

**MOLECULAR PHYLOGENY OF *GYMNOCALYCIUM* (CACTACEAE):
ASSESSMENT OF ALTERNATIVE INFRAGENERIC SYSTEMS, A NEW
SUBGENUS, AND TRENDS IN THE EVOLUTION OF THE GENUS¹**

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- **Premise of the study:** The South American genus *Gymnocalycium* (Cactoideae-Trichocereae) demonstrates how the sole use of morphological data in Cactaceae results in conflicts in assessing phylogeny, constructing a taxonomic system, and analyzing trends in the evolution of the genus.
- **Methods:** Molecular phylogenetic analysis was performed using parsimony and Bayesian methods on a 6195-bp data matrix of plastid DNA sequences (*atpI-atpH*, *petL-psbE*, *trnK-matK*, *trnT-trnL-trnF*) of 78 samples, including 52 species and infraspecific taxa representing all the subgenera of *Gymnocalycium*. We assessed morphological character evolution using likelihood methods to optimize characters on a Bayesian tree and to reconstruct possible ancestral states.
- **Key results:** The results of the phylogenetic study confirm the monophyly of the genus, while supporting overall the available infrageneric classification based on seed morphology. Analysis showed the subgenera *Microsemineum* and *Macrosemineum* to be polyphyletic and paraphyletic. Analysis of morphological characters showed a tendency toward reduction of stem size, reduction in quantity and hardness of spines, increment of seed size, development of napiform roots, and change from juicy and colorful fruits to dry and green fruits.
- **Conclusions:** *Gymnocalycium saglionis* is the only species of *Microsemineum* and a new name is required to identify the clade including the remaining species of *Microsemineum*; we propose the name *Scabrosemineum* in agreement with seed morphology. Identifying morphological trends and environmental features allows for a better understanding of the events that might have influenced the diversification of the genus.

Key words: *atpI-atpH*; *petL-psbE*; *trnK-matK*; *trnT-trnL-trnF*; Cactaceae; classification; *Gymnocalycium*; morphological trends; phylogeny.

With ca. 124 genera and 1500–1800 species almost exclusively native to the Americas, Cactaceae is one of the most remarkable plant families in the world, showing extraordinary adaptations to hot, arid environments that include morphological (e.g., water-storage tissues, thick wax-covered epidermis, expanded root systems) as well as physiological (CAM photosynthesis) features (Barcikowski and Nobel, 1984; Nobel, 2002). No less notable than their biological characteristics is their beauty, making cacti popular plants among collectors.

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In the last years, molecular-based studies have provided evidence supporting classifications of cacti at the family, subfamily, tribe, and subtribal level (Wallace, 1995; Wallace and Cota, 1996; Butterworth et al., 2002; Griffith, 2002; Nyffeler, 2002, 2007; Wallace and Dickie, 2002; Edwards et al., 2005; Ritz et al., 2007; Griffith and Porter, 2009; Korotkova et al., 2010; Hernández-Hernández et al., 2011; Bárcenas et al., 2011; Calvente et al., 2011). However, because of the high level of convergence of morphological characters in the family, controversy among different authors persist at generic and subgeneric levels (Hunt, 2006; Bárcenas et al., 2011).

Gymnocalycium Pfeiff. ex Mittler (Cactaceae: Cactoideae: Trichocereae) is a well-established genus comprising ca. 50 species characterized by its globular growth pattern and diurnal flowers with spineless receptacle. It is distributed in southern Bolivia, southwestern and northern Paraguay, southern Brazil, Uruguay, and Argentina except in southern Patagonia (Metzing et al., 1995; Pilbeam, 1995; Hunt, 2006; Charles, 2009) (Fig. 1).

