-trnT*, *trnL-trnT*, *petL-psbE*, *PhyC* and *ycf1*) produced for *Pilosocereus* and outgroups. Bootstrap values are shown above branches.

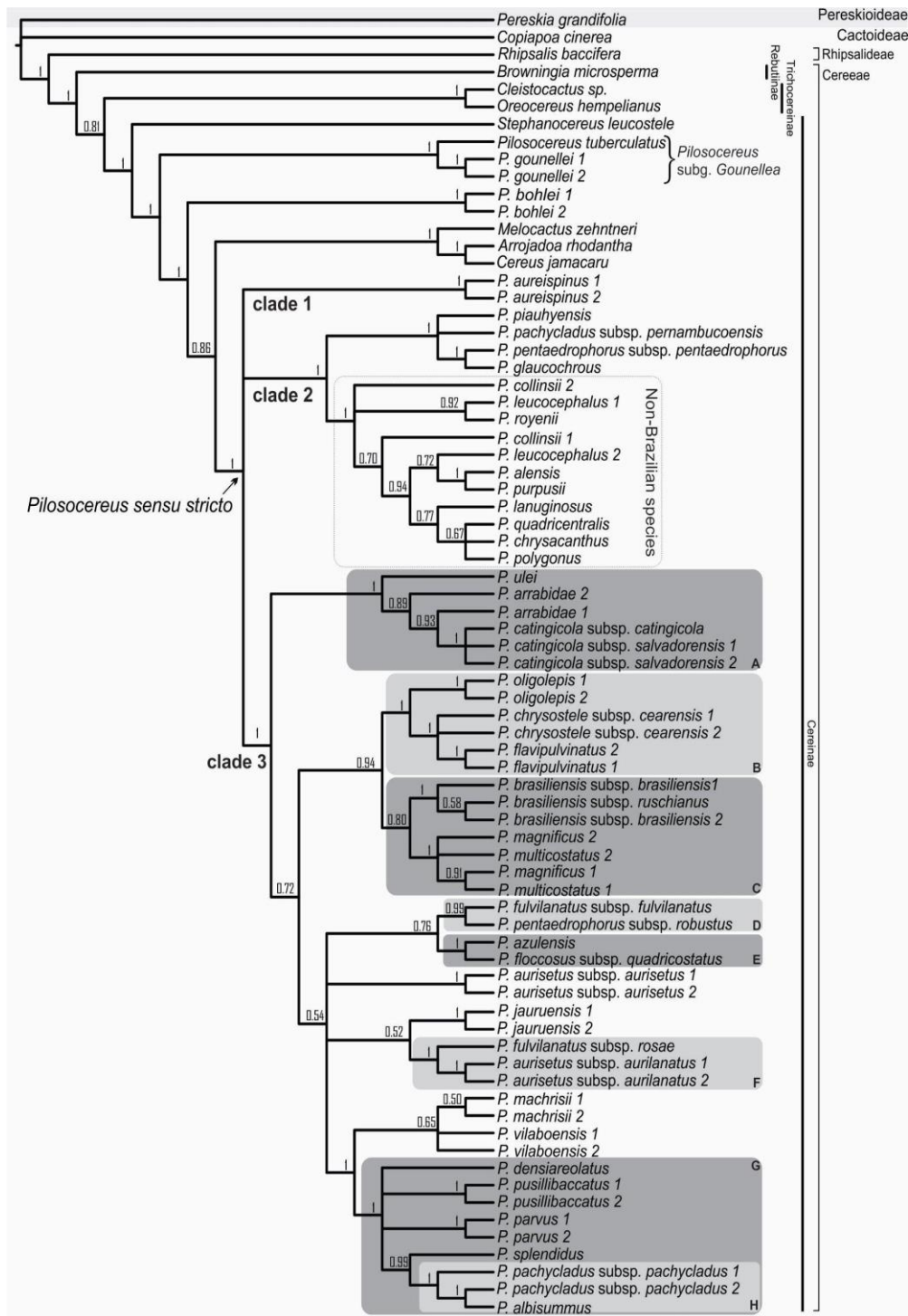


FIGURE 3. Majority rule consensus tree derived from Bayesian analyses of the combined dataset (from *trnS-trnG*, *psbD-trnT*, *trnL-trnT*, *petL-psbE*, *ycf1* and *PhyC*) produced for *Pilosocereus* and outgroups. Posterior probability values are shown above branches.

P. fulvilanatus subsp. *rosae*. *Pilosocereus pentaedrophorus* subsp. *pentaedrophorus* and *P. pachycladus* subsp. *pernambuconensis* are nested in clade 2 while *P. pentaedrophorus* subsp. *robustus* and *P. pachycladus* subsp. *pachycladus* are nested in clade D and H, respectively. The BI

results also indicate the non-monophyly of *P. leucocephalus*, *P. collinsii*, *P. arrabidae*, *P. magnificus* and *P. multicostatus* under moderate to low PP support. However, among those, only *P. leucocephalus*, *P. magnificus* and *P. multicostatus* are non-monophyletic under moderate to low BS support on the MP topology.

4. Discussion

4.1 Relationships within Cactoideae

The results from this work corroborate some relationships found in previous works treating the whole family (Arakaki et al. 2011; Hernández-Hernández et al. 2011, 2014). Despite the controversial positioning of *Browningia microsperma*, the relationships of subtribes within Cereeae (sensu Nyffeler and Eggli 2010) agree with previous results. We found differences in relationships within subfamily Cactoideae, mainly due to the inclusion of *Pilosocereus* subg. *Gounellea* and *P. bohlei*, which had not yet been sampled in any previous major Cactaceae or Cactoideae molecular phylogenetic hypothesis published so far, although similar results were found by Lavor et al. (2018).

