CLADISTIC ANALYSIS OF *TRICHOCEREUS* (CACTACEAE: CACTOIDEAE: TRICHOCEREEAE) BASED ON MORPHOLOGICAL DATA AND CHLOROPLAST DNA SEQUENCES

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Dedicated to Omar Emilio Ferrari (1936-2010)

Abstract: *Trichocereus* (Cactaceae: Cactoideae: Trichocereeae) is a South American genus primarily inhabiting arid and semiarid areas in the Andean region of Ecuador, Peru, Bolivia, Chile and Argentina. The phylogenetic relationships of *Trichocereus* were examined on the basis of 39 exomorphological characters and chloroplast DNA sequences *trnL-F* and *rpl16* for 17 species of *Trichocereus*, including three other genera of the tribe Trichocereeae (*Echinopsis*, *Lobivia*, *Setiechinopsis*), two of Notocacteae (*Eulychnia*, *Wigginsia*) and one of the Hylocereeae (*Harrisia*). The simultaneous phylogenetic analysis of both morphological data and noncoding DNA sequence data recovered *Trichocereus* as monophyletic if two species of *Harrisia* are part of it. Moreover, the *Trichocereus* clade is supported by three synapomorphies: basitonic growth with prostrate branches, imbricate scales along the floral tube, and subglobose fruits.

Resumen: *Trichocereus* (Cactaceae: Cactoideae: Trichocereeae) es un género sudamericano que habita principalmente las zonas áridas y semiáridas de la región andina de Ecuador, Perú, Bolivia, Chile y la Argentina. Las relaciones filogenéticas de *Trichocereus* fueron examinadas sobre la base de 39 caracteres exomorfológicos y secuencias de ADN del cloroplasto, *trnL-F* y *rpl16* de 17 especies de *Trichocereus*, incluyendo otros tres géneros de la tribu Trichocereeae (*Echinopsis*, *Lobivia*, *Setiechinopsis*), dos de Notocacteae (*Eulychnia*, *Wigginsia*) y uno de Hylocereeae (*Harrisia*). El análisis filogenético combinado con base en datos morfológicos y datos de sequencias de ADN, recuperó a *Trichocereus* como monofilético si las dos especies de *Harrisia* son por parte de él. Además, el clado *Trichocereus* fue apoyado por tres sinapomorfías: crecimiento basítono con ramas postradas, escamas imbricadas a lo largo del tubo floral y frutos subglobosos.

Keywords: chloroplast DNA, *Echinopsis*, character evolution, phylogeny, *Lobivia*, morphology, South America, *Trichocereus.*

INTRODUCTION

The genus *Trichocereus* (Cactaceae: Subfamily Cactoideae: Tribe Trichocereeae: Subtribe Trichocereinae) comprises around 45 species from the Andes of Ecuador, Peru, Bolivia, Chile and Argentina, reaching in this last country the extra-Andean provinces of Cordoba, La Pampa and Buenos Aires (Kiesling 1978, Brako and Zarucchi 1993, Navarro 1996, Kiesling 1999a, Navarro and Maldonado 2002, Hoffmann and Walter 2004, Anderson 2005, Hunt et al. 2006). The genus name *Trichocereus* derives from the fact that these plants are columnar cacti with pilosity at the flower areoles (Berger 1905). *Trichocereus* has been defined morphologically by cylindrical stems with shallow ribs, large flowers (ca. 13–30 cm) with a wide ovary (2–3 cm) and a dense covering of hairs (Kiesling 1978, Kiesling and Ferrari 2005).

The taxonomy and systematics of *Trichocereus* have been problematic, and acceptance of the genus has been questioned, as well as the number of its related species and groups. *Trichocereus* was proposed by Berger (1905) as a subgenus of *Cereus* (for 14 species), a genus which formerly grouped all columnar cacti. In 1909, Riccobono transfered it to the genus level, but with only two species (*T. macrogonus* and *T. spachianus*). Other species continued to be within

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Author	Taxonomic Categories	Species
Britton & Rose (1920)		
	Tribe: Cereeae	
	Subtribe: Cereanae	
	Genus: *Harrisia*	
	Genus: *Trichocereus*	Species: *T. bridgesii*, *T. candicans*, *T. chiloensis*, *T. coquimbanus*, *T. cuzcoensis*,*T. fascicularis*, *T. huascha*, *T. lamprochlorus*, *T. macrogonus*, *T. pachanoi*, *T. pasacana*, *T. peruvianus*, *T. schickendantzii*, *T. shaferi*, *T. spachianus*, *T. strigosus*, *T. terscheckii*, *T. thelegonoides*, *T. thelegonus*
	Subtribe: Echinocereanae	
	Genus: *Echinopsis* (*E. mirabilis*)	
	Genus: *Lobivia*	
Berger (1929)	Subfamily: Cereoideae	
	Tribe: Cereeae	
	Subtribe: Cereinae	
	Genus: *Cereus*	
	Section: Eucerei	
	Subsection: Nyctocerei	
	Subgenus: *Harrisia*	
	Subsection: Trichocerei	
	Subgenus: *Trichocereus*	Species: *C. bridgesii*, *C. candicans*, *C. coquimbanus*, *C. chiloensis*, *C. fascicularis*, *C. huascha*, *C. lamprochlorus*, *C. macrogonus*, *C. pasacana*, *C. schickendantzii*, *C. spachianus*, *C. strigosus*, *C. thelegonus*
Backeberg (1958)		
	Subfamily: Cereoideae	
	Tribe: Cereeae	
	Semitribe: Austrocereeae	
	Subtribe: Austrocereinae	
	Clan: Trichocerei	
	Subclan: Nyctotrichocerei	
	Genus: *Setiechinopsis*	
	Genus: *Trichocereus*	
	Subgenus: *Trichocereus* (*T. bridgesii*, *T. candicans, T. pachanoi*, *T. peruvianus*, *T. schickendantzii*, *T. terscheckii*, etc)	
	Subgenus: *Medioeulychnia* (*T. chiloensis*, *T. coquimbanus*, *T. deserticola*, *T. skottsbergii*, etc)	
	Genus: *Echinopsis*	
	Subclan: Heliotrichocerei	
	Genus: *Helianthocereus*	
	Subgenus: *Helianthocereus*: *H. pasacana* (*T. atacamensis*), *H. tarijensis*, etc.	
	Subgenus: *Neohelianthocereus*	
	Subtribe: Austrocactinae	
	Clan: Lobiviae	

Table 1: Classification of *Trichocereus* and related genera according to ten authors, indicating the species of *Trichocereus*.

Author	Taxonomic Categories	Species
Backeberg (*cont.*)	Subclan: Eriolobiviae Genus: *Lobivia* Subgenera: *Lobivia*, *Neolobivia* Semitribe: Boreocereeae Subtribe: Boreocereinae Clan: Nyctocerei Genus: *Harrisia*	
Buxbaum (1958)	Subfamily: Cereoideae Tribe: Hylocereae Subtribe: Nyctocereinae Linea: Harrisiae Genus: *Harrisia* Tribe: Trichocereeae Subtribe: Trichocereinae Genera: *Acanthocalycium*, *Arthrocereus* (incl. *Setiechinopsis*), *Echinopsis* (*Pseudolobivia*), *Espostoa* (*Pseudoespostoa*), *Haageocereus* (*Neobinghamia*, *Peruvocereus*), *Soehrensia* and *Trichocereus* (*Helianthocereus*, *Leucostele*, *Roseocereus* and *Weberbauerocereus*)	
Friedrich (1974), Rowley (1974)	Tribe Echinopsideae (new name given to replace Trichocereeae) Subtribe Echinopsidinae Genus: *Echinopsis* Subgenus: *Acanthocalycium* Subgenus: *Echinopsis* Section: *Echinopsis* Section: *Hymenorebutia* Section: *Pseudoechinopsis* Subgenus: *Trichocereus* Section: *Soehrensia* Section: *Trichocereus* (incl. *Helianthocereus*)	Species: *E. antezanae*, *E. atacamensis*, *E. bertramiana*, *E. camarguensis*, *E. candicans*, *E. cephalomacrostibas*, *E. chalaensis*, *E. chiloensis*, *E. conaconensis*, *E. coquimbana*, *E. courantii*, *E. cuzcoensis*, *E. deserticola*, *E. escayachensis*, *E. friedrichii*, *E. fulvilana*, *E. glauca*, *E. herzogiana*, *E. huascha*, *E. knuthiana*, *E. lagenaeformis*, *E. litoralis*, *E. macrogona*, *E. manguinii*, *E. narvaecensis*, *E. nigripilis*, *E. orurensis*, *E. pachanoi*, *E. pasacana*, *E. peruviana*, *E. poco*, *E. puquiensis*, *E. purpureopilosus*, *E. randallii*, *E. rivierei*, *E. rubinghiana*, *E. santaensis*, *E. santiaguensis*, *E. schoenii*, *E. skottsbergii*, *E. strigosa*, *E. tacaquirensis*, *E. taquimbalensis*, *E. toratensis*, *E. tarijensis*, *E. tarmaensis*, *E. terscheckii*, *E. thelegona*, *E. thelegonoides*, *E. trichosus*, *E. tulhuayacensis*, *E. tunariensis*, *E. uyupampensis*, *E. vollianus*, *E. werdermannianus*.

Table 1 (cont.) : Classification of *Trichocereus* and related genera according to ten authors, indicating the species of *Trichocereus*.

Author	Taxonomic Categories	Species
Kiesling (1978)	Subfamily: Cereoideae	
	Genus: *Echinopsis*	
	Genus: *Lobivia*	
	Genus: *Trichocereus*	
		Species: *T. andalgalensis*, *T. angelesii*, *T. candicans*, *T. cabrerae*, *T. fabrisii*, *T. huascha*, *T. lamprochlorus*, *T. pasacana*, *T. pseudocandicans*, *T. rowleyi*, *T. schickendantzii*, *T. smirzianus*, *T. spachianus*, *T. strigosus*, *T. tarijensis*, *T. terscheckii*, *T. thelegonoides*, *T. thelegonus*, *T. vatteri*.
Ritter (1980a, 1980b, 1981)		
	Genus: *Echinopsis* (incl. *Setiechinopsis*)	
	Genus: *Eriocereus* (incl. *Harrisia*)	
	Genus: *Trichocereus*	
		Species: *T. callianthus*, *T. caulescens*, *T. chuquisacanus*, *T. eremophilus*, *T. fulvilanus*, *T. glaucus*, *T. quadratiumbonatus*, *T. riomizquensis*, *T. scopulicola*, *T. serenanus*, *T. tacnaensis*, *T. terscheckioides*, *T. tenuispinus*, *T. torataensis*
		Varieties*:* *T. chiloensis* var. *australis*, *T. chiloensis* var. *borealis*, *T. chiloensis* var. *conjungens*, etc.
Gibson & Nobel (1986)	Subfamiliy: Cactoideae	
	Tribe: Hylocereeae	
	Genus: *Harrisia*	
	Tribe: Trichocereeae	
	Genus: *Echinopsis* (incl. *Setiechinopsis*)	
	Genus: *Lobivia*	
	Genus: *Trichocereus*	
Anderson (2005)	Subfamily: Cactoideae	
	Tribe: Trichocereeae	
	Genus: *Echinopsis* (incl. *Acantholobivia*, *Cinnabarinea*, *Chamaecereus*, *Cylindrolobivia*, *Furiolobivia*, *Helianthocereus*, *Hymenorebutia*, *Leucostele*, *Lobivia*, *Mesechinopsis*, *Neolobivia*, *Reicheocactus*, *Salpingolobivia*, *Setiechinopsis*, *Soehrensia*, *Trichocereus*)	
	Genus: *Harrisia*	
Hunt et al (2006)	Subfamiliy: Cactoideae	
	Genus: *Echinopsis* (incl. *Acanthocalycium*, *Acantholobivia*, *Chamaecereus*, *Helianthocereus*, *Lobivia*, *Pseudolobivia*, *Setiechinopsis*, *Soehrensia*, *Trichocereus*)	
	Genus: *Harrisia*	

Table 1 (cont.) : Classification of *Trichocereus* and related genera according to ten authors, indicating the species of *Trichocereus*.

other genera such as *Cereus* (*C. atacamensis, C. eriocarpus*), *Echinopsis* (*E. candicans, E. lamprochlorus*), and *Echinocereus* (*E. spinibarbis*). Later on, Britton and Rose (1920) proposed for the first time a key to distinguish the 19 species they recognized (Table 1). The Britton and Rose delineation of the genus underwent no modifications, except for some new species being added to it, until Backeberg (1949) proposed the genus *Helianthocereus* for species of *Trichocereus* with yellow, orange or red day-opening flowers. According to Backeberg, the species with white, night-opening flowers remained within *Trichocereus*.