Easy to grow and flowering in culture, *Gymnocalycium* has long been one of the most popular genera for cacti enthusiasts. Most new descriptions of taxa are made by private collectors and growers who usually have the tendency to overemphasize small morphological differences detected in every new form found in the wild and to describe it as a new taxon, thus overlooking

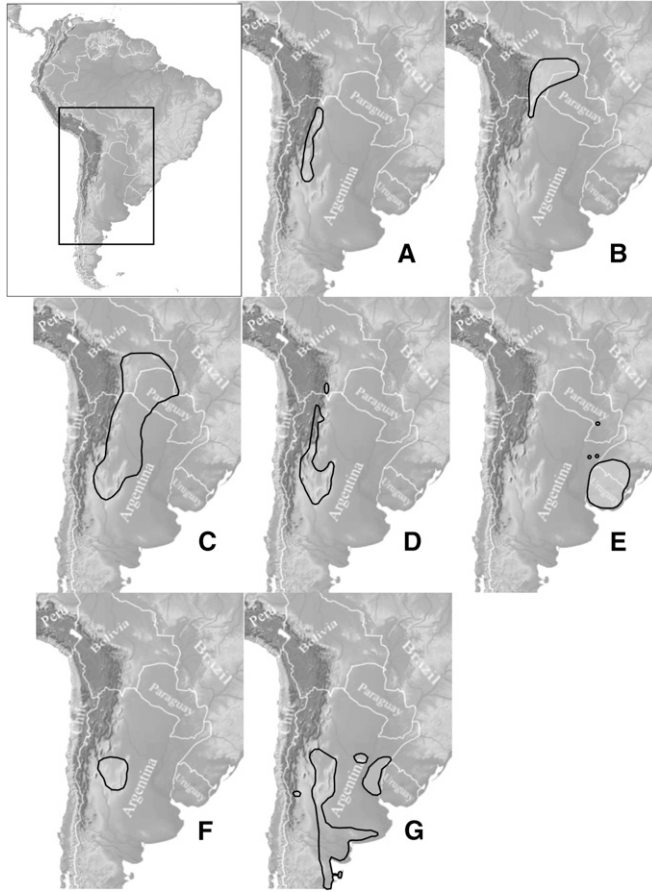


Fig. 1. Geographical distribution of the subgenera of *Gymnocalycium*: (A) *Microsemineum*; (B) *Pirisemineum*; (C) *Muscosemineum*; (D) *Scabrosimineum*; (E) *Macrosemineum*; (F) *Trichosemineum*; (G) *Gymnocalycium*.

the characteristically high morphological variability of cacti and ignoring modern criteria to delimit species. This has resulted in a steady proliferation of synonyms at or below the species rank (Metzing et al., 1995; Hunt, 2006; Charles, 2009) and the need for a stable infrageneric system of classification. The first attempt to establish infrageneric taxa in the genus was by Frič (Kreuzinger, 1935), who published an informal system of five groups based on seed characters: *Ovatiseminae*, *Macroseminae*, *Trichoseminalae*, *Microseminalae* and *Muscoseminalae*. Schütz (1968) published a valid (according to the ICBN rules; McNeill et al., 2006) system of five subgenera, following Frič's criteria: *Gymnocalycium* (= *Ovatisemineum* Schütz, nom. illeg.), *Macrosemineum* Schütz, *Trichosemineum* Schütz, *Microsemineum* Schütz, and *Muscosemineum* Schütz (Table 1). Buxbaum (1968) published another valid system, based primarily on seed morphology, with different taxonomic ranks (Table 2). Other authors such as Backeberg (1941, 1958), Ito (1950, 1957) and Pazout (1964) proposed different infrageneric taxa based on the morphology of stems and flowers, but which were deemed invalid (Metzing et al., 1995).

The system of Schütz (1968) modified by Till and Hesse (1985) and Metzing (1992), has been widely accepted by researchers and collectors during almost 30 yr, until 2001, when Till (2001) and Till et al. (2008) published a new classification system based on characters of fruits, flowers, and seeds (Table 3).

The systems of Schütz and Till differed greatly in the way the species were grouped (Tables 1, 3) and, until now, there was no definitive evidence to decide which system better reflected natural groups.