Our results show that *Arrojadoa rhodantha* and *Stephanocereus leucostele* do not group together in the same clade, supporting their positioning in separate genera. Due to conspicuous morphological similarities, such as the presence of an apical cephalium when young, supplanted by continuous vegetative growth, then forming cephalium rings, their segregation into two genera have always raised questions on whether these genera would or not form a single group, despite their different pollination mechanisms and floral morphology (Taylor and Zappi 1989). Hunt et al. (2006) even claimed that based on the circumscription they adopt for *Arrojadoa*, and on preliminary molecular evidence, they considered including *Stephanocereus leucostele* within *Arrojadoa*. Our results refute such hypothesis.

We recovered a paraphyletic *Pilosocereus* s.l. corroborating the results found in Calvente et al. (2017) and Lavor et al. (2018) as other genera of tribe Cereinae emerged clustered within the group. We found a clade with *Melocactus*, *Arrojadoa* and *Cereus* species more closely related to *Pilosocereus* s.s. than to *P. bohlei*, *P. gounellei* and *P. tuberculatus* (the two latter belong to *P.* subg. *Gounellea*). Although this relationship is not strongly supported in neither MP nor BI topologies (BS = 57, PP = 0.86), the clade corresponding to *P.* subgenus *Gounellea* is strongly supported as a distinct lineage from a clade including all the other *Pilosocereus* in the BI results (PP = 1, BS = 51).

Pilosocereus subg. *Gounellea* differs morphologically from *Pilosocereus* s.s. due to its candelabriform branching, sinuate ribs, and fruits with floral remnant frequently erect, not sunken into the apex of the pericarp (Zappi 1994, Fig. 1). *Pilosocereus frewenii* (not sampled in our study) more recently described and included in *P.* subg. *Gounellea*, has the same morphological features common to *P. gounellei* and *P. tuberculatus*, however it has smaller habit and seeds and has flowers with different coloration (Zappi and Taylor 2011). Zappi (1994) states that the remarkable features of the subgenus *Gounellea* segregating it from *P.* subg. *Pilosocereus* would be plesiomorphic within the genus. Such hypothesis was raised by the presence of features such as ribs with marked hexagonal podaria, which are also found in some species of the subtribe Trichocereinae (e.g. *Rauhocereus*

Backeb., *Weberbauerocereus* Backeb. and *Haageocereus* Backeb.) (Zappi 1994). Based on our results we believe that morphological characters shared by both subgenera are possibly homoplasious and developed independently in both groups.

According to Taylor and Zappi (2004), *P. bohlei* shares similarities with *P. gounellei* (such as the narrower apical part of the fertile stem) but has fruits with floral remnants inserted in the pericarp (which is a diagnostic character of *P.* subg. *Pilosocereus*). However, the flowers in *P. bohlei* are smaller and S-shaped, different from all the species of the genus. We cannot find satisfying evidence in this work to support either the inclusion or the exclusion of *P. bohlei* from *Pilosocereus* and its inclusion in another new genus is still premature. Further studies are needed to elucidate the positioning of *P. bohlei* using additional molecular and morphological data. An investigation using a broader sampling for Cereinae is currently under course and may lighten its positioning. We found support in our results and on overall morphological diagnostic characters to propose a generic status for *Pilosocereus* subg. *Gounellea*. This new genus accommodates *P. gounellei*, *P. tuberculatus* and *P. frewenii*.

4.2 Relationship in *Pilosocereus* s.s.

Pilosocereus aureispinus appears isolated from the remaining species of *Pilosocereus* s.s. corroborating other studies using molecular data (Bonatelli et al. 2014, Lavor et al. 2018). We cannot find a conspicuous overall morphological differentiation between *P. aureispinus* and the remainder of *Pilosocereus* s.s. species. The only consistent difference is the globose to depressed-globose fruit and the seed coat (Zappi 1994). Also, a biogeographic pattern that differentiates this species from the others in *Pilosocereus* s.s. is not clear, since the species occurs in the same region as most Brazilian species (eastern Brazil), in an area of Cerrado in the state of Bahia (Taylor and Zappi, 2004, Lavor et al. 2020), within the Caatinga Biome (sensu Olson et al. 2001).

In general, the major clades formed within *Pilosocereus* s.s. (clades 1-3; Fig. 2, 3) apparently do not reflect easily perceived diagnostic characters or clear biogeographic structuring of clades. As discussed by Calvente et al. (2017) it is not easy to find diagnostic characters (even through ancestral states reconstruction analyses) in this group or to explain phylogenetic relationships recovered. Relationships apparently reflect a niche conservatism pattern, with diversification constrained to the same geographic area and habitat type of ancestral lineages or, occasionally, driven by migration events (Lavor et al. 2018).

Clade 2 includes a subclade of Brazilian species. Zappi (1994) reports that *P. pentaedrophorus* is a species related to *P. glaucochorous* because of the shared glabrous flowering areoles, curved flowers and stem epidermis striking blue colored, glaucous. Some populations of *P. pachycladus* subsp. *pernambucoensis* also present glaucous epidermis and these three taxa occur in close (but not sympatric) localities in the Brazilian state of Bahia. Because *P. pachycladus* subsp. *pernambucoensis* has a wide distribution in the northeastern

region of Brazil and is found in localities close to *P. piauihyensis*, there is a misleading history of collections in the state of Piauí for *P. pachycladus* subsp. *pernambucoensis*, however the material referred to this taxa there actually belongs under *P. piauihyensis* (Zappi 1994). All these species are found in the northeastern region of Brazil, as well *P. aureispinus* and other species of clade 3.

The second subclade in clade 2 includes non-Brazilian species. *Pilosocereus collinsii* and *P. purpusii* occur in sympatry in Mexico. Zappi (1994) indicated the first as a synonym of the second. However, Hunt et al. (2006) included both as distinct and accepted species. Main morphological differences among them are the number of ribs (7-10 in the first and 12 in the second) and sizes of the flowers (5 and 7 cm, respectively). Our results corroborate Hunt et al. (2006) decision, since *P. collinsii* does not emerge as a sister species of *P. purpusii* in our analyses.