Buxbaum (1958) gave a classification for the subfamily Cereoideae, proposing the tribe Trichocereeae, denoting *Trichocereus* as type genus of the tribe. In addition, within the Trichocereeae, the subtribe Trichocereinae grouped the following genera (synonyms in parentheses): *Acanthocalycium*, *Arthrocereus* (*Setiechinopsis*), *Echinopsis* (*Pseudolobivia*), *Espostoa* (*Pseudoespostoa*), *Haageocereus* (*Neobinghamia*, *Peruvocereus*), *Soehrensia* and *Trichocereus* (*Helianthocereus*, *Leucostele*, *Roseocereus* and *Weberbauerocereus*) (Table 1). According to Buxbaum, the subtribe is characterized by "large and columnar stems and rarely globular; flowers radiate, campanulate to funnelform; perianth large, mostly white or whitish, sometimes brightly colored; nectar chamber lacking or present; stamen insertion beginning at base of the receptacle or above a nectar chamber".

Friedrich (1974) defined and merged several genera, including *Lobivia* and *Trichocereus,* with *Echinopsis* (Table 1), based only on two floral characters, hairs in the axils of the scales and disposition of the stamens in two groups—even though these characters were present in other genera of the tribe Trichocereeae (Buxbaum 1958) or were polymorphic in a few species, as in the case of *Lobivia grandiflora.* According to Friedrich (op. cit.), *Echinopsis* encompasses three subgenera: *Acanthocalycium*; *Echinopsis* with three sections – *Echinopsis*, *Hymenorebutia* and *Pseudoechinopsis*; and *Trichocereus* with sections *Trichocereus* and *Soehrensia*. Moreover the subtribe Echinopsidinae (new name given by Friedrich to replace Trichocereinae) is defined only by the nectar chamber without giving details of its characteristics. Rowley (1974) published the combinations and new names proposed by Friedrich and Rowley, without verifying synonymy. Kiesling (1978) improved the state of knowledge of the *Trichocereus* species from Argentina, by illustrating, describing, and providing a key to distinguish them. Moreover, he concluded that the genus proposed by Backeberg—*Helianthocereus*—is a synonym which should be assigned at most to the category of subgenus.

Ritter (1980a, 1980b, 1981) provided several treatments for the family Cactaceae in Argentina, Bolivia, Brazil, Chile, Paraguay, Peru and Uruguay, but without keys for the identification of genera and species, and with some taxonomic problems, e.g., the absence of some type specimens in the mentioned herbarium (U) or incomplete citation of the basionyms. In addition, Ritter proposed new species and combinations of *Trichocereus* (*T. eremophilus, T. glaucus, T. serenanus, T. atacamensis* var. *pasacana*, *T. tarijensis* var. *poco*, among others, Table 1).

Friedrich and Glaetzle (1983) studied the ultrastructure of the seed testa of *Echinopsis* sensu lato, using scanning electron microscopy (SEM), and proposed nine groups based on those data, where the first four (Ia, Ib, IIa, and IIb) correspond to species of the subgenus *Trichocereus,* and in group IIa they also included species of *Soehrensia*. Most recent proposals of classification (Hunt and Taylor 1986, Hunt 1999, Anderson 2005, Hunt et al. 2006) accept Friedrich's hypothesis (1974), where the genus *Echinopsis* (s.l.) includes *Acanthocalycium*, *Chamaecereus*, *Echinopsis* sensu stricto, *Helianthocereus*, *Hymenorebutia*, *Pseudolobivia*, *Soehrensia* and *Trichocereus,* as well as *Lobivia.* However, Kiesling (1978), Ritter (1980a, 1980b, 1981) and Gibson and Nobel (1986) consider that *Trichocereus* and *Echinopsis* should remain separate, because the flowers of the former are wide (floral tube and ovary wider than 2 cm in diameter) and with abundant pilosity.

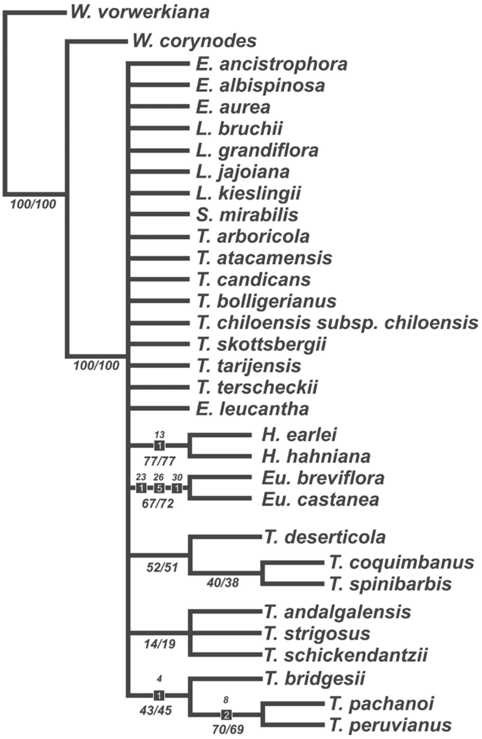


Figure 1. Strict consensus of 684 most parsimonious trees (L = 506, CI = 0.18, RI = 0.27), using morphological data set. Numbers below branches indicate bootstrap support values/ jackknife percentages. Numbers above branches are characters (Appendix 2), and character states appear within the square. Black squares are synapomorphies.

Several molecular phylogenetic studies of cacti have included members of the tribe Trichocereeae or the genus *Echinopsis* (s.l.). For example, Nyffeler (2002) in his study of the Cactaceae sequenced the chloroplast genes *trnK/matK* and *trnL-trnF* of 70 species belonging to 48 genera. His results showed that the tribe Trichocereeae was paraphyletic in nine species sampled, and its sister relationship with the tribes Cereeae, Browningieae and Notocacteae is not fully resolved. In addition, *Echinopsis* (s.l.) represented by three species did not form a monophyletic clade; *Harrisia pomanensis* was sister to *Echinopsis* (*Trichocereus*) *chiloensis* (Nyffeler 2002, his Fig. 2), and the *H. pomanensis-E.* (*T.*) *chiloensis* clade was sister to *Echinopsis pentlandii,* while *Echinopsis glaucina* remained unresolved. Ritz et al. (2007) studied the phylogeny of *Rebutia* and closely related genera of the tribe Trichocereeae, using three noncoding chloroplast regions (intergenic spacers *atpB-rbcL*, *trnK-rps16* and *trnL-trnF*). Their results (Ritz et al. 2007, Fig. 1) showed that the tribe Trichocereeae (represented by 20 genera of the 27 sensu Anderson's classification 2005) was paraphyletic, and that the genus *Echinopsis* (s.l.) represented by nine species sensu Barthlott and Hunt 1993, including *Echinopsis* (s.s.), *Lobivia*, *Setiechinopsis* and *Trichocereus,* was not monophyletic. Their results also confirm the close relationship between *Echinopsis* (s.l.), *Espostoa* and *Haageocereus,* as suggested by Anderson (2005). Korotkova et al. (2010) studied the relationships of *Pfeiffera* using sequence data of more than ten different genes. In their tree of Cactoideae inferred from Bayesian analysis, the tribe Trichocereeae (represented by six genera and seven species) was recovered as

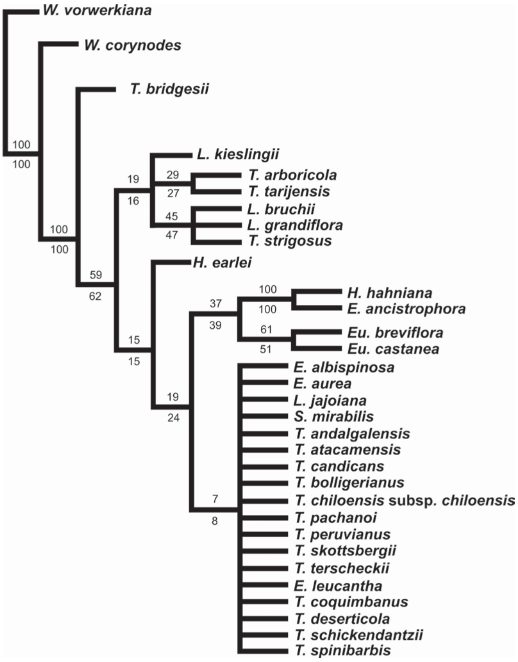


Figure 2. Strict consensus of 31371 most parsimonious trees (L = 313, CI = 0.47, RI = 0.58), using trnL-F and rpl16 combined data set. Numbers above and below branches indicate bootstrap support values and jackknife percentages, respectively

monophyletic (Korotkova et al. 2010, Fig. 1), but not *Echinopsis* (s.l.). Notably *Harrisia pomanensis* was sister to *Echinopsis pentlandii*.

In two recent molecular phylogenies for the Cactaceae contrasting results were recovered for the Trichocereeae and *Echinopsis* (s.l.). Hernández et al. (2011) analyzed 224 species of 108 genera by sequencing nuclear (*ppc*) and chloroplast markers (*matK*, the intron *rpl16*, and the two intergenic spacers *trnL-trnF* and *trnK/matK*), and found that both the Trichocereeae (represented by 18 genera and 33 species) and *Echinopsis* s.l. (seven species) were paraphyletic (Hernández et al. 2011, their Fig. 4). However, they recovered the three species studied of the subgenus *Trichocereus* (*E. chiloensis*, *E. pasacana* and *E. pachanoi*) as monophyletic, and *Harrisia* related to them as suggested by Nyffeler (2002, see above). Bárcenas et al. (2011), using nucleotide sequences from the plastid gene region *trnK-matK* of 532 species, found that the tribe Trichocereeae was monophyletic, but not the genus *Echinopsis* (s.l.); however, *Echinopsis chiloensis* was sister to *E. pentlandii,* and the *E. chiloensis-E. pentlandii* clade was sister to *H. pomanensis*.

Probably, the delimitation of this complex of genera of the Trichocereeae [*Echinopsis* (s.l.): *Acanthocalycium*, *Chamaecereus*, *Echinopsis* (s.s.), *Helianthocereus*, *Hymenorebutia*, *Lobivia*, *Pseudolobivia*, *Soehrensia*, and *Trichocereus*] and the assignment of each species to the corresponding genus is the greatest current challenge to the study of South American Cactaceae. No total-evidence phylogenetic analyses thus far have been conducted for most genera in this tribe. Therefore, the interest of this work lies in testing for the monophyly of *Trichocereus* based on morphological and molecular characters, proposing a phylogenetic hypothesis of its relationships with other close genera, suggested by Buxbaum (1958), and studying the evolution of some morphological characters.

MATERIALS AND METHODS

Ingroup and Outgroup Sampling: Morphological and molecular analyses included as the ingroup 17 of the approximately 45 species of *Trichocereus* (the total number of species corresponds to the taxonomic treatment of the genus, which is being revised—Albesiano, in preparation), four species of *Echinopsis* (s.s.), and four of *Lobivia*. In the case of *Trichocereus* we included representatives of the subgenera *Trichocereus* and *Medioeulychnia,* as well as the genus *Helianthocereus* (Table 1) proposed by Backeberg (1959). The 25 species selected for this study represent the generic morphological diversity in stem, flowers, fruit, and seed. To test the monophyly of *Trichocereus*, six species of related genera (*Eulychnia*, *Harrisia* and *Wigginsia*) were selected based on the Endler and Buxbaum (1958) classification and the topology of Nyffeler (2002). Also the monotypic genus *Setiechinopsis* (*S. mirabilis*) was included to determine whether it is part of *Echinopsis* (s.s.) or the sister genus to it. *Wigginsia vorwerkiana* and *W. corynodes* were used to root the tree. A total of 32 taxa were included in both morphological and molecular matrices. We are sure of species determinations and the type species of each genus was included except for *Echinopsis*. The species list, with information on the country, collector's name, specimen number and herbarium where specimens were housed, is given in Appendix 1.

Morphological Data: Several botanical explorations were conducted in arid and semiarid areas of Argentina, Bolivia, Chile, Colombia, and Uruguay, with the aim to collect and record information about *Echinopsis*, *Eulychnia*, *Lobivia*, *Setiechinopsis*, *Trichocereus,* and *Wigginsia*. Most measurements were made in the field and supplemented with material studied in the following herbaria: BAB, CTES, LIL, LP, LPB, MERL, MEXU, NY, SGO, SI, and U (Holmgren et al. 1990). For two species morphological information was taken from the original descriptions, because we were unable to collect material of *Harrisia earlei,* and most reproductive features of *H. hahniana* were corroborated with those observed in the herbarium material.

Seeds were observed under a scanning electron microscope (JSM-6610LV). Seeds were cleaned with acetone using ultrasound equipment for one or two minutes, then the air-dried seeds were fixed to aluminum specimen holders with double-sided tape and coated with metal using a Denton Vacuum Desk IV. Magnifications of 80x were used. The terminol-

Primer name	Sequences (5´ to 3´)	Reference	Used for
	trnL-F		
C Forward	CGAAATCGGTAGACGCTACG	Taberlet et al. 1991	Ampl.
F Reverse	ATTTGAACTGGTGACACGAG	Taberlet et al. 1991	Ampl.
E Forward	GGTTCAAGTCCCTCTATCCC	Taberlet et al. 1991	Seq.
D Reverse	GGGGATAGAGGGACTTGAAC	Taberlet et al. 1991	Seq.
Cii Forward	TAGACGCTACGGACTTGATTG	Cialdella et al. 2007	Seq.
Fdw Reverse	CAGTCCTCTGCTCTACCAGC	Cialdella et al. 2007	Seq.
	rpl16		
71 Forward	GCTATGCTTAGTGTGTGTCTC	Jordan et al. 1996	Ampl.
1661 Reverse	CGTACCCATATTTTTCCACCACGAC	Jordan et al. 1996, Applequist & Wallace 2000	Ampl.-Seq.
584 Reverse	TTCCGCCATCCCACCCAATGAA	Applequist & Wallace 2000, Cialdella et al. 2007	Ampl.-Seq.
584 Forward	TTCATTGGGTGGGATGGCGGAA	Cialdella et al. 2007	Seq.