Controversy in the infrageneric classification of *Gymnocalycium* illustrates how systematic problems in *Cactaceae* are difficult to resolve using only morphology-based methods; a stable classification can be achieved, however, using both morphological and molecular data. Use of DNA sequence data to estimate the phylogenetic relationships of *Gymnocalycium* started with an analysis of the phylogeny of *Cactaceae* (Nyffeler, 2002). This study showed *Gymnocalycium* (represented by the single species *G. denudatum*) in a polytomy with *Cereaceae*, *Browningia*, and *Trichocereaceae* forming the *Browningieae-Cereaceae-Trichocereaceae* (BCT) clade, while *Stetsonia* and *Uebelmannia* were the basal clade. A study by Ritz et al. (2007) focused on the BCT clade to clarify relationships among the globose and short cylindrical cacti of the clade, especially *Gymnocalycium*, *Rebutia*, *Sulcorebutia*, and *Weingartia*. They included 10 species of *Gymnocalycium* that had a sister relationship with a clade including *Echinopsis*, *Cleistocactus*, *Espostoa*, *Denmoza*, *Samaipaticereus*, *Matucana*, *Oroya*, *Oreocereus*, *Haageocereus*, and *Rauhocereus* and formed a well-supported clade in both parsimony and Bayesian analyses. Other globose and short, cylindrical genera (e.g., *Rebutia*, *Sulcorebutia*, *Weingartia*) were resolved to be more distantly related. The limited number of *Gymnocalycium* included, however, was not enough to address phylogenetic relationships within the genus in detail. Recently, Demaio et al. (2010) published a molecular-based phylogeny of the genus and its consequences for infrageneric classification, using *atpI-atpH*, *petL-psbE*, *trnK-matK*, and *trnT-trnL-trnF* as markers and 58 species. Shortly after, Meregalli et al. (2010) published another molecular study, using *atpB-rbcL*, *trnK-rps16*, and *trnL-trnF* as markers and 31 species, with slightly different results. None of these studies analyzed how morphological characters might have evolved in the genus.

To deepen our knowledge of the phylogeny and evolution of *Gymnocalycium* and to establish a robust infrageneric system, we carried out a study to (1) estimate the phylogenetic structure of the genus, (2) compare the phylogeny with existing infrageneric classification systems, and (3) analyze morphological trends in the evolution of the genus. Considering the difficulties reported with nuclear markers in *Cactaceae* (Hartmann et al., 2001; Harpke and Peterson, 2006), we used four plastid markers, including coding and noncoding regions: (1) *trnK-matK* and

TABLE 1. Classification of the genus *Gymnocalycium* according to Schütz (1968), modified by Till and Hesse (1985) and Metzing (1992).

Subgenus	Sections
<i>Gymnocalycium</i> (= <i>Ovatisemineum</i> Schütz, nom. illeg.)	<i>Gymnocalycium</i> <i>Lafaldensia</i> <i>Calochlora</i> <i>Denudata</i>
<i>Macrosemineum</i>	<i>Paraguayensia</i> <i>Microsemineum</i> <i>Hybopleura</i> <i>Loricata</i> <i>Mazanensia</i>
<i>Microsemineum</i>	<i>Muscosemineum</i> <i>Periferalia</i>
<i>Muscosemineum</i>	
<i>Pirisemineum</i>	
<i>Trichosemineum</i>	

TABLE 2. Classification of the genus *Gymnocalycium* according to Buxbaum (1968)

Series	Subseries
<i>Uruguayenses</i>	<i>Uruguayenses</i> <i>Denudata</i>
<i>Baldiana</i>	
<i>Lafaldensia</i>	
<i>Mostiana</i>	
<i>Pileisperma</i>	
<i>Chiquitana</i>	
<i>Castellanosiana</i>	
<i>Horridispina</i>	
<i>Saglionia</i>	
<i>Pflanziana</i>	
<i>Schickendantzianae</i>	<i>Schickendantzianae</i> <i>Mihanovichiana</i> <i>Marsoneriana</i>

trnT-trnL-trnF were previously used in Cactaceae (Nyffeler, 2002; Ritz et al., 2007), allowing comparisons to prior work. (2) *atpI-atpH* and *petL-psbE* have high levels of variability and have been proposed as useful at low taxonomic levels (Shaw et al., 2007).