Also, in this clade *P. royenii* and *P. polygonus* apparently require further studies at the population level and possibly a delimitation reevaluation. *Pilosocereus royenii* occurs in Mexico and in the Caribbean Islands, but some authors believe that Mexican populations (specifically in the Yucatán peninsula) are morphologically distinct from the Caribbean ones [these usually identified as *P. gaumeri* (Britton & Rose) Backeb., a synonym of *P. royenii* according to Hunt et al. 2006]. However, the Caribbean populations are sympatric with *P. polygonus*, and these two species are differentiated only by the abundant presence of hairs in the areoles in *P. royenii*, which are absent in *P. polygonus* (Zappi 1994). Hunt et al. (2006) argue that perhaps the populations for these two species occurring in the Caribbean could be the same species that present a variation in the amount of hairs in the areoles. Our results do not serve to refute this idea, since our sample of *P. royenii* is from a population that occurs in the Yucatán peninsula (Mexico). The only biogeographic pattern found in this subclade is that all species occur outside Brazil. As expected, none of them emerge grouped with *P. oligolepis*, which occurs in northern Brazil in the state of Roraima and Guyana (Lavor et al. 2016).

The largest clade of Brazilian species (clade 3), includes clade "A" with three taxa of *P. arrabidaea* group (sensu Zappi 1994): *P. catingicola* subsp. *catingicola*, *P. catingicola* subsp. *salvadorensis* and *P. arrabidaea*. These three taxa present large flowers, fruit and seeds, small numbers of ribs, and are found in forest formations or sand dunes (*restinga*) on the Brazilian coast. The other species belonging to this clade, *P. ulei*, although presenting a completely different morphology from the species mentioned above is also found restricted to areas of *restinga*, where it grows in sympatry with *P. arrabidaea* (Zappi 1994).

Clade "B" presents three taxa: *Pilosocereus flavipulvinatus*, *P. oligolepis* and *P. chrysostele* subsp. *cearensis*. Zappi (1994) suggested a close relationship between the first two based on their morphology. This relationship would be an evidence of a past northern-northeastern Brazil phytogeographical linkage (Taylor and Zappi 2004), since *P. flavipulvinatus* is found in ecotonal areas of Caatinga/Cerrado and Carnaubais (i.e. a *Copernicia*

palm dominated vegetation between Amazon and Caatinga in Brazil) in the Brazilian northeast (Menezes et al. 2013), while *P. oligolepis* is found in rock outcrops in savanna areas in the north of Brazil and Guyana (Lavor et al. 2016). Endemic to the state of Ceará *P. chrysostele* subsp. *cearensis* occurs in savanna formations and rock outcrops (Menezes et al. 2013) in localities close to those of *P. flavipulvinatus*. The similar habitat connection seems to be the link between these closely related taxa. *Pilosocereus chrysostele* subsp. *cearensis* is treated by Taylor and Zappi (2004) and Hunt et al. (2006) as a synonym of *P. piauihyensis*, what is refuted by our results.

Clade "C" is composed by *P. brasiliensis*, *P. magnificus* and *P. multicosatus*. Previous studies based on morphology and habitat evidence have not approximated them. *Pilosocereus magnificus* and *P. multicosatus* have sympatric and restricted distribution in the state of Minas Gerais, while *P. brasiliensis* subsp. *ruschianus* has a wider distribution (occurring in Minas Gerais, Bahia and Espírito Santo states) and *P. brasiliensis* subsp. *brasiliensis* occurs only in the states of Espírito Santo and Rio de Janeiro (Zappi 1994). The geographical proximity seems to be the only connection among these species, however further studies focusing on this clade can elucidate more aspects of its complexity.

We assume the same for clades "D" and "E". Species on these clades although not morphologically similar occur close to each other or in sympatric localities. We find *P. fulvilanatus* subsp. *fulvilanatus* in the north of Minas Gerais state and *P. pentaedrophorus* subsp. *robustus* in the middle east and south of Bahia state and northeast of Minas Gerais, Brazil. Also, in Minas Gerais, *P. azulensis* and *P. floccosus* subsp. *quadricostatus* both occur in the municipality of Pedra Azul (northern Minas Gerais), however *P. floccosus* subsp. *quadricostatus* expands to northeastern Minas Gerais, along the drainage basins of the Pardo and Jequitinhonha Rivers (Zappi 1994). The species of clade "F", *P. fulvilanatus* subsp. *rosae* and *P. aurisetus* subsp. *aurilanatus*, are also morphologically distinct but not sympatric. Both are found in rocky outcrops in the center-north of the Minas Gerais State with restricted distributions (Zappi 1994; Taylor and Zappi 2004).

The species *P. parvus* and *P. pusillibaccatus*, which make up the clade "G", belong to the species complex *P. aurisetus* (sensu Zappi 1994), and have already been synonymized (together with *P. jauruensis*) in Zappi (1994) as *P. machrisii*, but they were accepted by Hunt et al. (2006), delimitation corroborated by our results. *Pilosocereus aurisetus* group is composed of *P. aureispinus*, *P. aurisetus*, *P. bohlei*, *P. jauruensis*, *P. machrisii*, *P. parvus*, *P. pusillibaccatus* and *P. vilaboensis* presenting great morphological variation. It is distributed throughout the central region of Brazil and presents a history of controversial classification (Zappi 1994; Taylor and Zappi 2004; Bonatelli et al. 2013). In our results this group did not appear as monophyletic.

Also, in clade G, *P. splendidus* is considered by Zappi (1994) as a synonym of *P. pachycladus* but is accepted by Hunt et al. (2006). It appears as sister to the clade H, composed of *P. pachycladus* subsp. *pachycladus* and *P. albisummus*, corroborating the classification of Hunt et al.

(2006). The species *P. albisummus* is reported by several authors as a poorly known taxon (e.g. Zappi 1994; Taylor and Zappi 2004; Hunt *et al.* 2006). The close relationship found in our work for *P. pachycladus* subsp. *pachycladus* and *P. albisummus*, may give a hint to the history of this species.