Table 2: Primers used for PCR amplification and sequencing of the *trnL-F* and *rpl16* regions.

ogy used follows Friedrich and Glaetzle (1983) and Barthlott and Hunt (2000).

Selection and study of characters: Thirty-nine morphological characters were recognized (Appendix 2), which are proposed as primary homology hypotheses, following conjunction and similarity criteria (position, shape and function, per De Pinna 1991). Of those 39 characters, 17 are related to vegetative structures, 13 to floral structures, 4 to fruits, and 5 to seeds (numbering of characters starts at zero). The matrix was created using Winclada (Nixon 1999), including only informative characters (autapomorphies were excluded). Unknown character states were coded as (-) and polymorphic characters as (*; $). The matrix contains 13 binary characters and 26 multistate characters (3–6 states); the latter were coded as nonadditive (because no prior information was available about transition between states) and equally weighted. Each character represents a homology hypothesis, where the state present in two or more taxa is interpreted as similarity due to a common ancestry (Nixon and Ochoterena 2000). The characters of spines and areoles (color, difference between radial and central spines, position, number, and shape) were excluded from the cladistic analysis for not being variable or for presenting continuous variation, and for failing to meet similarity and conjunction criteria (De Pinna, 1991).

DNA extraction, amplification and sequencing: Chloroplast DNA was extracted using the CTAB method (Doyle and Doyle 1987), following the modifications described by Hartmann et al. (2001) to prevent problems of mucilage or other polysaccharides present in cactus tissues. A 1 cm x 1 cm cut was made on the surface of the stem of each individual, and we kept as the tissue sample only the outer layers from epidermis to chlorenchyma. Each tissue sample was placed in a mortar, and liquid nitrogen was added to facilitate its crushing with a pestle. Crushed tissue samples were placed inside their respective 1.5 ml Eppendorf tubes, which contained 500 µl of CTAB buffer (2.5 % CTAB, 1.5 M NaCl, 50 mM ETDA, 250 mM Tris HCl pH 8). After incubation at 50° C for 60 min, 500 µl of phenol:chloroform:isoamyl alcohol (25:24:1) were added and gently mixed. The mixture was centrifuged at 14000 rpm for 5 min. The aqueous supernatant phase was transferred to a new Eppendorf tube, and the old tubes (with pellet) were discarded. Then 250 µl of chloroform:isoamyl alcohol (24:1) were added. The tubes were again centrifuged for 5 min at 14000 rpm. The aqueous phase was transferred to new Eppendorf tubes, and DNA was precipitated by adding 900 µl of 100% ethanol. The tubes were centrifuged for 10 min at 13000 rpm, then the ethanol was discarded. The tubes were left to dry upside down for 20 min. For resuspension, 100 µl of TE buffer were added, and the tubes were gently shaken and left in a rack for 3 h. In order to aid resuspension, the tubes were heated in a double boiler for 30 min at 35–40° C. A 1:10 dilution was then made to measure DNA concentration and purity on a Biophotometer. Absorbance of each taxon fell within the range from 1.6 to 2.2 (at 260 and 200 nm), indicating that the purity of the extracted DNA was suitable for continuing the process. DNA from two cpDNA regions was amplified using the polymerase chain reaction (PCR); the first region (*trnL-F*) consisted of the *trnL* intron and the *trnL-trnF* intergenic spacer, and the second region (*rpl16*) consisted of the *rpl16* intron. The primers used for each of these two sequenced regions of cpDNA are described in Table 2. The *trnL-F* region was amplified by using primers

Data	No. of taxa	No. of characters + gaps coded (total)	No. of informative characters	No. of mpt in 60 repetitions	No. of mpt (optimal)						
						mpt			ct		
						l	ci	ri	l	ci	ri
morphological	32	-	39	684	19	272	0.34	0.68	506	0.18	0.27
trnL-F	32	1022 + 23 (1045)	39	958	71	61	0.67	0.87	67	0.61	0.83
rpl16	27	1122 + 27 (1149)	94	300	25	145	0.74	0.84	152	0.71	0.81
trnL-F and rpl16	32	2144 + 50 (2194)	133	31371	1497	313	0.47	0.58	247	0.60	0.58
morphological, trnL-F and rpl16	32	172	172	1166	1	603	0.40	0.62	-	-	-

Table 3: Statistical results of parsimony analysis of the individual and combined matrices. Mpt (Maximum parsimony trees), ct (Consensus tree), l (length=steps), ci (consistency index), ri (retention index).

C and F, and Cii and Fdw were used for sequencing; in those taxa where these primers failed, primers D and E were used, following Taberlet et al. (1991) and Cialdella et al. (2007). Amplification of the *rpl16* intron was accompanied by use of primers F71 and R1661, whereas primers R584 and F584 were used for sequencing; in those taxa where these primers failed, primers R1661 and R584 were used, as described by Applequist and Wallace (2000).

For PCR, the reaction volume of 25 µl contained the following: 3 µl of diluted (1:10) DNA, 0.25 µl Taq DNA, 0.25 µl dNTPs (0.025 mM of each), 2.5 µl Buffer minus Mg (1X), 2 µl $MgCl_2$ (5 mM), 1 µl of each primer (5µM) and 15 µl of ultrapure water. This reaction mixture was prepared for each taxon. PCR was performed in an Eppendorf Mastercycler gradient thermal cycler, using the following protocols: For *trnL-F:* 1 cycle of 5 min at 94° C, 34 cycles of 30 sec at 94° C, 1 min at 48° C, and 1 min 30 sec at 72° C, and a final extension cycle of 7 min at 72° C. For *rpl16*: 1 cycle of 4 min at 94° C, 34 cycles of 1 min at 94° C, 1 min at 55° C, and 2 min 30 sec at 72° C, and a final extension cycle of 7 min at 72° C. PCR product was obtained at a concentration of 30–60 ng/µl from each taxon, and was purified with a Promega Wizard kit, following the manufacturer's instructions. Sequencing reactions were prepared at the Genomic Unit of INTA Castelar (Buenos Aires, Argentina), using Sanger's technique and electrophoresis in an automatic capillary sequencer (Applied Biosystems 3500xL Genetic Analyzer).

Molecular Data Analysis: Sequence editing and assembly were performed using the BioEdit program (version 7.0.5.1, Hall 1999), using as standards nine sequences obtained from GenBank (Appendix 1). Automatic alignment was carried out with the Muscle program (Edgar 2004), and manually with Mesquite (version 2.74, Maddison and Maddison 2010). Insertions and deletions were coded following the simple coding method (Simmons and Ochoterena 2000). The matrix with sequences was joined to the two gap matrices (corresponding to *trnL-F* and *rpl16*), which yielded 133 informative characters out of a total of 2194 characters (Table 3).

Phylogenetic Analysis: The parsimony analysis was carried out for three data sets: (a) morphological, (b) molecular (*trnL-F*+*rpl16*+gaps), and (c) combining morphological with molecular information (32 taxa and 172 characters). Data matrices were edited using Winclada, eliminating autapomorphies and constant (non-informative) data. All characters used were equally weighted and non-additive.

The parsimony analysis was conducted with TNT version 1.1 (Goloboff et al. 2008). Searches were made under the following parameters: max. trees: 10000; random seed: 0; and the following strategies: sectorial searches, ratchet, tree drifting and tree fusing, with 1000 random additional sequences, making 12 searches. All searches were replicated five times (total 60), in order to verify that taxa with missing data did not affect the length of cladograms. Subsequently, the trees obtained from all searches in TNT were transferred to Winclada, eliminating suboptimal trees (of equal length but less parsimonious, in comparison to optimal trees). The strict consensus tree was estimated using the option "Consensus (strict)", and saving it as a metafile. The Acctran character optimization criterion was selected, and tree length together with consistency and retention indices were calculated with Winclada. Bootstrap values in data matrices calculated in TNT were used as branch support measures, with the following options: max. trees = 10000; random seed = 0; number of replicas = 1000; search trees with ratchet, tree drifting and tree fusing. The same procedure was used for jackknife values. All trees were edited in Corel Draw X3.

The use of molecular data has reframed the taxonomic congruence problem, i.e., the degree of correspondence between different classifications or group-

Figure 3 (opposite): The most parsimonious tree (L=603, CI=0.40, RI=0.62), based on total evidence, morphological and molecular (trnL-F and rpl16). The solid squares represent synapomorphies, and the white squares, homoplasies, that are present unambiguously. The numbers on the squares correspond to the morphological characters, and the numbers inside the squares indicate the character states. Numbers in italics separated by a virgule are bootstrap support values / jackknife percentages.


W. vorwerkiana
W. corynodes
100/100
99/99
Eu. breviflora
Eu. castanea
64/73
S. mirabilis
L. jajoiana
E. aurea
E. albispinosa
E. leucantha
E. ancistrophora
33/40
L. kieslingii
L. bruchii
L. grandiflora
T. strigosus
T. andalgalensis
T. schickendantzii
T. arboricola
23/36
H. earlei
H. hahniana
T. tarijensis
T. macrogonus
61/63
T. pachanoi
T. peruvianus
T. chiloensis subsp. chiloensis
T. bolligerianus
T. skottsbergii
T. terscheckii
T. atacamensis
T. candicans
37/33
T. deserticola
15/11
T. coquimbanus
T. spinibarbis
Echinopsis
Lobivia
Trichocereus

ings, produced by different data sets (Lanteri et al. 2005). Various measures of congruence have been proposed, one of which is the incongruence length difference (ILD) (Mickevich and Farris 1981) as we calculated in this study. The ILD for the data set is: ILD= $L_{(morphological)(trnL\text{-}F)(rpl16)}$ - ($L_{(morphological)}$ + $L_{(trnL\text{-}F)(rpl16)}$) / $L_{(morphological)(trnL\text{-}F)(rpl16)}$, where $L_{(morphological)(trnL\text{-}F)(rpl16)}$ is the length of the shortest tree from the combined data set, $L_{(morphological)}$ is the length of the shortest tree from the morphological data set, and $L_{(trnL\text{-}F)(rpl16)}$ is the length of the shortest tree of the molecular data set.

RESULTS: The number of informative characters and statistics for separate and combined analyses are given in Table 3. Neither the parsimony analysis with only morphological characters nor the parsimony analysis with only molecular characters recovered the tribe Trichocereeae or the genus *Trichocereus* as monophyletic (Figs. 1, 2).

Morphology: The strict consensus of 684 most parsimonious trees (L = 506; CI = 0.18; RI = 0.27), revealed a large polytomy (Fig. 1). Within this large polytomy five clades with low support (< 80%) were recovered and supported by few synapomorphies. For example, *T. pachanoi* and *T. peruvianus* are sister taxa sharing the blue-green stems, and the *T. pachanoi-T. peruvianus* clade is sister to *T. bridgesii*, supported by the occurrence of an interareolar furrow. *Harrisia earlei* and *H. hahniana* are sisters supported by the alternate or diagonal areole position. *Eulychnia breviflora* and *E. castanea* are sisters supported by the presence of hairs in the axils of the floral tube scales, receptacle diameter 4–5 cm at the ovary level, and semidry fruits. *T. deserticola*, *T. coquimbanus* and *T. spinibarbis* are closely related with low bootstrap and jackknife support values, while *T. andalgalensis*, *T. strigosus* and *T. schickendantzii* form a polytomy in a weakly supported clade (Fig. 1).

Molecular: The molecular strict consensus of 31371 most parsimonious trees (L = 313; CI = 0.47; RI = 0.58; Fig. 2) shows that *T. bridgesii* is the first branching species. Among the clades the one with the highest number of species is unresolved. The sister relationship of *H. hahniana* and *E. ancistrophora* has strong support values (100/100). The sister relationship of *Eulychnia breviflora* and *E. castanea*—found also in the morphological tree—is confirmed even though it has low support values of 61/51. At the molecular level, *T. strigosus* relates to *L. bruchii* and *L. grandiflora*, equally, and *T. arboricola* forms a clade with *T. tarijensis* as sister.

Combined Molecular and Morphological Data: The combined parsimony analysis of morphological and molecular data yielded a single 603-step tree with CI = 0.40 and RI = 0.62 (Fig. 3). The ILD results indicated that the morphological and molecular partitions were incongruent (ILD = 0.67). However, when the morphological and molecular data are combined, the most parsimonious tree is more resolved. The members of the tribe Trichocereeae are recovered by four synapomorphies (slightly protruding tuberculate ribs, total length of flower 4 to 12 times the diameter of ovary, receptacle scales triangular-ovate, and 20 to 40 hairs on receptacle areoles), and by four homoplasies (small flowers 6 to 9 cm long, ovary and throat diameters almost the same, seeds ovate, and seeds medium).