MATERIALS AND METHODS

Taxon sampling—The ingroup taxa comprise 78 samples, representing 52 species and subspecies of *Gymnocalycium*. *Opuntia sulphurea*, *Uebelmannia pectinifera*, *Matucana polzii*, *Oreocereus celsianus*, and *Stetsonia coryne* were used as outgroups, following Nyffeler (2002), Ritz et al. (2007), Hernández-Hernández et al. (2011), and Bárcenas et al. (2011). Nomenclature of the species, subspecies, and varieties mostly follows Hunt (2006) updated by Charles (2009). Sampling details and EMBL accession numbers are given in Appendix 1.

DNA isolation, amplification, and sequencing—Total DNA was extracted from fresh flower or stem tissues of living plants or from material collected and stored at -80°C using a modified sorbitol/CTAB procedure for plant material with high content in polysaccharides (Tel-Zur et al., 1999), and verified in 1.5% agarose gels prepared with 0.5× Tris-acetate-EDTA (TAE) buffer. A detailed protocol is available in Appendix S1 (see Supplemental Data with the online version of this article). PCR amplifications of double-stranded DNA were performed using universal PCR conditions described in Shaw et al. (2007). PCR reactions of 20 μL contained 18 μL of 1.1× ReddyMix PCR Master Mix (Thermo Fisher Scientific, ABgene House, Epsom, UK), 0.4 μL of each primer (20 mmol/L), 0.2 μL BSA (20 mg/mL) and 1 μL of DNA template. PCR products were checked on 1.5% agarose gels with 0.5× TAE buffer and purified using the two-enzyme clean-up method described by Werle et al. (1994). Enzymes Exo I (Exonuclease I) and FastAP (Thermosensitive Alkaline Phosphatase) were used according to manufacturers' instructions (Fermentas, St. Leon-Rot, Germany). Amplification primer sequences for *atpI-atpH* and *petL-psbE* follow Shaw et al. (2007), primers for amplifying the *trnT-trnL-trnF* region from Taberlet et al. (1991, 2007), *trnK-matK* from Samuel et al. (2005) and newly designed primers (Table 4). Enzyme-cleaned PCR products were direct sequenced from both ends using the same primers as for PCR amplification (*atpI-atpH*, *petL-psbE*) and additional internal sequencing primers (*trnT-trnL-trnF*, *trnK-matK*). For cycle sequencing reactions, we used 0.5 μL of Big Dye Terminator v3.1 Cycle Sequencing Kit Mix (Applied Biosystems, Life Technologies, Vienna, Austria), 1.75 μL of self-made 5× sequencing buffer (350 mM Tris-HCl, pH 9.0; 2.5 mM MgCl_2), 1 μL of primer (4 mmol/L), 4–6.75 μL of cleaned PCR product, and 0–2.75 μL ddH₂O to make the total volume 10 μL . Cycle sequencing follow manufacturer's instructions. Sephadex-purified sequencing reactions were run on a 3730 DNA Analyzer (Applied Biosystems).