Within clade 3, we observe some biogeographic patterns, related to the major biomes of the central/eastern region of Brazil (Caatinga, Cerrado, and Atlantic Forest). Some subgroups in this clade present many species restricted to some of these biomes (such as Cerrado) while other species are found in different regions too. The historical biogeography of *Pilosocereus* (Lavor *et al.* 2018) showed that this pattern could be associated with historical factors (such as the Pleistocene climatic fluctuations). In a recent analysis (Lavor *et al.* 2020) no correlation was found between the current distribution of species and environmental variables such as vegetation, climate and soil, suggesting that historical factors possibly had a more significant role on shaping the current distribution and impacting phylogenetic relationships observed for the group.

4.3 Intraspecific monophyly

We have evidence for the monophyly of *P. catingicola*, *P. brasiliensis*, *P. gounellei*, *P. bohlei*, *P. aureispinus*, *P. oligolepis*, *P. flavipulvinatus*, *P. jauruensis*, *P. pusillibaccatus*, and *P. parvus* (corresponding near to 23 % of the species of *Pilosocereus* included in this study). Among those, we sampled different subspecies for the first two. *Pilosocereus catingicola* subsp. *catingicola* and *P. catingicola* subsp. *salvadorensis* clustered in our phylogeny in clade A. Although both subspecies are distinct morphologically, they are not found in sympatry as the dry vegetation along the valley of the São Francisco river basin (Brazil) is separating both (Zappi 1994). Similar fact occurs with *P. brasiliensis* subsp. *brasiliensis* and *P. brasiliensis* subsp. *ruschianus* (clade C). Few morphological differences may be noted between these two subspecies, which according to Zappi (1994) would reflect their adaptation to distinct habitats (*P. brasiliensis* subsp. *brasiliensis* occurs in rock outcrops inside restinga formations of the Atlantic Forest domain in Brazil, while *P. brasiliensis* subsp. *ruschianus* occurs in areas of dry forest rock outcrops also in Brazil).

Among the four consistently non-monophyletic species highlighted in this study, *P. aurisetus*, *P. fulvilanatus*, *P. pachycladus* and *P. pentaedrophorus* have two subspecies each, one homotypic and the other heterotypic. For the first three species, the heterotypic subspecies arose from synonyms and lowering of the rank of other species of *Pilosocereus* to the subspecies level by Zappi (1994). Only *P. pentaedrophorus* subsp. *robustus* was described initially as a subspecies. Based on this phylogenetic evidence together with consistencies of morphological and geographical characters we propose to elevate the heterotypic subspecies to the species rank. As the specific epithet “*robustus*” is already taken in *Pilosocereus* for a different taxon, we propose a new name. The other three binomials already exist at the species rank.

Each taxonomic change proposed in this work is discussed below.

5. Taxonomic treatment

We propose a new monophyletic genus including species formerly placed in *Pilosocereus* subg. *Gounellea*, and the elevation of four heterotypic subspecies to the species rank. Following these changes *Pilosocereus* is now composed by the species grouped in *Pilosocereus* s.s. clade plus *P. bohlei*, including a total of 42 species and four heterotypic subspecies.

XIQUEXIQUE Lavor, Calvente & Versieux **nom. nov.** *Pilosocereus* subg. *Gounellea* Zappi, Succ. Pl. Res., 3:36 (1994). TYPE: *Pilosocereus gounellei* (F. A. C. Weber) Byles & G. D. Rowley in Cact. Succ. J. Gr. Brit. 19: 67 (1957). (= *Xiquexique gounellei* (F. A. C. Weber) Lavor & Calvente).

Tree-like to shrubby cacti, main stem upright, mature branches arched, running more or less parallel to the ground, apices ascending, new axes arising subapically (branching candelabriform); ribs 4–15, sinuses sinuate with conspicuous podaria beneath the areoles; fruit with floral remnant frequently erect, not sunken into the apex of the pericarp, circular at point of attachment.

Distribution — In the Eastern region of Brazil (along the entire Northeast region and in the central-north portion of Minas Gerais state).

Etymology — Named in reference to the vernacular name of *Pilosocereus gounellei*, the xique-xique, broadly known in Brazil and one of the most emblematic cacti of the Brazilian semi-arid Northeastern region.

Notes — We publish this new name as a replacement name for *Pilosocereus* subg. *Gounellea* to avoid a generic name derived from one of the species of the genus (recommendation 20A1 from the Melbourne code; McNeill *et al.* 2012a). We kept the generic name in masculine to maintain the gender of *Pilosocereus* (recommendation 62A from the Melbourne code; McNeill *et al.* 2012b).

Three species and one heterotypic subspecies that were included within *Pilosocereus* subgenus *Gounellea*, are now transferred to *Xiquexique*.

XIQUEXIQUE GOUNELLEI (F. A. C. Weber) Lavor & Calvente, comb. nov. *Pilosocereus gounellei* F. A. C. Weber in K. Schum., Gesamtbeschr. Kakt.: 188 (1897). *Pilosocereus gounellei* (F. A. C. Weber) Byles & G. D. Rowley in Cact. Succ. J. Gr. Brit. 19: 67 (1957), syn. nov. TYPE: BRAZIL. Pernambuco, ‘Certão’, *Gounelle* s.n. (P†). Neotype (Zappi 1994): Brazil, Paraíba, Várzia, São Gonçalo (Várzea de Souza), Jan. 1936, *P. Luetzelburg* 26921 (M!, K [photo], IPA).

XIQUEXIQUE GOUNELLEI subsp. ZEHNTNERI (Britton & Rose) Lavor & Calvente comb. nov. *Cephalocereus zehntneri* Britton & Rose, Cact. 2: 35 (1920). *Pilosocereus*

gounellei subsp. *zehntneri* (Britton & Rose) Zappi in Succ. Pl. Res. 3: 43 (1994), *syn. nov.* TYPE: BRAZIL. Bahia, district of Chique-Chique (Xique-Xique), Serra de Tiririca, Nov. 1917, *Zehntner s. n.* (Zappi 1994) (US, K, US [photo], lectoparatype: NY).