Based on the species samples (Appendix 1) *Trichocereus* is monophyletic if the two *Harrisia* species considered are part of it (Fig. 3). This clade is recovered by three synapomorphies (basitonic growth and prostrate branches [1/3 Appendix 2], imbricate scales along the flower receptacle [24/3], and globose fruits, flattened at the ends [30/4]), and eight homoplasies: (1) maximum stem length between 60 and 100 cm, (2) vegetative apex of stems sharp, forming a 45° to 90° cone, (3) stems cylindrical, more than twice as long as wide, (4) absence of tuberculate ribs, (5) nocturnal flower opening, (6) average receptacle diameter at ovary level 2 cm, (7) flower throat twice as wide as ovary, and (8) more than 11 scales along the fruit). The genus *Trichocereus* is sister to three *Lobivia* species. The occurrence of acute ribs and scales close to each other in the floral receptacle define this sister relationship. Moreover, *Lobivia* and *Echinopsis* (s.s.) are recovered as paraphyletic in our analysis. *Setiechinopsis mirabilis* is distinctive by virtue of the presence of 13 autapomorphies, two of them exclusive (ellipsoidal shape of stems and margin of the apex of inner tepals ending in an angle less than 45 degrees).

DISCUSSION

The results of separate and simultaneous analyses for the group of Cactaceae studied corroborate what was recorded for other groups of plants (Simmons et al. 2001, Cialdella et al. 2007, Ruíz et al. 2008, Lehnert et al. 2009), namely that it is the simultaneous analysis that provides a more informative and explanatory account of the data (Nixon and Carpenter 1996, Gravendeel and De Vogel 2002, Gravendeel et al. 2004). In the simultaneous or total-evidence analysis (morphology + DNA), both the tribe Trichocereeae and the genus *Trichocereus* were recovered as monophyletic, and are supported by a unique combination of characters including some synapomorphies (Fig. 3). These results differ from the findings by Nyffeler (2002) and Ritz et al. (2007), who used exclusively molecular data.

In our total-evidence analysis, *Lobivia* turned out to be paraphyletic. It is worthy of mention that three species of *Lobivia* were grouped together in a poorly supported clade, sharing a unique combination of five homoplastic characters (flowers small, from 6 to 9 cm long, lanceolate scales in the floral receptacle, red tepals, semidry fruits, and bright seeds). The value of this unique combination of features will be enhanced with the inclusion of more species of *Lobivia*. Friedrich (1974) suggested a hypothesis (based on morphology), whereby *Lobivia* has a different origin than *Echinopsis* s.s. and *Rebutia* (sister groups) due to the occasional presence of spines on fruit areoles and to the structure of the flower, which is similar to that of the flower of the ancestor of the tribe Echinopsideae. Our results do not allow us to reject this hypothesis because of the limited sample of *Echinopsis* s.s. and *Rebutia*. All four species of *Echinopsis* s.s. included in our analysis represent extremes of morphological variation and are not recovered as monophyletic; therefore more species of the genus need to be studied to understand the limits of this genus.

Setiechinopsis mirabilis was the first branching taxon in our simultaneous analysis with a high number of autapomorphies not closely related to any species of *Echinopsis* s.s., *Lobivia* or *Trichocereus*. Ritz et al. (2007) found that *S. mirabilis* is the sister species of *Echinopsis huotii* and *Cleistocactus strausii* in a clade with *Espostoa guentheri* as the first branching species. Las Peñas et al. (2011) found cytogenetic differences (in chromosome studies) between *S. mirabilis* and taxa of *Echinopsis* s.l., which, they concluded, supported the proposition that *Setiechinopsis* stands as a valid genus, and our results add further support to this assertion.

Trichocereus and relationships among its species

Trichocereus is monophyletic if *Harrisia earlei* and *H. hahniana* are included, which is supported by three synapomorphies and eight homoplasies. Riccobono (1909) recognized *Trichocereus* as genus because of its columnar-shaped stems and the pilosity of its flowers. The manner of growth—columnar—proposed by Riccobono to define the genus was not recovered as a synapomorphy, but the basitonic branching pattern was recovered as such. The second character he relied upon—flower pilosity—was not recovered as a synapomorphy, either, but the presence of imbricate scales on the flower receptacle was recovered as such.

Several clades were recovered within the genus *Trichocereus* (Fig. 3). The first is comprised by *T. strigosus*, *T. andalgalensis* and *T. schickendantzii*, defined by seven homoplastic characters, including basitonic branching with arching branches (1/5) (Appendix 2), obtuse ribs (11/0), high number of ribs (12/7), long flowers (21/3), orbicular seeds (34/2), very small seeds (35/0), and non-shiny seeds (36/0). Ritz et al. (2007, Fig. 1) found that *Echinopsis* (*Trichocereus*) *schickendantzii* is the sister species of *Samaipaticereus corroanus.* Our results do not support his assertion, as *T. schickendantzii* is here grouped with the other species of *Trichocereus*. *Trichocereus schickendantzii* has an autapomorphy, narrowly ovate seeds (34/3), a character omitted in the original description by Weber (1896), but noted by Kiesling (1978). Additionally, this taxon exhibits seven homoplasies: branches 30 cm tall (5/0), with apex sunken (6/0), ribs sharp (11/1), position of flowers apical (17/0) , receptacle diameter at ovary height 2.5 cm (character 26/3), seeds medium-sized (35/2) and seeds shiny (36/2). Receptacle diameter at ovary height is the only character not mentioned, either in the original description (Weber 1896) or in the treatment of the genus by Kiesling (1978). *Trichocereus andalgalensis* is supported by the unique combination of the following characters: maximum number of ribs in middle part of stems (15), flowers 10 to 17 cm long, and seeds ovate. Of these three characters, only the shape of the seed was included in the extended description of the species (Kiesling 1978).

The second clade, composed of *T. arboricola, H. earlei* and *H. hahniana*, is supported by four synapomorphies: presence of adventitious roots (2/1), stems totally exposed on the ground surface (3/2), with ribs broad, with an angle greater than 135° (11/2), and seeds large, from 2.0 to 2.9 mm (35/3). Moreover, two homoplasies relative to the flower are present (Fig. 3): similar diameter of throat and ovary (27/1) and margin of apex of inner tepals ending in an angle of between 45º and 90º. Leuenberger (1976) first suggested the close relationship between *Harrisia* and *Trichocereus,* based on pollen features. This close relationship is supported by our analysis, in agreement with other molecular findings (Wallace 1997; Nyffeler 2002; Korotkova et al. 2010; Hernandez et al. 2011), although more species of *Trichocereus* and *Harrisia* need to be included in future simultaneous phylogenetic analyses to support this finding. *Harrisia earlei* and *H. hahniana* are supported by two synapomorphies, viz., the alternating arrangement of areoles on adjacent ribs (13/1), and globose fruits (30/3), mentioned by Britton and Rose (1920) in their extended description of the genus. *Harrisia earlei* has four homoplasies: stem length between 2 and 3 m (5/3), flowers up to 24 cm long (21/3), margin of inner tepals concave (29/0), and presence of a keel in seed (character 38/1). The two first characters were mentioned in the original description by Britton and Rose (1920).

The third clade is composed of *T. pachanoi* and *T. peruvianus*, defined by the synapomorphy of glaucous green branches (8/2) and two homoplasies: non-shiny seeds (36/0) and presence of a keel on the seed (38/1).The glaucous green branches were diagnostic in the original description (Britton and Rose 1920) and were mentioned by Madsen (1989) in his extended description of *T. pachanoi* from Ecuador. (For the nomenclature of these species, see the other paper by Albesiano and Kiesling in this issue of *Haseltonia*.)

The fourth clade consists of four species, for one of which a particular subspecies was examined. *T. chiloensis* subsp. *chiloensis* and *T. bolligerianus* form a grade with *T. skottsbergii* and *T. terscheckii,* which are defined as sisters by four homoplasies: arborescent aspect (0/1), presence of trunk with lateral branching (1/6) (Fig. 3), branches up to 15 cm in diameter (9/4), and presence of a keel in seeds (38/1). Both *T. skottsbergii* and *T. terscheckii* share with *T. bolligerianus* the stem length of 2–3 m (5/3), and the three of them share with *T. chiloensis* subsp. *chiloensis* ribs1.1–1.5 cm high (10/2) and more than 20 ribs in the middle part of branches (12/7). *T. terscheckii* is

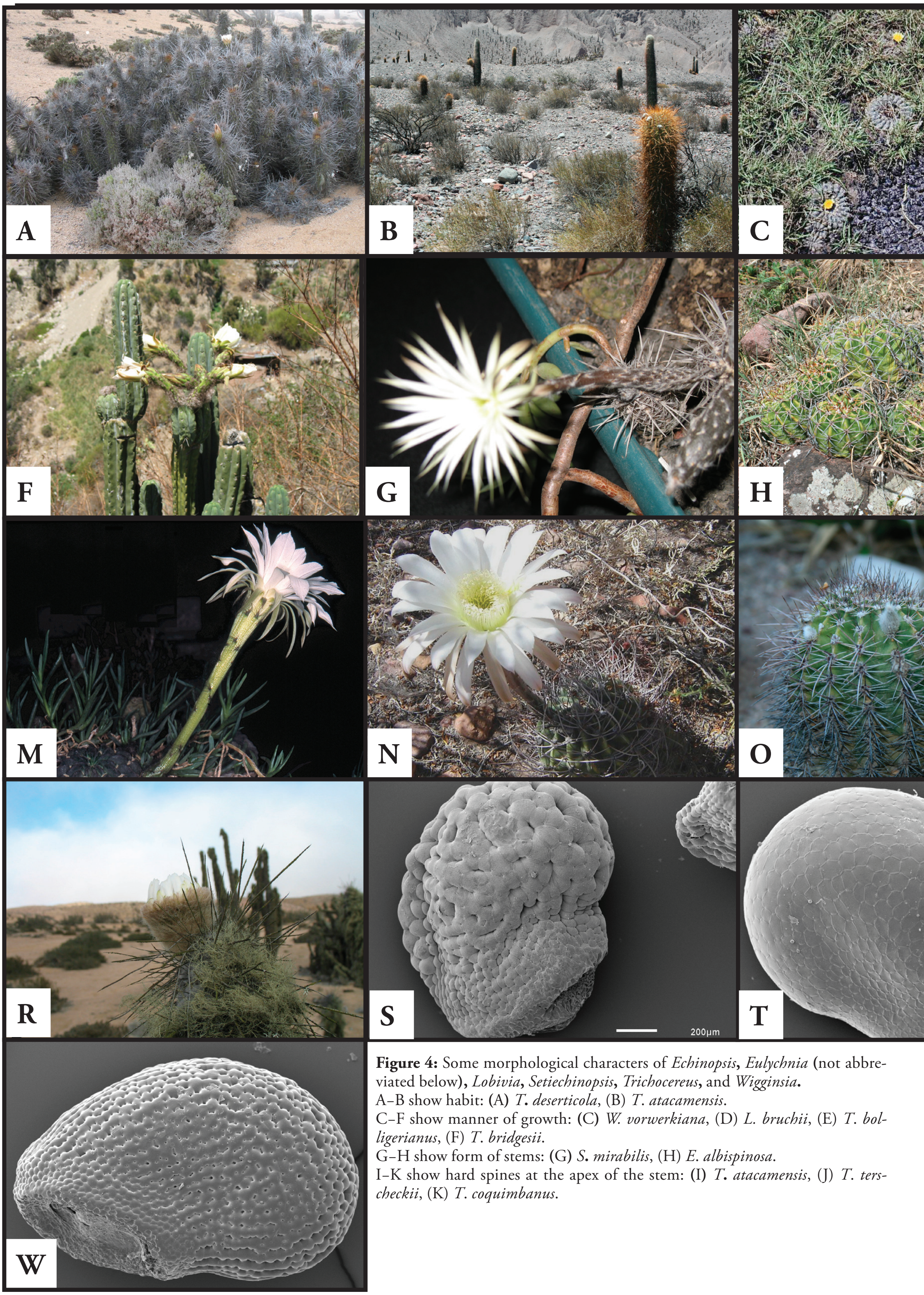
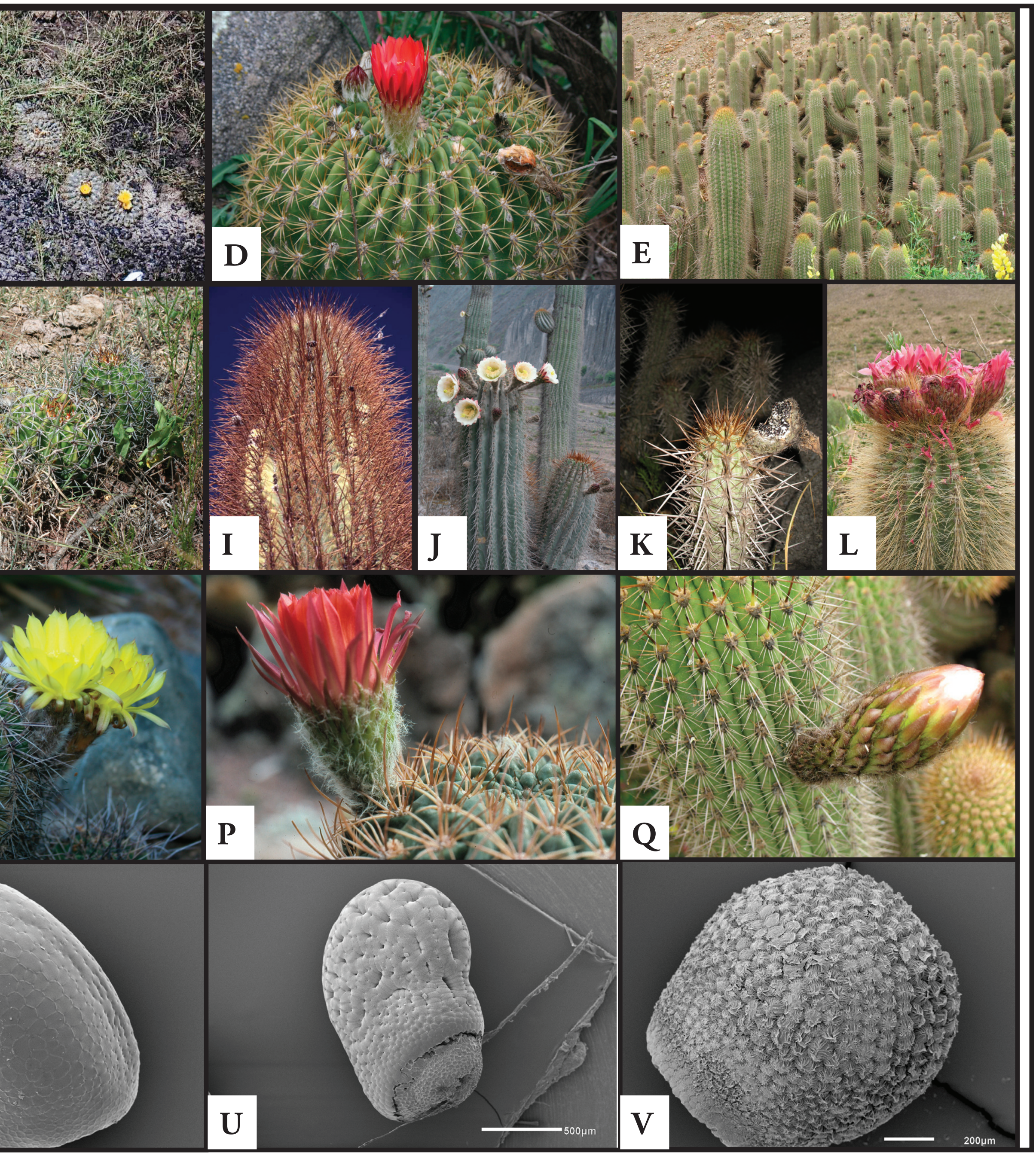