Phylogenetic analyses—Sequences of the four markers were aligned using the program CLUSTAL_X (Thompson et al., 1997) and manually adjusted according to the guidelines of Kelchner (2000) and Borsch and Quandt (2009). Phylogenetic analyses were performed using Bayesian inference and maximum parsimony. Bayesian analysis was done with the program MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001). Four incrementally heated simultaneous Markov chain Monte Carlo (MCMC) simulations were run over 3 000 000 generations. Akaike information criterion (AIC) and Bayesian information

TABLE 3. Classification of the genus *Gymnocalycium* according to Till et al. (2008)

Subgenus	Section	Subsection	Series	Aggregate
<i>Gymnocalycium</i>			<i>Gymnocalycium</i>	<i>Gymnocalycia</i> <i>Reducta</i> <i>Hyptiacantha</i> <i>Lafaldensia</i> <i>Quehliana</i> <i>Capillensia</i> <i>Parvula</i> <i>Kieslingia</i> <i>Borthiana</i> <i>Berchtiana</i> <i>Amerhauseriana</i>
			<i>Mostiana</i>	<i>Mostiana</i> <i>Hosseiana</i> <i>Oenantha</i> <i>Rhodanthera</i> <i>Spegazziniana</i> <i>Monvilleiana</i> <i>Saglionia</i> <i>Chiquitana</i> <i>Chacoensia</i> <i>Uruguayensia</i> <i>Multiflora</i> <i>Horstiana</i> <i>Pileisperma</i> <i>Castellanosia</i> <i>Terminalia</i> <i>Schickendantziana</i>
			<i>Saglionia</i>	<i>Saglionia</i> <i>Chiquitana</i> <i>Chacoensia</i>
			<i>Chiquitana</i>	<i>Chiquitana</i> <i>Chacoensia</i>
			<i>Chacoensia</i>	<i>Chacoensia</i>
			<i>Uruguayensia</i>	<i>Uruguayensia</i>
			<i>Multiflora</i>	<i>Multiflora</i>
			<i>Horstiana</i>	<i>Horstiana</i>
			<i>Pileisperma</i>	<i>Pileisperma</i>
			<i>Castellanosia</i>	<i>Castellanosia</i>
<i>Terminalia</i>	<i>Terminalia</i>			
<i>Schickendantziana</i>	<i>Schickendantziana</i>			
<i>Periferalia</i>	<i>Periferalia</i>			

TABLE 4. Primer sequences used for amplification and direct sequencing. Primer name extensions for *trnK-matK* are: an=angiosperms, di = dicots, ca = Cactaceae

Primer name	Sequence (5'–3')	Author
<i>trnK-matK</i>		
matK-50Fdi	GTTTGGACTGTATCGCACTATGTATC	This study
matK550Fdi	CTRGAAATCTTGGTTCAARCTCTTCG	This study
matK750Rca	TGATCGTAAATGAGAGGATTGGTTACAG	This study
matK1200Fca	CTCTGGTGGATCGTTGGCTAAAG	This study
trnKR3an	TCGAACCCGGAAGTAGTCGG	Russell et al., 2010
<i>trnT-trnL-trnF</i>		
a	CATTACAAATGCGATGCTCT	Taberlet et al., 1991
b	TCTACCGATTTCCGCATATC	Taberlet et al., 1991
c	CGAAATCGGTAGACGCTACG	Taberlet et al., 1991
d	GGGATAGAGGACTTGAAC	Taberlet et al., 1991
e	GGTTCAAGTCCCTCTATCCC	Taberlet et al., 1991
f	ATTTGAAGTGGTGACACGAG	Taberlet et al., 1991
h*	CCTTTGAGTCTCTGCACCTTTC	mod. after Taberlet et al., 2007
<i>atpI-atpH</i>		
atpI	TATTTACAAGYGGTATTCAAGCT	Shaw et al., 2007
atpH	CCAAAYCCAGCAGCAATAAC	Shaw et al., 2007
<i>petL-psbE</i>		
petL	AGTAGAAAACCGAAATAACTAGTTA	Shaw et al., 2007
psbE	TATCGAACTGGTAATAATATCAGC	Shaw et al., 2007

criterion (BIC), as implemented in the program JModelTest 0.1.1 (Posada, 2008) were used to choose the TPM1uf+G substitution model. Trees were sampled every 2000 generations resulting in an overall sampling of 1500 trees. A majority rule consensus tree showing all compatible partitions was computed to obtain estimates for the posterior probabilities (PP). Branch lengths were estimated as mean values over the sampled trees.