XIQUEXIQUE FREWENII (Zappi & Taylor) Lavor & Calvente *comb. nov.* *Pilosocereus frewenii* Zappi & Taylor in Bradleya 29: 131 – 136 (2011), *syn. nov.* TYPE: BRAZIL. Minas Gerais, Mun. Santana de Pirapama, distrito de Coberto, north of Inhame, Bambuí limestone outcrop in dry forest at the western foot of the Serra do Cipó, 777 meters above sea level, 19 July 2009, *Zappi & Taylor 2208* (holotype: SPF, isotype: RB).

XIQUEXIQUE TUBERCULATUS (Werderm.) Lavor & Calvente *comb. nov.* *Pilosocereus tuberculatus* Werderm., Bras. Säulenkakt.: 101 (1933). *Pilosocereus tuberculatus* (Werderm.) Byles & G. D. Rowley in Cact. Succ. J. Gr. Brit. 19(3): 69 (1957), *syn. nov.* TYPE: BRAZIL. Pernambuco, Serra Negra, '900 m', Mar. 1932, Werdermann (B†). (lectotype: Werdermann l. c. infra [photo] p. 21 (Zappi 1994)).

5.1 New Names and Synonyms for *Pilosocereus*

PILOSOCEREUS AURILANATUS F. Ritter, Kakt. Südamer. 1: 77–78, Abb. 50 (1979). *Pilosocereus aurisetus* subsp. *aurilanatus* (Ritter) Zappi in Succ. Pl. Res. 3: 123 (1994), *syn. nov.* TYPE: BRAZIL. Minas Gerais, Joaquim Felício, 1964, *Ritter 1325* (holotype: U).

To propose *P. aurisetus* subsp. *aurilanatus*, Zappi (1994) downgraded *P. aurilanatus* Ritter to the category of subspecies under *P. aurisetus*, based on both taxa morphological similarities (as their very distinct fruit that divides in the apex breaking the floral remnants and by the very smooth seed coats). According to Zappi's (1994) understanding they would compose a continuous distribution for *P. aurisetus* as they occur isolated from each other in rocky outcrops in Minas Gerais (Brazil). The subspecies differ in the flower-bearing areoles, with golden hairs in *P. aurisetus* subsp. *aurilanatus* and with white hairs in *P. aurisetus* subsp. *aurisetus*; and stems size 4.0–7.0 cm diam. in *P. aurisetus* subsp. *aurilanatus* and < 6 cm in *P. aurisetus* subsp. *aurisetus* (Ritter 1979; Zappi 1994; Hunt et al. 2006).

PILOSOCEREUS PERNAMBUCOENSIS F. Ritter, Kakt. Südamer. 1: 65 (1979). *Pilosocereus pachycladus* subsp. *pernambucoensis* (F. Ritter) Zappi in Succ. Pl. Res. 3: 109 (1994), *syn. nov.* TYPE: BRAZIL. Pernambuco, Araripina, 1963, *Ritter 1219* (holotype: U).

Pilosocereus pachycladus is one of the most widely distributed species in Northeastern Brazil, with great morphological diversity. The many heterotypic synonyms recognized for this species indicate an extensive history of nomenclatural and taxonomic confusion (Zappi 1994). Zappi (1994) proposed *P. pachycladus* subsp. *pernambucoensis*, synonymizing and lowering *Pilosocereus pernambucoensis* Ritter to the subspecies rank, based on the existence of

morphologically intermediate populations in the region of Juazeiro and Sento Sé (Brazil). These intermediate populations occur to the north of the São Francisco river basin presenting 10–15 ribs and relatively fine spination (Zappi 1994; Taylor and Zappi 2004). However, *P. pachycladus* subsp. *pernambucoensis* differs from *P. pachycladus* subsp. *pachycladus* by a considerable number of features as the greater number of ribs (13–19 in the first and 5–12 in the latter), which are lower and closer to each other; the overall smaller spines (reaching 1,8 cm in the first and 3 cm in the latter); and the flower-bearing areoles (with scarce wool in the first and with abundant wool in the latter) (Ritter 1979; Zappi 1994; Taylor and Zappi 2004).

PILOSOCEREUS ROSAE P. J. Braun in Kakt. and. Sukk. 35(8): 178–181 (1984). *Pilosocereus fulvilanatus* subsp. *rosae* (P. J. Braun) Zappi in Succ. Pl. Res. 3: 100 (1994), *syn. nov.* TYPE: BRAZIL. Minas Gerais: Mun. Augusto de Lima, near Santa Bárbara, 6 km from road BR 135, west slopes of the Serra do Espinhaço, 800 m, 1983, *Horst & Uebelmann 546* (ZSS, isotype: K!).

Zappi (1994) and Taylor and Zappi (2004) state that the two subspecies of *P. fulvilanatus* are very similar morphologically, differing in the less robust habit of *P. fulvilanatus* subsp. *rosae*, which has branches smaller than 5.5 cm in diameter (8–12 cm in *P. fulvilanatus* subsp. *fulvilanatus*) and more ribs (5–) 6–8 compared to 4–7 in *P. fulvilanatus* subsp. *fulvilanatus* (Buining and Brederoo 1973; Braun 1984; Hunt et al. 2006). These taxa occur separated on different sides of the Espinhaço mountain Range (Minas Gerais state, Brazil) (Zappi 1994). The proposition of *P. fulvilanatus* subsp. *rosae* by Zappi (1994) is based on the lowering of *Pilosocereus rosae* P. J. Braun to the subspecies rank.