Figure 4: Some morphological characters of *Echinopsis*, *Eulychnia* (not abbreviated below), *Lobivia*, *Setiechinopsis*, *Trichocereus*, and *Wigginsia***.**
A–B show habit: (A) *T. deserticola*, (B) *T. atacamensis*.
C–F show manner of growth: (C) *W. vorwerkiana*, (D) *L. bruchii*, (E) *T. bolligerianus*, (F) *T. bridgesii*.
G–H show form of stems: (G) *S. mirabilis*, (H) *E. albispinosa*.
I–K show hard spines at the apex of the stem: (I) *T. atacamensis*, (J) *T. terscheckii*, (K) *T. coquimbanus*.



(L) Crown of flowers: *T. tarijensis*.
M–R show hairiness of areoles on receptacles: (M) *E. ancistrophora*, (N) *E. leucantha*, (O) *E. aurea*, (P) *L. kieslingii*, (Q) *T. chiloensis* subsp. *chiloensis*, (R) *Eulychnia breviflora*.
S–V show form of the anticlinal wall of the cells of the testa and keel: (S) *T. pachanoi*, (T) *T. strigosus*, (U) *T. atacamensis*, (V) *E. aurea*.
(W) Shininess of seed: *L. jajoiana*.

defined by the unique combination of five homoplasies: stems longer than 8 m (5/5) and up to 20 cm in diameter (9/5), ribs 2.0–2.5 cm high (10/3), flowers long, 20 to 24 cm (21/3), and seeds circular-orbicular (34/2). These character states were not mentioned by Pfeiffer (1837), but they were commented upon by Britton and Rose (1920) and by Kiesling (1978). *Trichocereus skottsbergii* has a unique combination of six homoplasies: light green branches (8/1), numerous ribs (17) (12/6), receptacle diameter at the level of the ovary 2 cm (26/2), non-shiny seeds (36/0), anticlinal walls of the testa cells S-shaped (37/1), and flexible spines on the apex of the vegetative branches (15/1), a character mentioned by Backeberg (1950). Our results do not agree with those of Backeberg (1950), who suggested that *T. skottsbergii* and *T. deserticola* were synonyms, or with those of Charles (2005), who proposed *T. skottsbergii* to be a subspecies of *Trichocereus* (*Echinopsis*) *chiloensis.* The species *T. bolligerianus* is defined by the unique combination of two homoplasies: ribs low (10/0) and ribs sharp (11/1). Our findings do not support those of Kiesling et al. (2008), who considered *T. bolligerianus* to be a synonym of *T. chiloensis* subsp. *litoralis*.

The fifth clade includes the sister species *T. coquimbanus* and *T. spinibarbis*, defined by two homoplasies: adult branches 12 cm in diameter (9/1), and seeds ovate (34/1). In addition, these two species form a clade that shows a sister relationship with *T. deserticola*, through six homoplasies: vegetative apex obtuse (6/2), adult branches 6 cm in diameter (9/3), ribs 1.1–1.5 cm high (10/2), ribs sharp (11/1), flowers apical (17/0), and absence of shininess in seeds (36/0). In turn, *T. candicans* is related to the three previous species by three homoplasies: branches up to one meter long (5/1), receptacle at ovary level wide, 2.5 cm in diameter (26/3), and anticlinal walls of testa cells S-shaped (37/1). Finally, *T. atacamensis* is recovered as the sister species of the remaining taxa of this clade, whose ancestor was characterized by lateral branches arched at the base, then straightening up, and adult branches thick, 15 cm in diameter. It is interesting to mention that *T. spinibarbis* (Otto ex Pfeiff.) F. Ritter exhibits a homoplasy, namely flexible spines on the apex of the vegetative branches (15/1). This species clearly belongs to the genus *Trichocereus*; thus previous studies suggesting it belonged to *Cereus* (Schumann1897) or *Eulychnia* (Britton and Rose 1920; Ritter 1980b) were not supported in our analysis.

Echinopsis, *Lobivia* and *Trichocereus*

Curiously, the European authors that favor uniting *Trichocereus* and *Lobivia* with *Echinopsis* (e.g., Hunt et al. 2006), recognize *Haageocereus* and *Weberbauerocereus*—exclusively from Peru—which exhibit smaller morphological differences from *Trichocereus* than does *Trichocereus* from *Echinopsis* or *Lobivia*. However, Hunt et al. (2006: 90), who support joining the genera, do not seem to be convinced of their position, either: “Current botanical opinion favors uniting several popularly recognized but closely interrelated genera under *Echinopsis*, pending a better understanding of the group as whole.” Thus, while Hunt et al. appear to be suggesting that uniting a collection of hitherto poorly understood genera under *Echinopsis* s.l. is beneficial—based on the unaccountable source cited as “Current botanical opinion”—the nature of any such benefit is both nonobvious and unexplained.

Phylogenetic studies on the Cactaceae (Nyffeler 2002, Hernández et al. 2011, Bárcenas et al. 2011) and on the genera *Rebutia* and *Pfeiffera* (Ritz et al. 2007, Korotkova et al. 2010), based on chloroplast and nuclear sequences, suggest that relationships within the tribe Trichocereeae remain unresolved, and that *Echinopsis*, in its broad sense, is not monophyletic and is closely related to the genus *Harrisia*. Our findings as discussed above agree with Kiesling (1978), Ritter (1980a, 1980b, 1981), and Gibson and Nobel (1986), who suggest that *Echinopsis* and *Trichocereus* should remain separate. Geographically, *Trichocereus* and *Echinopsis* overlap only marginally, since *Echinopsis* is distributed from the Atlantic to the Andes foothills, whereas most species of *Trichocereus* occur at higher altitudes in the Andes or the pre-Andean mountains. Kiesling (1978) considers that similarities are proof of a common origin but that they have evolved independently enough to constitute two separate genera. *Trichocereus* is also close to *Lobivia*, with an intermediate group that was raised to genus: *Soehrensia* (Kiesling 1978). The species of *Soehrensia* are considered by Kiesling (1999b) as part of *Lobivia*. Our results support this assertion, based on the sister taxa relationship of *L. grandiflora* and *L. bruchii* (*S. bruchii*). However, as mentioned above, a larger number of species must be included in future phylogenetic studies to support these results.

Helianthocereus-Trichocereus

Backeberg (1949) proposed *Helianthocereus* for species of *Trichocereus* and in his publication of 1959 he listed 13 species of *Helianthocereus.* However, Kiesling (1978) proposed that *Helianthocereus* should be considered a synonym of *Trichocereus,* because otherwise the two very closely related taxa *H. pseudocandicans* and *T. candicans*, would be treated as belonging to separate genera. A similar case is that of *T. vatteri*, which owing to its colorful and diurnal flowers should be included in *Helianthocereus*, whereas its morphological characters are very similar to *T. strigosus*, except for the size of its flowers. *Helianthocereus andalgalensis, H. atacamensis* and *H. tarijensis* do not form a monophyletic clade; they belong to three different clades within *Trichocereus* in our simultaneous analysis (Fig. 3). Based on the results presented here, we accept the proposal of Kiesling et al. (2008), in which *Helianthocereus* should be considered a synonym of *Trichocereus*.

Character evolution

The present analysis reveals that several of the characters analyzed are of high informative value for identifying relationships among genera and species of the tribe Trichocereeae. Among the characters lacking homoplasy are adventitious roots (2), position of areoles on adjacent ribs (13), pseudocephalium (16), and fruits with remnants of the floral tube (33). Homoplastic character states appearing more than two or three times in an independent manner within the tribe or the genus are, for stems, arborescent habit, presence of trunk with lateral, non-basal branching, ribs low, and flexible spines on the apex of vegetative branches; for flowers, throat and ovary similar, flowers long (between 20 and 24 cm), and margin of apex of inner tepals acute); and, for seeds, shape, size, brightness, and anticlinal testa walls S-shaped. Three character states occur in an independent manner more than three times (vegetative apex sunken, position of flowers apical, and presence of keel in seed). However, many character states correspond to autapomorphies and do not contribute to understanding relationships among species or genera.

On character transformation

Habit and manner of growth: The species of *Trichocereus* show different manners of growth, depending on alterations occurring in the apical dominance (Gibson and Nobel 1986). There are arborescent plants, with trunk and branching above the base, such as *T. atacamensis* (from Argentina, Bolivia and Chile, Fig. 4B), *T. skottsbergii* (from Chile) and *T. terscheckii* (from Argentina, Fig. 4J). These species have thick stems (15 cm) due to the presence of thick pith and to an anatomical development which produces increased number and breadth of ribs (Gibson and Nobel 1986). There are plants with stems emerging from the base, in an upright and parallel position, such as *T. bridgesii* (from Bolivia, Fig. 4F), *T. pachanoi*, *T. peruvianus* (from Ecuador and Peru), *T. chiloensis* and *T. bolligerianus* (from Chile, Fig. 4E). There are low erect shrubs with abundant thin stems, for instance *T. candicans* (from Argentina), *T. coquimbanus* (Fig. 4K), *T. deserticola* (Fig. 4A) and *T. spinibarbis* (from Chile), which rarely exceed 2 m in height. These low caespitose plants exhibit less apical dominance compared to arborescent Cactaceae, because basal branches begin to differentiate early during the growing process of the plant, and their development is accompanied by a reduction of pith diameter and development of a few narrow ribs (Gibson and Nobel 1986). Another growth habit is columnar or barrel-shaped like *T. tarijensis* (from Argentina and Bolivia, Fig. 4L), in which no basal branches are produced, and all the energy is concentrated in the development of a single stem (Gibson and Nobel 1986). Finally, when *T. bolligerianus* grows near the Chilean coast, its stems hang from the cliffs. The same habit was observed by Gibson and Nobel (1986) for *T. peruvianus* on the Peruvian coast.