Incongruence between markers was assessed with the incongruence length difference (ILD) test (Farris et al., 1994), implemented as the partition homogeneity test in the program PAUP* version 4.0b10 (Swofford, 2002). Parsimony analyses were performed using the heuristic search mode in PAUP* with 100 random addition sequence replicates and tree-bisection-reconnection (TBR) branch swapping. All character states were treated as unordered and equally weighted. Gaps were treated as missing data. Branch support was evaluated as bootstrap support (BS) from 1000 bootstrap replicates with TBR branching on and MulTrees off (Felsenstein, 1985; Spangler and Olmstead, 1999). Although not included in phylogenetic analysis, indels for each taxon were coded with the simple indel coding procedure of Simmons and Ochoterena (2000). Ingvarsson et al. (2003) highlighted the potential of indels as tools for inferring relationships among closely related taxa.

The final alignment is deposited in the database TreeBase (<http://www.treebase.org>), accession number 11627.

Morphological trends in *Gymnocalycium*—To assess character evolution in *Gymnocalycium*, we used Bayesian reconstruction to trace morphological characters with the Ancestral State Reconstruction Package as implemented in the program Mesquite (Maddison and Maddison, 2005), using likelihood as the reconstruction method. We used this approach in a morphological data matrix containing characters scored for each species, following criteria of Buxbaum (1951), Gibson and Nobel (1986), Barthlott and Hunt (2000), and Hernández-Hernández et al. (2011): main growth form (barrel, columnar, globose solitary, globose cespitose, flattened); roots (fasciculate, napiform); spines (strong, slender);

fruit form (globular, fusiform); mature fruit color (colored [reddish], green), fruit pulp (juicy, dry), seed size (very small [0.3–0.8 mm], small [0.9–1.1 mm], medium size [1.2–1.9 mm], large [2–2.9 mm]); seed shape (mussel-shaped, ovoid-truncate, hat-shaped). Data were obtained from previously published descriptions and pictures of the taxa (Kiesling, 1980; Pilbeam, 1995; Hunt, 2006; Charles, 2009; Demaio et al., 2010).

RESULTS

Descriptive data on the plastid markers—The *trnK-matK*, *trnT-trnL-trnF*, *atpI-atpH*, and *petL-psbE* were successfully sequenced for all taxa, with the exception of *atpI-atpH* for *Stetsonia coryne* (Table 5). The partition homogeneity test detected no significant incongruence among the four data sets ($P = 0.82$), thus allowing for its combination into a single data matrix with a total length of 6195 bp.

Phylogenetic analyses—The topologies of the trees obtained by parsimony and Bayesian analyses are largely congruent, but differ slightly in the position of clade A (Figs. 2, 3). The monophyly of *Gymnocalycium* is supported by both PP and BS values of 100%. Bayesian and parsimony analyses detected the same major clades in the ingroup (clades A–B–C–D in Figs. 2, 3). Clade A (PP = 100%, BS = 99%) comprises only *Gymnocalycium saglionis* and clade B (PP = 100%, BS = 93%) comprises two monophyletic groups, clade B1 (PP = 100%, BS = 100%), corresponding to subgen. *Pirisemineum* and clade B2 (PP = 100%, BS = 98%), corresponding to subgen. *Mucosemineum* (Figs. 2, 3).

In the parsimony analysis, clades A and B are unresolved and sister to a clade comprising clades C and D, whereas the Bayesian approach shows clade A as basal to the remaining species, which are grouped in a well-supported clade (PP = 94%) (Fig. 3). Clade C (PP = 100%, BS = 95%) comprises a group of species assigned to subgen. *Microsemineum*.

Clade D (PP = 100%, BS = 79%) includes species traditionally assigned to subgenera *Gymnocalycium*, *Macrosemineum*, and *Trichosemineum*. Subclade D1 corresponds to species of the subgen. *Trichosemineum* (PP = 100%, BS = 100%) and D2 include all the species of subgen. *Gymnocalycium* (PP = 88%, BS = 57%). Subgenus *Macrosemineum* appears as paraphyletic, since both subclades D1 and D2 are nested within this clade.