PILOSOCEREUS ZAPPIAE Lavor & Calvente *nom. nov.* *Pilosocereus pentaedrophorus* subsp. *robustus* Zappi in Succ. Pl. Res. 3: 74 (1994), *syn. nov.* HOLOTYPE: BRAZIL. Bahia, Mun. Livramento do Brumado, 11 km S of town on road to Brumado, 450 m, 13°45'S, 41°49'W, 23 Nov. 1988, *Taylor & Zappi in Harley 25544* (SPF, isotype: CEPEC!, K!).

Etymology — Named in honor of the botanist Daniela Zappi. Her extensive taxonomic work with Brazilian cacti and *Pilosocereus* has pushed the limits of the knowledge of the genus and of the Brazilian Cactaceae, as a whole.

Zappi (1994) described *Pilosocereus pentaedrophorus* subsp. *robustus* based on observed morphological discontinuities among the populations of *P. pentaedrophorus*. *Pilosocereus pentaedrophorus* subsp. *pentaedrophorus* occurs from the northern of Bahia state to Pernambuco (Brazil) and *P. pentaedrophorus* subsp. *robustus* occurs in the center-south of Bahia to the northeast of Minas Gerais (mainly in the drainage basin of the Contas and Pardo rivers). Besides the geographical separation, *P. zappiae* differs from *P. pentaedrophorus* subsp. *pentaedrophorus* by the stouter branches (never leaning) up to 7.5 cm diam. (up to 4.5 cm in

the latter), with (5-) 6-10 acute ribs (4-6 in the latter) (Zappi 1994).

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Appendixes

APPENDIX 1. Vouchers and GenBank accession numbers for species used in the phylogenetic analyses of *Pilosocereus* (new sequences generated in this study are printed in bold).

Species: collector/voucher herbarium/location (country) - accession numbers *trnS-trnG*, *psbD-trnT*, *trnL-trnT*, *petL-psbE*, *PhyC* and *ycf1* (Abbreviations — BHCB: Herbário Universidade Federal de Minas Gerais, Brazil; CCTS: Herbário da Universidade Federal de São Carlos (Campus Sorocaba), Brazil; CEPEC: Herbário CEPEC, Brazil; HUEFS: Herbário Universidade Federal de Feira de Santana, Brazil; QCNE: Museo Ecuatoriano de Ciencias Naturales del Instituto Nacional de Biodiversidad, Ecuador; SORO: Herbário do Centro de Ciências e Tecnologia para Sustentabilidade, Universidade Federal de São Carlos (Campus Sorocaba), Brazil; UFRN: Herbário Universidade Federal do Rio Grande do Norte, Brazil; MA: Vascular Plant Herbarium, Real Jardín Botánico de Madrid, Espanha; MEXU: Univ. Nacional Autónoma de México).

Arrojadoa rhodantha: Machado M. 777/HUEFS107367/Bahia (Brazil) – KX301205, KX30108, KX301167, KX301129, KX301244, **MF614893**; *Browningia microsperma*: Madsen JE. 7311/MA752144/Loja (Ecuador) – **KX387798**, **KX387705**, **KX387770**, **KX387739**, **MF614857**, **MF614884**; *Cereus jamacaru*: Calvente A. 461/UFRN/Bahia (Brazil) – KX301200, KX301076, KX301162, KX301119, KX301238, **MF614874**; *Cleistocactus* sp: Aedo C. 14512/MA759900/Cochabamba (Bolivia) – **KX387795**, **KX387701**, **KX387766**, **KX387735**, **MF614853**, **MF614880**; *Copiapoa cinerea*: Rodrigues R. 3120/MA702058-11019/Antofagasta (Chile) – -, **KX387702**, **KX387767**, **KX387736**, **MF614854**, **MF614881**; *Melocactus zehntneri*: Calvente A. 462/UFRN/ Rio Grande do Norte (Brazil) – KX301198, KX301074, KX301160, KX301117, KX301236, **MF614872**; *Oreocereus hempelianus*: Aedo C. 11369/MA728565/Moquegua (Peru) – **KX387796**, **KX387703**, **KX387768**, **KX387737**, **MF614855**, **MF614882**; *Pereskia grandifolia*: Damaso P./UFRN 7796/Rio Grande do Norte (Brazil) – -, **KX387707**, **KX387772**, **KX387741**, **MF614858**, **MF614886**; *Pilosocereus albissimus*: Moraes E.M. S141/SORO4530 /Minas Gerais (Brazil) – KX301216, KX301097, KX301178, KX301140, KX301255, **MF614902**; *P. alensis*: Sanchez-Mejorada H. 4449/MEXU/Jalisco (Mexico) – KX301188, KX301064, KX301150, KX301107, KX301226, **MF614866**; *P. arrabidae* (1): Machado MC. S35MI/SORO4488/Bahia (Brazil) – **KX387801**, **KX387714**, **KX387774**, **KX387747**, **MF614861**, **MF614891**; *P. arrabidae* (2): Franco FF S79B1/SORO 2656/ Rio de Janeiro (Brazil) – KX301222, KX301103, KX301184, KX301146, KX301261, **MF962471**; *P. aureispinus* (1): Moraes EM. S21/HUFS642/Bahia (Brazil) – KX301201, **KX387712**, JN035609, -, KC779308.1, **MF962458**; *P. aureispinus* (2): Moraes EM. S21/HUFS642/Bahia (Brazil) – JN035414/JN035456, KX301080, KX301163, KX301123, KX301240, -, *P. aurisetus* subsp. *aurisetus* (1): Moraes EM. S11/HUFS646/ Minas Gerais (Brazil) – JN035403 /JN035437, **KX387709**, JN035585, **KX387743**, KC779292.1, **MF614887**; *P. aurisetus* subsp. *aurisetus* (2): Moraes EM. S30/ SORO2651/Minas Gerais (Brazil) – KC779380.1, KX301082, KC779380.1, KX301125, KX301241, **MF962460**; *P. aurisetus* subsp. *aurilanatus* (1): Moraes EM. S7/HUFS639/Minas Gerais (Brazil) – KC779423.1, **KX387708**, KC621246.1, **KX387742**, **MF962439**, **MF962454**; *P. aurisetus* subsp. *aurilanatus* (2): Moraes EM. S7/HUFS639/Minas Gerais (Brazil) – KC779425.1, KX301077, KC621248.1, KX301120, KC779288, **MF962455**; *P. azulensis*: Olsthoorn G. 253/ SORO 4531/ Minas Gerais (Brazil) – KX301214, KX301095, KX301176, KX301138, KX301253, **MF614901**; *P. bohlei* (1): Moraes EM. S35/CCTS3000/Bahia (Brazil) – **KX387802**, **KX387715**, **KX387775**, **KX387748**, **MF962441**, **MF962461**; *P. bohlei* (2): Moraes EM. S51/CCTS3000/Bahia (Brazil) – KX301211, KX301092, KX301173, KX301135, KX301250, **MF614899**; *P. brasiliensis* subsp. *brasiliensis* (1):