From the phylogenetic standpoint, the plesiomorphic condition within the tribe Trichocereeae, globose and branchless, is present in *Setiechinopsis, Echinopsis*, and *Lobivia*. In *Trichocereus*, where the plesiomorphic character state is basitonic with prostrate branches (1/3), the following changes are observed: (i) basitonic with arching branches (1/5) in the clades of *T. strigosus, T. andalgalensis, T. schickendantzii* and *T. candicans, T. deserticola, T. coquimbanus, T. spinibarbis*, (ii) columnar (1/2) in *T. tarijensis*, (iii) basitonic with erect branches (1/4), and (iv) mesotonic arching (1/6) in *T. atacamensis* and in the clade of *T. skottsbergii* and *T. terscheckii*. The shrub habit in the tribe Trichocereeae changes to arborescent in the genus *Trichocereus*, as autapomorphies in *T. atacamensis* and *T. tarijensis* and as a synapomorphy in the clade of *T. terscheckii* and *T. skottsbergii*. In the case of *T. chiloensis* subsp. *chiloensis*, its manner of growth, basitonic with erect branches, is derived from basitonic with prostrate branches, and not from a barrel shape (plants with spherical stems taller than 50 cm) as suggested by Hernández et al. (2011), whose conclusion is based on observation of this character in a phylogenetic tree obtained from molecular sequences, but not on a simultaneous analysis including morphological data.

Flowers and pollination: Flower opening, fragrance, color of tepals, and length, thickness and pubescence of the floral tube are some of the characters influenced by the type of pollinators (Mandujano et al. 2010). The presence of hairs and absence of spines on the receptacle prevent damage to pollinators like hummingbirds and moths, which feed on flower nectar (Gibson and Nobel 1986).

The flowers pollinated by animals will have higher seed production per fruit, compared to anemophily, ensuring a greater number of pollen grains in the stigma of a flower and, moreover, ensuring that descendants show higher genetic variability, with correspondingly increased possibilities for adapting to new environments and for competing with other species (Gibson and Nobel 1986). Also cross-pollination (outcrossing) is favored by herkogamy (different degrees of separation between anthers and stigma in the same flower), which is very frequent in Cactaceae, for example in *Ariocarpus fissuratus, Opuntia imbricata, Pilosocereus lanuginosus, P. moritzianus,* and *Stenocereus queretaroensis*, among others (Mandujano et al. 2010). Several species of *Trichocereus* have styles longer than anthers (*T. bolligerianus*, *T. chiloensis* subsp. *chiloensis, T. pachanoi,* and *T. tarijensis*).

Flower opening: In *Trichocereus*, the plesiomorphic character is its night-opening flowers (19/1). Diurnal flowers (19/0) appear as a reversal in the clade of *T. andalgalensis-T. schickendantzii* and in *T. tarijensis.* In the Trichocereeae, nocturnal flowers appear independently in *Setiechinopsis mirabilis* and in the clade of *Echinopsis albispinosa* and *E. leucantha*.

Neotropical columnar cacti of North America, such as *Stenocereus griseus*, are primarily pollinated by bats, whereas major pollinators of subtropical columnar cacti of South America are insects and birds. The breeding system in *T. atacamensis* is xenogamic (its ovules can be fertilized only by pollen from other plants), so its fertilization depends on external agents like bees (*Apis mellifera*), wasps (*Polybia ruficeps*), moths (*Manduca diffissa*), and hummingbirds (*Patagona gigas*) (Badano and Schlumpberger 2001, Schlumpberger and Badano 2005). Those investigators also recorded the presence of beetles, which consume flower parts and remain on the flower for long periods of time, even after the flower has withered, and of ants, which feed on nectar by drilling a hole in the base of the nectary. In neither case does pollination play a major role. Roig and Schlumpberger (2008) found a mutualistic relationship between species of Opuntioideae and Cactoideae and species of the genus *Brachyglossula* (Hymenoptera: Colletidae), the distribution range of these bees overlapping with that of the pollinated species. *Trichocereus tarijensis* is visited by *B. martinezi*, distributed from northern Argentina, in the Province of Jujuy (Tilcara, Humahuaca), to southern Bolivia, in the Department of Potosí (Villazón), which agrees with the location of *T. tarijensis* in the Puna phytogeographic province. In a different geographic region, *Echinopsis ancistrophora* and *T. candicans* are pollinated by *B. communis*, which has been recorded for Argentina in the provinces of Salta, Tucumán, Catamarca, La Rioja, San Juan, and Mendoza. Finally, *Lobivia grandiflora* is visited by *B. ancasti*, endemic to the Sierra de Ancasti, in Catamarca province (Argentina). There are pollinators like *Manduca sexta* (Lepidoptera) that select certain flowers to pollinate because of the presence of a certain compound in the floral scent (Schlumpberger and Raguso 2008). In the case of *Setiechinopsis mirabilis*, its flowers produce fragrances whose main constituent is methyl benzoate, which attracts certain nocturnal pollinators, such as moths, in search of nectar.

Flower length: The ancestor of the tribe Trichocereeae had small flowers (6–9 cm). In *Trichocereus*, the plesiomorphic character state is medium–sized flowers (10–17 cm), and long flowers (20–24 cm) appear three times independently. An inverse relationship exists between flower size and rate of floral scent emission in *Echinopsis ancistrophora*, which has flowers with a short tube (8–10 cm) and high emission of strong floral scents; the flowers are pollinated by bees (Schlumpberger and Raguso 2008). Flower size is no doubt related to the change of pollinating agent.

NEW COMBINATION

Trichocereus bolligerianus (Mächler & Helmut Walter) S. Albesiano, comb. nov.

Echinopsis bolligeriana Mächler & Helmut Walter. *Kakteen Sukk.* 54 (10): 269, ill. 2003. TYPE: Chile, VI Región del Libertador General Bernardo O'Higgins, Prov. Cardenal Caro, Punta de Lobos, Ene. 2003. Walter 213 (holotype, SGO!).

Trichocereus chiloensis var. *conjungens* F. Ritter, *Kakteen Südamerika* 3: 1109, ill. 1980b. TYPE: V Región de Valparaíso, desde la zona costera, hacia el este de Valparaíso, en Limanche, Olmué y Granizo, FR 228c (holotype, U 160273! (Sub "*T. chilensis* var. *conjungens*").

Iconographs: Ritter (1980b), photograph 1062: 1221. Hoffmann and Walter (2004), plate 15: 89. Hunt et al. (2006), Fig. 232.4: 232.

Extended description: *Plants* erect or pendulous, 2 to 6 m long. *Branches* 18 cm in diameter. *Apical ribs* 17, obtuse, 0.6–2 cm wide, 3 mm high; with *areoles* ovate, 2 mm high, 6–8 mm wide, apex yellow, base gray or black; with 1–4 *central spines*, 0.3 to 2 cm long, acicular, base light green, middle part and apex brown; and with 16 *radial spines*, 0.5 to 2 cm long, acicular, base light grey or green, middle part green and apex brown. *Basal ribs* up to 20, obtuse, 2 cm wide, 10 mm high; with *areoles* ovate, 2 mm high, 6 mm wide, gray or brown; with 4–6 *central spines*, 0.2–6 cm long, smaller than 0.7 mm in diameter, acicular, olive green or dark brown; 15 *radial spines*, 1–2 cm long, acicular, flexible, diameter less than 0.7 mm, olive green or light grey. *Flowers* subapical, 1 or 3, infundibuliform, 14 cm long, with abundant brown pilosity on ovary and floral tube, *corolla* 7 cm in diameter, *ovary* 2–3 cm wide, *floral scales of the pericarpel* 2 cm long, green with apex brown, *floral scales of tube* 3 cm long, green or yellowish green with apex brown, *tepals* 4–6 cm long, greenish yellow or yellowish white with longitudinal lines brown, *style* green, 8 cm long, *stigmas* yellow, 1.5 cm long. *Fruit* berry umbilicate, dark green, subapical, 2–3 cm long and 3–4 cm in diameter, covered with triangular scales, from whose axils emerge abundant brown and grey hairs, white pulp. *Seeds* black, 1.8 mm long and 1.0 mm wide.

Distribution and habitat: It grows in Regions V and VI, on hills of the Coastal Cordillera, next to the sea, and on steep slopes (15–20°), where part of the vegetation has been altered by tourist activities.

Taxonomic and nomenclatural comments: In analyzing the original descriptions of *Trichocereus chiloensis* var. *conjungens* and *Echinopsis bolligeriana*, high similarity is observed in the number of ribs and in the number and morphology of spines, for which reasons we propose to unite these two taxa.

Trichocereus chiloensis var. *conjungens* has been considered synonymous with *Echinopsis chiloensis* (Anderson 2005), and lately with *T. chiloensis* subsp. *chiloensis* (Kiesling et al. 2008), but on analyzing the phylogeny of the genus *Trichocereus* (Fig. 3), morphological (ribs low and sharp) and molecular characters were found that allow its differentiation.

The individuals in populations of *Trichocereus bolligerianus* observed in Region VI are smaller in size (2 m) because of the drastic environmental conditions (higher influence of the cold Humboldt current), compared to the Valparaiso region where populations develop under benign conditions and in more sheltered places.

Herbarium material studied: Chile, VI Región del Libertador General Bernardo O'Higgins, Prov. Cardenal Caro, Punta Lobos, 34º25'33"S; 72º02'36"W; 3 m, 2 Nov. 2008, S. Albesiano, et al. 2039 (AGUCH, MERL); Costa de Pichilemú, 34º27'19"S; 72º01'05"W; 7 m, 2 Nov. 2008, S. Albesiano, et al. 2040 (AGUCH, MERL).

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LITERATURE CITED

Anderson EF. 2005. *Das große Kakteen-Lexikon.* Germany: Eugen Ulmer.

Applequist WL, Wallace RS. 2000. Phylogeny of the Madagascan endemic family Didiereaceae. *Plant Syst. Evol.* 221: 157–166.

Arias S, Terrazas T. 2006. Análisis cladístico del género *Pachycereus* (Cactaceae) con caracteres morfológicos. *Brittonia* 58 (3): 197–216.

Backeberg C. 1949. The Natio Lobiviae. *Cact. Succ. Journ. Gr. Brit.* 11(3): 53.

Backeberg C. 1950. *Trichocereus skottsbergii*. Acta Horti Gothob. 18: 146.

Backeberg C. 1958. *Die Cactaceae: Handbuch der Kakteenkunde I.* Jena (DDR): VEB Gustav Fischer Verlag.

Backeberg C. 1959. *Die Cactaceae: Handbuch der Kakteenkunde II.* Jena (DDR): VEB Gustav Fischer Verlag.

Badano EI, Schlumpberger BO. 2001. Sistema de cruzamiento y estimaciones en la eficiencia de polinización sobre *Trichocereus pasacana* (Cactaceae) en dos poblaciones del noroeste Argentino. *Gayana Bot.* 58 (2): 115–122.

Bárcenas RT, Yesson Ch, Hawkins JA. 2011. Molecular systematics of the Cactaceae. *Cladistics* 27 (5): 470–489.

Barthlott W, Hunt DR. 1993. Cactaceae, in K. Kubitzki, JG. Rohwer and V. Bittrich (eds.), *The families and genera of vascular plants*, pp. 161–197. Germany: Springer Verlag.

Barthlott W, Hunt DR, editors. 2000. Seed diversity in the Cactaceae. *Succulent Plant Research* 5: 1–173.

Berger A. 1905. A systematic revision of the genus Cereus. *Rep. (Annual) Missouri Bot. Gard.* 16: 57–86.

Berger A. 1929. Kakteen. Stuttgart: Eugen Ulmer.

Brako L, Zarucchi JL. 1993. *Catalogue of the Flowering Plants and Gymnosperms of Peru.* 45. St. Louis: Missouri Bot. Gard.

Bravo H, Sánchez H. 1991. *Las cactáceas de México III. México D.F.*: Univ. Nac. Autón. México.

Britton NL, Rose JN. 1920. *The Cactaceae: Descriptions and illustrations of plants of the cactus family. II.* Washington: The Carnegie Institution.

Buxbaum F. 1955. *Morphology of cacti. Section III. Fruits and seeds.* Pasadena: Abbey Garden Press.

Buxbaum F. 1958. The phylogenetic division of the subfamily Cereoideae, Cactaceae. *Madroño* 14: 177–206.

Buxbaum F. 1964. Was ist ein Cephalium? *Kakteen And. Sukk.* 15: 43–48.

Charles G. 2005. *Echinopsis chiloensis* subsp. *skottsbergii*, in D. Hunt and N. Taylor (eds.), Notulae Systematicae Lexicon Cactacearum Spectantes V. *Cactaceae Syst. Init.* 19: 13.

Cialdella AM, Giussani LM, Aagesen L, Zuloaga F, Morrone O. 2007. A phylogeny of *Piptochaetium* (Poaceae: Pooideae: Stipeae) and related genera based on a combined analysis including trnL-F, rpl16, and morphology. *Syst. Bot.* 32 (3): 545–559.

De Pinna MC. 1991. Concepts and test of homology in the cladistics paradigm. *Cladistics* 7: 367–394.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.

Edgar RC. 2004. Muscle: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Research* 32 (5): 1792–1797.

Endler J, Buxbaum F. 1958. *Die pflanzenfamilie der Kakteen.* A. Minden: Philler Verlag.

Friedrich H. 1974. Zur Taxonomie und Phylogenie der Echinopsidinae (Trichocereinae). *IOS Bulletin* 3 (3): 79–92.

Friedrich H, Glaetzle W. 1983. Seed morphology as an aid to classifying the genus *Echinopsis* Zucc. *Bradleya* 1: 91–104.

Gibson AC, Nobel PS. 1986. *The cactus primer.* Cambridge: Harvard University Press.