Franco FF. S86/SORO 4568/Espírito Santo (Brazil) – KX387810, KX387723, KX387782, KX387755, MF962446, MF962472; P. brasiliensis subsp. brasiliensis (2): Franco FF. S79E/SORO2654/Espírito Santo (Brazil) – KX301223, KX301104, KX301185, KX301147, KX301262, MF614907; P. brasiliensis subsp. ruschianus: Olsthoorn G. 188/SORO 4540/Minas Gerais (Brazil) – KX387809, KX387722, KX387781, -, MF962445, MF962470; P. catingicola subsp. catingicola: Olsthoorn G. 1026/SORO4532/Bahia (Brazil) – KX301217, KX301098, KX301179, KX301141, KX301256, MF614903; P. catingicola subsp. salvadorensis (1): Menezes MOT. 378/EAC57091/Bahia (Brazil) – KX387803, KX387716, -, KX387749, MF962442, MF962462; P. catingicola subsp. salvadorensis (2): Menezes MOT. 152/EAC44189/Ceará (Brazil) – KX301218, KX301099, KX301180, KX301142, KX301257, MF962469; P. chrysacanthus: Arias S. 858/ MEXU/Oaxaca (Mexico) – KX301190, KX301066, KX301152, KX301109, KX301228, MF614868; P. chrysostele subsp. cearenses (1): Lavor P. 36/ UFRN17127/Ceará (Brazil) – KX387789, KX387695, KX387761, KX387729, -, MF614875; P. chrysostele subsp. cearenses (2): Menezes MOT. 161/ EAC44385/ Ceará (Brazil) – KX387804, KX387717, KX387776, KX387750, MF962443, MF962463; P. collinsii (1): Arias S. 1658/MEXU/Chiapas (Mexico) – KX387784, KX387690, KX387756, KX387724, -, MF614869; P. collinsii (2): Arias S. 1635/_ – KX301191, KX301067, KX301153, KX301110, KX301229, MF962448; P. densiareolatus: Moraes EM. S43/ SORO2650/Minas Gerais (Brazil) – KX301208, KX301089, KX301170, KX301132, KX301247, MF614896; P. flavipulvinatus (1): Lavor P. 71/_/Ceará (Brazil) – KX387799, KX387706, KX387771, KX387740, -, MF614885; P. flavipulvinatus (2): Menezes MOT. 259/EAC48762/Ceará (Brazil) – KX301224, KX301105, KX301186, KX301148, KX301263, -; P. floccosus subsp. quadricostatus: Olsthoorn G. 42/SORO4558/Minas Gerais (Brazil) – KX301220, KX301101, KX301182, KX301144, KX301259, MF614905; P. fulvilanatus subsp. fulvilanatus: Moraes EM. S42/SORO2655/Minas Gerais (Brazil) – KX301207, KX301088, KX301169, KX301131, KX301246, MF614895; P. fulvilanatus subsp. rosae: Olsthoorn G. 263/ SORO 4534/Minas Gerais (Brazil) – KX301215, KX301096, KX301177, KX301139, KX301254, MF962467; P. glaucochrous: Machado MC. S35M2/SORO 4536/Bahia (Brazil) – KX301202, KX301083, KX301164, KX301126, -, -; P. gounellei (1): Lavor P. 18/UFRN16223/Piauí (Brazil) – KX387787, KX387693, KX387759, KX387727, MF614848, MF614870; P. gounellei (2): Lavor P. 22/ UFRN16227/Piauí (Brazil) – KX387788, KX387694, KX387760, KX387728, MF962437, MF962451; P. jauruensis (1): Moraes EM. S23/HUFS 638/Mato Grosso do Sul (Brazil) – KC779348.1, KX387713, KC779348.1, KX387746, MF614860, MF614890; P. jauruensis (2): Moraes EM. S25/SORO 2646/Mato Grosso do Sul (Brazil) – KC779358.1, KX301081, KC779358.1, KX301124, KC779302, MF962459; P. lanuginosus: Mero 96/QCNE/_ – KX387791, KX387697, KX387763, KX387731, MF614850, MF614877; P. leucocephalus (1): Arias S. 1621/_/_ – KX387785, KX387691, KX387757, KX387725, MF962436, MF962449; P. leucocephalus (2): Arias S. 1654/MEXU/Chiapas (Mexico) – KX301193, KX301069, KX301155, KX301112, KX301231, -; P. machrisii (1): Moraes EM. S18/HUFS 648/Goiás (Brazil) – KC779332.1, KX387710, KC621149.1, KX387744, MF614859, MF614888; P. machrisii (2): Moraes EM. S17/HUFS 645/Goiás (Brazil) – KC779262.1/JN035400, KX301078, JN035602, KX301121, KX301239, MF962456; P. magnificus (1): Moraes EM. S37/SORO4550/Minas Gerais (Brazil) – KX387805, KX387718, KX387777, KX387751, MF614862, MF614892; P. magnificus (2): Taylor NP. & Zappi DC. 755/BHCB20954/ Minas Gerais (Brazil) – KX301204, KX301085, KX301166, KX301128, KX301243, MF962464; P. multicostatus (1): Moraes EM. S41/SORO2649/Minas Gerais (Brazil) – KX387806, KX387719, KX387778, KX387752, MF614863, MF614894; P. multicostatus (2): Moraes EM. S39/SORO2653/Minas Gerais (Brazil) – KX301206, KX301087, KX301168, KX301130, KX301245, MF962465; P. oligolepis (1): Lavor et al. 60/UFRN 18663/Roraima (Brazil) – KX387792, KX387698, KX387764, KX387732, MF614851, MF614878; P. oligolepis (2): Lavor P. & Lavor J. 69/UFRN18670/ Roraima (Brazil) – KX387793, KX387699, KX387765, KX387733, MF962438, MF962453; P. pachycladus subsp. pachycladus (1): Taylor NP. 1434/ CEPEC 50888/Bahia (Brazil) – KX387807, KX387720, KX387779, KX387753, MF962444, MF962466; P. pachycladus subsp. pachycladus (2): Moraes EM. S45/SORO2647/ Minas Gerais (Brazil) – KX301209, KX301090, KX301171, KX301133, KX301248, MF614897; P. pachycladus subsp. pernambucoensis: Lavor P. 23/UFRN16228/Piauí (Brazil) – KX301197, KX301073, KX301159, KX301116, KX301235, MF962452; P. parvus (1): Moraes EM. S47/SORO2648/Goiás (Brazil) – KX387808, KX387721, KX387780, KX387754, MF614864, MF614898; P. parvus (2): Moraes EM. S47/SORO2648/Goiás (Brazil) – KX301210, KX301091, KX301172, KX301134, KX301249, -; P. pentaedrophorus subsp. pentaedrophorus: Calvente A. 409/UFRN 13193/Bahia (Brazil) – KX387783, KX387689, -, -, -, MF962447; P. pentaedrophorus subsp. robustus: Olsthoorn G. 172/SORO4538/Bahia (Brazil) – KX301212, KX301093, KX301174, KX301136, KX301251, MF962467; P. piauihyensis: Lavor P. 14/UFRN16219/Piauí (Brazil) – KX387786, KX387692, KX387758, KX387726, -, -; P. polygonus: DNA Bank Kew 45353/_/_ – KX387794, KX387700, -, KX387734,

MF614852, MF614879; *P. purpusii*: Blancas Vazquez JJ. 119/MEXU/Nayarit (Mexico) – KX301189, KX301065, KX301151, KX301108, KX301227, **MF614867; *P. pusillibaccatus* (1):** Lavor P. 20/UFRN16225/ Piauí (Brazil) – KX301195, KX301071, KX301072, KX301157, KX301114, KX301233, **MF614871; *P. pusillibaccatus* (2):** Lavor P. 21/UFRN 16226/Piauí (Brazil) – KX301196, KX301072, KX301158, KX301115, KX301234, **MF962450; *P. quadricentralis*:** Arias S. 2180/MEXU/Oaxaca (Mexico) – KX301187, KX301063, KX301149, KX301106, KX301225, **MF614865; *P. royenii*:** S Arias 1098/MEXU/ Yucatán (Mexico) – KX301192, KX301068, KX301154, KX301111, KX301230, -; ***P. splendidus*:** Moraes EM. S139/ SORO4539/Bahia (Brazil) – KX301213, KX301094, KX301175, KX301137, KX301252, **MF614900; *P. tuberculatus*:** Lavor P. 47/UFRN18650/Pernambuco (Brazil) – **KX387790, KX387696, KX387762, KX387730, MF614849, MF614876; *P. ulei*:** Franco FF. S79/SORO4557/Rio de Janeiro (Brazil) – KX301221, KX301102, KX301183, KX301145, KX301260, **MF614906; *P. vilaboensis* (1):** Moraes EM. S20/HUFS 641/Goiás (Brazil) – **KX387800, KX387711, KX387773, KX387745, MF962440, MF962457; *P. vilaboensis* (2):** Moraes EM. S19/CCTS3001/Goiás (Brazil) – KC779340.1, KX301079, KC621157.1, KX301122, KC779305.1, **MF614889; *Rhipsalis baccifera*:** Rodriguez A. 5318/MA733603/Limón (Costa Rica) – **KX387797, KX387704, KX387769, KX387738, MF614856, MF614883; *Stephanocereus leucostele*:** Calvente A. 413/UFRN 13195/Bahia (Brazil) – KX301199, KX301075, KX301161, KX301118, KX301237, **MF614873.**

APPENDIX 2. Primers of the different genomic regions used in this study.

Region name	Primers	Source
<i>petL-psbE</i>	petL: AGTAGAAAACCGAAATAACTAGTT A psbE: TATCGAATACTGGTAATAATATCAGC	Shaw et al. 2007
<i>psbD-trnT^{GGU}</i>	psbD: CTCCGTARCCAGTCATCCATA trnT(GGU)-R: CCCTTTTAACTCAGTGGTAG	Shaw et al. 2007
<i>trnL-trnT</i>	5'trnLUAAR(TabB): TCTACCGATTTTCGCCATATC trnT ^{UGU} F (TabA): CATTACAAATGCGATGCTCT	Taberlet et al. 1991
<i>trnS-trnG</i>	5'trnG2S: TTTTACCACTAAACTATACCCGC SGFwd2: CACCCATGGTTCCTTAGA trnS ^{GCU} : AGATAGGGATTTCGAACCCTCGGT SGRev2: TCCGCTCATTAGCTCTCCTC	Shaw et al. 2005 Bonatelli et al. 2013
<i>PhyC</i>	PhyF: AGCTGGGGCTTTCAAATCTT PhyR: TCCTCCACTTGACCACCTCT	Helsen et al. 2009
<i>ycf1</i>	ycf1-4182F*: AAATAYRRATAGAAAATATTTKGATT ycf1-5248R*: GAATTCTYAATTCTCTACGACG	Franck et al. 2012

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