Goloboff PA, Farris JS, Nixon KC. 2008. TNT, a free program for phylogenetic analysis cladistics. *Cladistics* 24: 774–786.

Gravendeel B, De Vogel EF. 2002. Revision of *Coelogyne* section *Moniliformes* (Orchidaceae) based on morphology, plastid and nrDNA ITS sequences. *Blumea* 47 (3): 409–462.

Gravendeel B, Eurlings M, Berg C, Cribb P. 2004. Phylogeny of *Pleione* (Orchidaceae) and parentage analysis of its wild hybrids based on plastid and nuclear ribosomal ITS sequences and morphological data. *Syst. Bot.* 29 (1): 50–63.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41: 95–98.

Hartmann S, Nason JD, Bhattacharya D. 2001. Extensive ribosomal DNA genic variation in the columnar cactus *Lophocereus*. *J. Mol. Evolution* 53: 124–134.

Hernández T, Hernández H, De Nova A, Puente R, Eguiarte L, Magallón S. 2011. Phylogenetic relationships and evolution of growth form in Cactaceae (Caryophyllales, Eudicotyledoneae). *Amer. J. Bot.* 98 (1): 44–61.

Hoffmann AE, Walter H. 2004. *Cactáceas en la flora silvestre de Chile.* Santiago de Chile: Fundación Claudio Gay.

Holmgren PK, Holmgren NH, Barnett LC. 1990. *Index Herbariorum. Part I: the herbaria of the world, 8th edition.* New York: New York Botanical Garden.

Hunt DR. 1999. *Cites Cactaceae Checklist.* 2nd ed. England: Royal Botanic Gardens, Kew/International Organization for Succulent Plant Study.

Hunt DR, Taylor N, editors 1986. The genera of the Cactaceae: towards a new consensus. *Bradleya* 4: 65–78.

Hunt DR, Taylor N, Charles G, International Cactaceae Systematics Group. 2006. *The new Cactus Lexicon.* England: Text. Dh books.

Kiesling R. 1978. El género *Trichocereus* (Cactaceae) I: Las especies de la Rep. Argentina. *Darwiniana* 21 (2-4): 263–330.

Kiesling R. 1999a. The Cacti of Bolivia. *Succulentes* (numere speciele 1999) 22: 3–16.

Kiesling R. 1999b. Cactaceae, in F. Zuloaga and O. Morrone (eds.), *Catálogo de las Plantas Vasculares de la República Argentina 2*, pp. 423–489; 1245–1246. St. Louis: Missouri Bot. Gard.

Kiesling R, Ferrari O. 2005. *100 Cactus Argentinos.* Buenos Aires: Albatros.

Kiesling R, Larocca J, Faúndez L, Metzing D, Albesiano S. 2008. Cactaceae, in F. Zuloaga and O. Morrone (eds.), *Catálogo de la Flora Vascular del Cono Sur de Sudamérica*, pp. 1715–1830. St. Louis: Instituto Darwinion (Arg.)–Missouri Bot. Gard.

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

Lanteri A, Cigliano M, Margaría C. 2005. Análisis filogenético de datos moleculares. Congruencia taxonómica, soporte y confianza estadística de grupos y árboles, in A. Lanteri and M. Cigliano (eds.), *Sistemática Biológica*: Fundamentos teóricos y ejercitaciones, pp. 155–173. La Plata: Edulp.

Las Peñas ML, Kiesling R, Bernardello G. 2011. Karyotype, heterochromatin, and physical mapping of 5S and 18-5.8-26S rDNA genes in *Setiechinopsis* (Cactaceae), an Argentine endemic genus. *Haseltonia* 16(1): 83–90.

Lehnert M, Kessler M, Schmidt A, Klimas S, Fehlberg S, Ranker T. 2009. Phylogeny of the fern genus *Melpomene* (Polypodiaceae) inferred from morphology and chloroplast DNA analysis. *Syst. Bot.* 34 (1): 17–27.

Leuenberger BE. 1976. *Die Pollenmorphologie der Cactaceae und ihre Bedeutung fur die Systematik.* Vaduz, Liechtenstein: J. Cramer.

Maddison WP, Maddison DR. 2010. *Mesquite: a modular system for evolutionary analysis.* Version 2.73 http://mesquiteproject.org

Madsen JE. 1989. Cactaceae 45, in G. Harling and L. Andersson (eds.), *Flora of Ecuador*, no. 35, pp. 1–79. Quito: Götenborg, Stockholm, University of Götenborg, Riksmuseum, Pontificia Universidad Católica del Ecuador.

Mandujano MC, Carrillo I, Martinez C, Golubov J. 2010. Reproductive Biology of Cactaceae, in K. Ramawat (ed.), *Desert Plant: Biology and Biotechnology*, pp. 197–230. London: Springer Link.

Mauseth JD. 1993. Anatomía de Cactus VI. *Quepo* 7: 4–11.

Mickevich ML, Farris JS. 1981. The implications of congruence in Menidia. *Syst. Zool.* 30 (3): 351–370.

Moreno N. 1984. *Glosario Botánico Ilustrado.* México: Continental.

Navarro G. 1996. Catálogo ecológico preliminar de las cactáceas de Bolivia. *Lazaroa* 11: 33–84.

Navarro G, Maldonado M. 2002. *Geografía ecológica de Bolivia: vegetación y ambientes acuáticos. Bolivia: Centro de Ecología Simón I.* Patiño–Departamento de difusión.

Nixon KC. 1999. Winclada (BETA) ver. 0.9.9. New York: Ithaca.

Nixon KC, Carpenter JM. 1996. On simultaneous analysis. *Cladistics* 12: 221–241. Nixon KC, Ochoterena H. 2000. Taxonomía tradicional, cladística y construcción de hipótesis filogenéticas, in HM. Hernández, AN. García, F. Álvarez, M. Ulloa (eds.), *Enfoques contemporáneos para el estudio de la biodiversidad*, pp. 15–37. México: IBUNAM Fondo de Cultura Económica.

Nyffeler R. 2002. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from trnK/matK and trnL-trnF sequences. *Amer. J. Bot.* 89 (2): 312–326.

Pfeiffer L. 1837. *Cereus terscheckii* Parm., in F. Otto, A. Dietrich (eds.), *Allgemeine Gartenzeitung* 5: 370.

Riccobono V. 1909. Studii Aulle Cattee. *Boll. Ort. Bot. Palermo* 8 (4): 236–237.

Ritter F. 1980a. *Kakteen Südamerika 2 Argentinien/Bolivien*. Germany.

Ritter F. 1980b. *Kakteen Südamerika 3 Chile.* Germany.

Ritter F. 1981. *Kakteen Südamerika 4 Peru.* Germany.

Ritz C, Martins L, Mecklenburg R, Goremykin V, Hellwig F. 2007. The molecular phylogeny of *Rebutia* (Cactaceae) and its allies demonstrates the influence of paleography on the evolution of South American mountain cacti. *Amer. J. Bot.* 94(8): 1321–1332.

Roig A, Schlumpberger BO. 2008. The Cactus-Specialist Bees of the Genus *Brachyglossula* Hedicke (Hymenoptera: Colletidae): Notes on Host Associations and Description of a New Species. *J. Kansas Entomol. Soc.* 81(2): 84–91.

Rowley GD. 1974. Reunion of the genus *Echinopsis*. *IOS Bulletin* 3 (3): 93–99.

Ruíz E, Sosa V, Mejía M. 2008. Phylogenetics of *Otatea* inferred from morphology and chloroplast DNA sequence data, and recircumscription of Guaduinae (Poaceae: Bambusoideae). *Syst. Bot.* 33 (2): 277–283.

Schlumpberger BO, Badano EI. 2005. Diversity of floral visitors to *Echinopsis atacamensis* subsp. *pasacana* (Cactaceae). *Haseltonia* 11: 18–26.

Schlumpberger BO, Raguso RA. 2008. Geographic variation in floral scent of *Echinopsis ancistrophora* (Cactaceae); evidence for constraints on hawkmoth attraction. *Oikos* 117 (6): 801–814.

Schumann K. 1897. *Gesamtbeschreibung der Kakteen.* Berlin: Neudamm.

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

Simmons MP, Savolainen V, Clevinger C, Archer R, Davis J. 2001. Phylogeny of the Celastraceae inferred from 26S nuclear ribosomal DNA, phytochrome B, *rbcl, atpB* and morphology. *Molec. Phylogen. Evol.* 19 (3): 353–366.

Stuessy TF. 1990. *Plant Taxonomy.* New York: Columbia University Press.

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

Wallace R. 1997. The phylogenetic position of *Mediocactus hahnianus*. *Cactaceae Consensus Init.* 4: 11–12.

Weber A. 1896. Cactées in D. Boissier dictionnaire d'horticulture: *Dictionnaire d'horticulture illustré.* Paris: Paul Klincksieck.

Appendix 1.

Taxa, with information on the country, collector's name, specimen number and herbarium where it is kept, GenBank accessions (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=nucleotide). *Abbreviations:* Lc: from a living collection; BAB: Instituto Nacional de Tecnología Agropecuaria, Argentina; COL: Herbario Nacional Colombiano; LIL: Fundación Miguel Lillo; LP: Museo de La Plata; LPB: Herbario Nacional de Bolivia; SI: Instituto de Botánica Darwinion, Argentina; CTES: Instituto de Botánica del Nordeste, Argentina; MERL: Instituto Argentino de Investigaciones de las Zonas Áridas; SGO: Museo de Historia Natural, Chile; NY: New York Botanical Garden; U: Herbarium Utrecht, Netherlands.

Tribe Notocacteae

Wigginsia corynodes (Otto ex Pfeiff.) D.M.Porter. Uruguay. R. Kiesling 10231 (SI), 10232 (SI). *W. vorwerkiana* (Werderm.) D.M.Porter. Colombia. S. Albesiano et al. 1735 (COL). *Eulychnia breviflora* Phil. Chile. U. Eggli et al. 2868 (SI). AY566662, DQ100007. *E. castanea* Phil. Chile. U. Eggli & B. Leuenberger 3083 (SI). AY566662, FN673680.

Tribe Hylocereeae

Harrisia earlei Britton & Rose. Cuba. DQ099939, DQ100008. *H. hahniana* (Backeb.) Kimnach & Hutchison. Paraguay. T. Rojas 8499 (SI). Lc O. Ferrari. The specimen had been send us for M. Kinmach and there is a clonotype; i.e. vegetative reproduction from the specimen partially used as the type.

Tribe Trichocereeae

Echinopsis albispinosa K.Schum. Argentina. R. Kiesling I-83. Lc O. Ferrari. *E. ancistrophora* Speg. Argentina. A. Krapovickas & A. Schinini 31416 (CTES), A. Schinini 16161 (CTES). A. Burkart 14458 (SI). Lc O. Ferrari. *E. aurea* Britton & Rose. Argentina. B. Piccinini & J. Hilfer 3854 (BAB). Lc O. Ferrari. *E. leucantha* Walp. Argentina. F. Biurrum et al. 920 (SI), E. Haene 440 (SI), D. Leguiza 28 (SI), R. Sanzin 616 (SI), R. Sanzín s.n. (SI). Lc O. Ferrari. *Lobivia bruchii* Britton & Rose. Argentina. Castellanos 1 (SI), A. Cocucci 2001 (SI), R. Kiesling 8709 (SI), M. Saravia 33 (SI), F. Zuluaga 10504 (SI). Lc O. Ferrari. *L. grandiflora* Britton & Rose. Argentina. Lc O. Ferrari. *L. jajoiana* Backeb. Argentina. R. Kiesling 8915 (SI), W. Rausch 217 (SI). Lc O. Ferrari. *L. kieslingii* Rausch. Argentina. W. Rausch 573 (SI). Lc O. Ferrari. *Setiechinopsis mirabilis* Backeb. ex de Haas. Argentina. R. Kiesling 9321 (SI). Lc R. Kiesling 9321. *Trichocereus andalgalensis* (F.A.C.Weber ex K.Schum.) Hosseus. Argentina. H. Sleumer 1671 (LIL), 1951 (LIL), Peirano 9906 (LIL), Schreiter 10389 (LIL), Vervoorst 3404 (LIL). P. Cantino 593 (SI), R. Kiesling 1068 (SI). *T. arboricola* Kimnach. Argentina. Hilgert 1510 (SI). Lc Hilgert 1510. *T. atacamensis* (Phil.) W.T. Marshall & T.M. Bock. Argentina. D. Drogheti s.n. (LIL), Peirano 9797 (LIL), Schreiter 7204 (LIL). Herbario 66736 (LIL). A.L. Cabrera 16340 (LP). A. Burkart 17616 (SI), M.M. Costa 2547 (SI), R. Kiesling 802 (LP). Bolivia. M. Arakaki & N. Quispe 1723 (LPB). Chile. H.C. Martin 516, 517 (SI). Lc O. Ferrari. *T. bridgesii* Britton & Rose. Bolivia. S. Albesiano & N. Quispe 2094 (LPB), St. G. Beck 4039, 17906 (LPB), R. Kiesling, et al. 10031a (LPB), J.C. Solomon 9458 (LPB), 9460 (LPB), 15576 (LPB), 15778 (LPB), 1709 (LPB). *T. candicans* Britton & Rose. Argentina. R. Kiesling 9 (LP), 799 (LP), 801 (LP), 808 (LP), Stukert s/n (LP), C. Spegazzini s/n (LP). A. Castellanos s/n (LIL), J. Fortuna s/n (LIL), H. Sleumer 335 (LIL), 352 (LIL), 355 (LIL). S. Albesiano & R. Kiesling 2092 (MERL). A. Burkart 15966 (SI), Gerling 19 (SI). *T. chiloensis* subsp. *chiloensis* Chile. U. Eggli & B. E. Leunberger 2570 (SGO), 3042 (SGO), 3052 (SGO). *T. bolligerianus* (Mächler & Helmut Walter) S. Albesiano. Chile. S. Albesiano et al. 2039 (MERL), 2040 (MERL). Walter 213 (SGO). *T. coquimbanus* Britton & Rose. Chile. S. Albesiano et al. 2053 (MERL), 2084 (MERL), 2085 (MERL), 2086 (MERL). U. Eggli & B. E. Leuenberger 2574 (SGO). AY566654. *T. deserticola* (Werderm.) Looser. Chile. U. Eggli & B. Leuenberger 2653 (SGO), 2664 (SGO), 2999 (SGO); U. Eggli 2888, 2915 (SGO); S. Albesiano et al. 2055 (MERL), 2058 (MERL), 2060 (MERL), 2061 (MERL), 2089 (MERL). AY566655. *T. pachanoi* Britton & Rose. Perú. J. N. Rose et al. 22806 (NY). F. Ritter 1467 (U). *T. peruvianus* Britton & Rose. Bolivia. L. Cayola et al. 1533 (LPB), 1534 (LPB), R. Kiesling et al. 10041 (LPB). *T. schickendantzii* Britton & Rose. Argentina. J. Schreiter 6450 (LIL), 9742 (LIL). *T. skottsbergii* Backeb. Chile. S. Albesiano et al. 2050 (MERL). Eggli & B. E. Leuenberger 2579 (SGO). E. Werdermann 885 (SI, U). *T. spinibarbis* (Otto ex Pfeiff.) F. Ritter. Chile. S. Albesiano et al. 2087 (MERL), 2088 (MERL), 2091 (MERL). AY566654. *T. strigosus* Britton & Rose. Argentina. K.J. Hayward 210 (LIL), H. Sleumer 354 (LIL). H.A Fabris & F.O. Zuluaga 8329 (LP), R. Kiesling 82 (LP), 808

(LP), C. Volponi 250 (LP). F. Biurrum 2825 (SI), 3063 (SI), A.L. Cabrera et al. 27122 (SI), E. Haene 124 (SI), R. Kiesling 1061 (SI), 1206 (SI), Zanzin 544, s/n (SI). *T. tarijensis* (Vaupel) Werderm. Argentina. H. Sleumer 3577, 4100, 4101 (LIL). H.A. Fabris 6366 (LP), 6388 (LP), R. Kiesling 26 (LP), D. Muhr 35 (LP). Dell'Prete 2330 (SI), A.L. Cabrera & L. Constance 18993 (SI), M. Cárdenas 1 (SI), K. Fiebrig 2770 (SI), R. Kiesling 4014 (SI), H. Sleumer 3135 (SI). Bolivia. G. Bourdy 2829 (LPB), R. Kiesling & D. Metzing 8393 (LPB), R.P. López 03 (LPB), J.C. Solomon 11334 (LPB). S. Albesiano 2065 (MERL), 2071 (MERL), 2074 (MERL). *T. terscheckii* (J.Parm. ex Pfeiff.) Britton & Rose. Argentina. F. Biurrum 3157 (LP). R. Kiesling 802 (LP), S. Venturi 2071 (LP). F.A. Roig 47177 (MERL), S. Albesiano et al. 2062 (MERL), 2064 (MERL). F. Biurrum 1203 (SI), 2517 (SI), 3060 (SI), P. Cantino 460 (SI), E. Haene 121 (SI), Joergensen 1528 (SI), R. Kiesling 142 (SI), E. Ulibarri 424 (SI).

Appendix 2.

List of morphological characters and character states.

0. Habit: (0) shrubs; (1) arborescent plants. Arias and Terrazas (2006) gave some categories to define the habit for the genus Pachycereus, based on the presence or absence of trunk and the position of branches on the trunk. Based on the previous, we propose the following status: Shrub with woody tissue, no trunk and branched from the base. Arborescent plants have woody tissue, with trunk and branching above the base.

1. Growth form: (0) geophyte; (1) unbranched globose; (2) columnar (with an erect trunk and thick); (3) basitonic prostrate branches (branches are produced from the base, with no central trunk); (4) basitonic erect branches (branches emerging from the base in an upright position and parallel to each other); (5) basitonic with upwardly arching branches (lateral branches are curved upward at the base, becoming erect), (6) mesotonic (with trunk and side branches that arch upward).

2. Adventitious roots (roots growing from non-root tissue, Moreno 1984): (0) absent; (1) present. In the family Cactaceae these roots grow from the areoles or the stem epidermis, especially where these are in contact with the soil (Bravo and Sánchez 1978).

3. Degree of stem exposure: (0) 1/3 of the stems are on the soil surface, with only the top visible, from where flowers and fruits appear (*Wigginsia*); (1) with 2/3 of the stems displayed on the surface (*Echinopsis*, *Eulychnia*, *Lobivia* and *Trichocereus*); (2) stems completely exposed (*Harrisia*, *T. arboricola*).

VEGETATIVE CHARACTERS

4. Interareolar furrow: (0) absent; (1) present. Species like *T. bridgesii*, *T. pachanoi* and *T. peruvianus* have a very deep furrow over each areole.

5. Maximum length of stems: (0) 10–30 cm; (1) 60–100 cm; (2) 150 cm; (3) 2–3 m; (4) 4–7 m; (5) > 8 m. It refers to the length of the stems when the plant has reached adulthood.

6. Growing point looks like: (0) sunken; (1) acute (margins ending at an angle of 45–90º); (2) obtuse (angles bigger than 90º).

7. Form of stems: (0) obconic, the upper part more or less flat, the lower part mostly subterranean; (1) more or less globular; (2) shortly cylindrical, the length less than twice the diameter; (3) cylindrical, length greater than twice the diameter; (4) fusiform (spindle-shaped).

8. Color of stems: (0) dark green; (1) clear green; (2) blue green. The stems' coloration can be influenced by the wax accumulation or by the abundance of chloroplasts (Mauseth 1993). In this group three green tones can be noted in adult plants.

9. Diameter of branches in adult plants: (0) 9 cm; (1) 6 cm; (2) 3 cm; (3) 12 cm; (4) 15 cm; (5) 20 cm. These measurements indicate the mode diameter, whose value does not overlap significantly between species.

10. Height of ribs at the middle part of the stems: (0) low, 0.6–1.0 cm; (1) very low, 0–0.3 cm; (2) medium, 1.1–1.5 cm; (3) high, 2.0–2.5cm.

11. Form of ribs: (0) obtuse (between 90° and 135°); (1) acute (45° to 90°; (2) wide (when the angle is greater than 135°). These observations were made from the front.

12. Maximum number of ribs at the middle part of the stems: (0) 23; (1) 28; (2) 6; (3) 10; (4) 13; (5) 15; (6) 17; (7) 20. The rib number in the different species can vary with maturity, but reach up to a definite maximum number. In the case of the studied species each value used as a character state corresponds to the mode value at maturity; which did not overlap significantly between species.

13. Position of the areoles in adjacent ribs: (0) opposite; (1) alternate. In *Harrisia earlei* and *H. hahniana* the areoles are at different (alternate) levels in adjacent ribs, whereas in the species of *Echinopsis* s.s., *Lobivia* and *Trichocereus*, the areoles are at the same level (opposite arrangement).

14. Tubercles of the ribs: (0) very protruding; (1) slightly protruding; (2) absent.

15. Hardness of spines at the apex of the stems: (0) rigid; (1) flexible.

REPRODUCTIVE CHARACTERS

16. Pseudocephalium; (0) present; (1) absent. At the top of the stems, the young areoles are forming a hairy structure from which the flowers appear. The fact the stem keeps its photosynthetic function, plus the absence of peridermis, are the two characteristics which define the pseudocephalium according to Buxbaum (1964).

17. Position of flowers: (0) apical; (1) lateral.

18. Crown of flowers: (0) absent; (1) present. (Refers to the occurrence of several flowers in a circle around the stem apex.).

FLOWERS

19. Time of opening: (0) diurnal (1) nocturnal.

20. Ratio of the total length of the flower to the diameter of the receptacle at the level of the ovary: (0) similar length of flower and diameter of receptacle at the level of the ovary; (1) the length of flower 4 to 12 times the diameter of the receptacle at the level of the ovary; (2) length of flower 15–20 times diameter of receptacle at the level of the ovary. The species of the Notocacteae tribe have similar length of flower and diameter of receptacle at the level of the ovary. In the tribes Hylocereeae and Trichocereeae, on the other hand, normally the flowers are longer than they are wide.

21. Flower length: (0) very small, from 3 to 6 cm; (1) small, from 6 to 9 cm; (2) medium-sized, from 10 to 17 cm; (3) large, from 20 to 24 cm. These measurements correspond to the mode values, which did not overlap between species.

22. Axils of the floral tube scales: (0) with hairs and bristles; (1) with only hairs. In the species studied, hairs or bristles have always been limited to the floral tube areoles.

23. Floral receptacle scales: (0) absent or inconspicuous; (1) linear (narrow, short and opposite margins parallel); (2) lanceolate (or narrowly elliptical, the width decreasing at each end); (3) triangular-ovate (elliptical with wide base). The scales of the floral receptacle can be inconspicuous as in *Wigginsia*, or very evident as in most of the studied taxa. Their forms are defined by the ratio of length to width (Stuessy 1990: 221).

24. Arrangement of scales along the floral receptacle: (0) absent or inconspicuous; (1) dispersed, (2) close to each other; (3) overlapping. The arrangement of closely spaced scales is imbricate.

25. Hairiness of receptacle areoles: (0) more than 40 hairs at each areole; (1) between 20 and 40 hairs/areole; (2) less than 20 hairs/areole.

26. Receptacle diameter at the ovary level (cm): (0) 1.0; (1) 1.5; (2) 2.0; (3) 2.5; (4) 3.0; (5) 4–5. The value is the mode, and its value it does not overlap significantly between species.

27. Relationship between the diameter of the throat and the ovary: (0) throat two times wider than the ovary; (1) throat and ovary almost same width. In a cactus flower, the throat is the middle part between the tube and the limb of the tepals (Moreno 1984).

28. Tepals color: (0) yellow; (1) white; (2) red.

29. Angle of the apex of the inner tepals: (0) Obtuse, the margins are concave and ending at an angle greater than 90°; (1) acute, the borders ending at an angle of 45° to 90°; (2) acuminate, the margins can be straight or convex, ending in an angle less than 45°.

FRUIT

30. Form: (0) oblong; (1) obconic (conical, with the apex wider); (2) ovoid (egg-shaped, the wider part near the base); (3) globular (approximately spherical); (4) subglobular (like a sphere that has been flattened from opposite sides).

31. Fruit consistency: (0) Dry; (1) semidry; (2) juicy. The consistency of the fruit is determined by the amount of succulence (internal water) presenting the funiculus.

32. Scale number on the fruit: (0) absent; (1) less than 7; (2) from 8 to 10; (3) more than 11. When the fruit was ripe, the scales were counted from one side. The mode value did not overlap significantly between species.

33. Floral tube remnants on fruit: (0) absent; (1) present. Buxbaum (1955), as well Arias and Terrazas (2006), mentioned that on the apical part of the cactus genus *Pachycereus* the fruits retain remnants of the perianth, style and stamens. In the Trichocereeae remnants of the tepals and style fall off upon full maturation of the fruit, leaving a scar called the umbilical depression.

SEEDS

34. Form: (0) widely oval (length/width ratio: (1.1–1.4); (1) ovate (1.5–2.0); (2) circular-orbicular (less than 1.09) and (3) narrowly ovate (2–3). We follow Barthlott and Hunt (2000) in defining the shapes of the seeds in terms of their length/width ratio.

35. Size: (0) very small (0.3–0.8 mm); (1) small (0.9–1.1 mm); (2) medium (1.2–1.9 mm); (3) large (2–2.9 mm). As per Barthlott and Hunt (2000), the seed size is determined as the length of the longer axis. In this study we recognized five states of this character, which did not overlap.

36. Brightness: (0) without shininess; (1) semi-shiny; (2) shiny. The ornamentations of the testa produce the degree of brightness of the seeds.

37. Form of the anticlinal wall of the cells of the testa: (0) straight; (1) S-shaped; (2) U-shaped. Periclinal walls of the cells of the testa can be straight or have different degrees of folding, here recognized as wavy, S-type or U-type.

38. Keel: (0) absent; (1) present. Some seeds have a protruding border on the dorsal side, which corresponds to the folding of the upper cells of the testa (Barthlott and Hunt 2000; Arias and Terrazas 2006).