Both parsimony and Bayesian reconstructions show clades C and D strongly nested in a larger clade (PP = 100%, BS = 100%). This clade is sister to clade B in Bayesian analysis, whereas in the parsimony reconstruction it appears as sister to a clade formed by clades A and B.

Descriptions of indel events—Major indel events associated with *Gymnocalycium* are found in *trnT-trnL-trnF* (indels 33 and 57, positions 2552–2564 and 3287–3291 of the aligned matrix) and one indel in *matK* (indel 63, position 4970–4975) (for details of indels and positions see Table 6).

TABLE 5. Sequence information for the different gene partitions. The combined data set includes the data for all four markers.

Sequence characteristics	<i>atpI-atpH</i>	<i>trnT-trnL-trnF</i>	<i>trnK-matK</i>	<i>petL-psbE</i>	Combined data set
Length of aligned matrix (sites)	871	2531	1786	1007	6195
Length of sequences (bp)	275–825	1150–2821	1721–1768	400–750	3365–4078
Number of constant sites (excluding gaps)	800	2330	1648	910	5688
Number of variable sites (excluding gaps)	71	201	138	97	507
Number of parsimony informative sites (excluding gaps)	40	114	72	70	298

Clade A (Fig. 2)—Indels associated with this clade are found in *trnT-trnL-trnF* (indels 24, 25, and 50; positions 2170–2179, 2203–2206, and 3001–3264).

Clade B (Fig. 2)—Indels associated with this clade are found in *trnT-trnL-trnF* (indel 19, position 2134–2144) and *matK* (indel 65, position 5037). Clade B1 is associated with indels in *trnT-trnL-trnF* (indels 13, 17, 42, and 55; positions 1275–1284, 2123–2127, 2812–2825, 3187–3197), *matK* (indel 60, position 3688–3693) and *petL-psbE* (indels 88 and 92, positions 5954–5958 and 6052–6064). Clade B2 is associated with indels in *trnT-trnL-trnF* (indels 1, 2, 4, 16, and 34; positions 80–400, 85–122, 208–293, 2064–2069, and 2645–2661) and *petL-psbE* (indels 70, 71, and 89; positions 5241–5245, 5262–5267, and 5269–5273).

Clade C (Fig. 2)—This clade is associated with indels in *trnT-trnL-trnF* (indel 18, position 2122–2156) and *matK* (indels 67 and 68, positions 5095–5103 and 5104–5112).

Clade D (Fig. 2)—This clade is associated with an indel in *trnT-trnL-trnF* (indel 45, position 2881–2885). Clade D1 is associated with an indel in *matK* (indel 64, position 5031–5037). Clade D2 is associated with an indel in *trnT-trnL-trnF* (indel 46, position 2969–2973).

DISCUSSION

Taxonomy: Division into subgenera—*Clade A: Microsemineum s.s.*—Our results suggest that *G. saglionis* is the earliest-diverging taxon of *Gymnocalycium*. Bayesian reconstruction (Fig. 3) presents this species as sister to all other, while parsimony methods (Fig. 2) place it as a sister to clade B. Recently, Hernández-Hernández et al. (2011), albeit with a reduced number of species of the genus, showed the same position for this species. *Gymnocalycium saglionis* has large stems compared to the other species of the genus and colorful, juicy, sweet fruits that attract birds and other vertebrates to contribute to seed dispersal. These fruit characteristics are similar to the species in clade B of our reconstruction (*G. pflanzii* and related taxa), while most of the other species of the genus produce fruits and seeds adapted for dispersal by ants. Juicy fruits are also common in many genera of the BCT clade of Nyffeler (2002, i.e., *Trichocereus*, *Stetsonia*, and *Echinopsis*) where *Gymnocalycium* is placed.

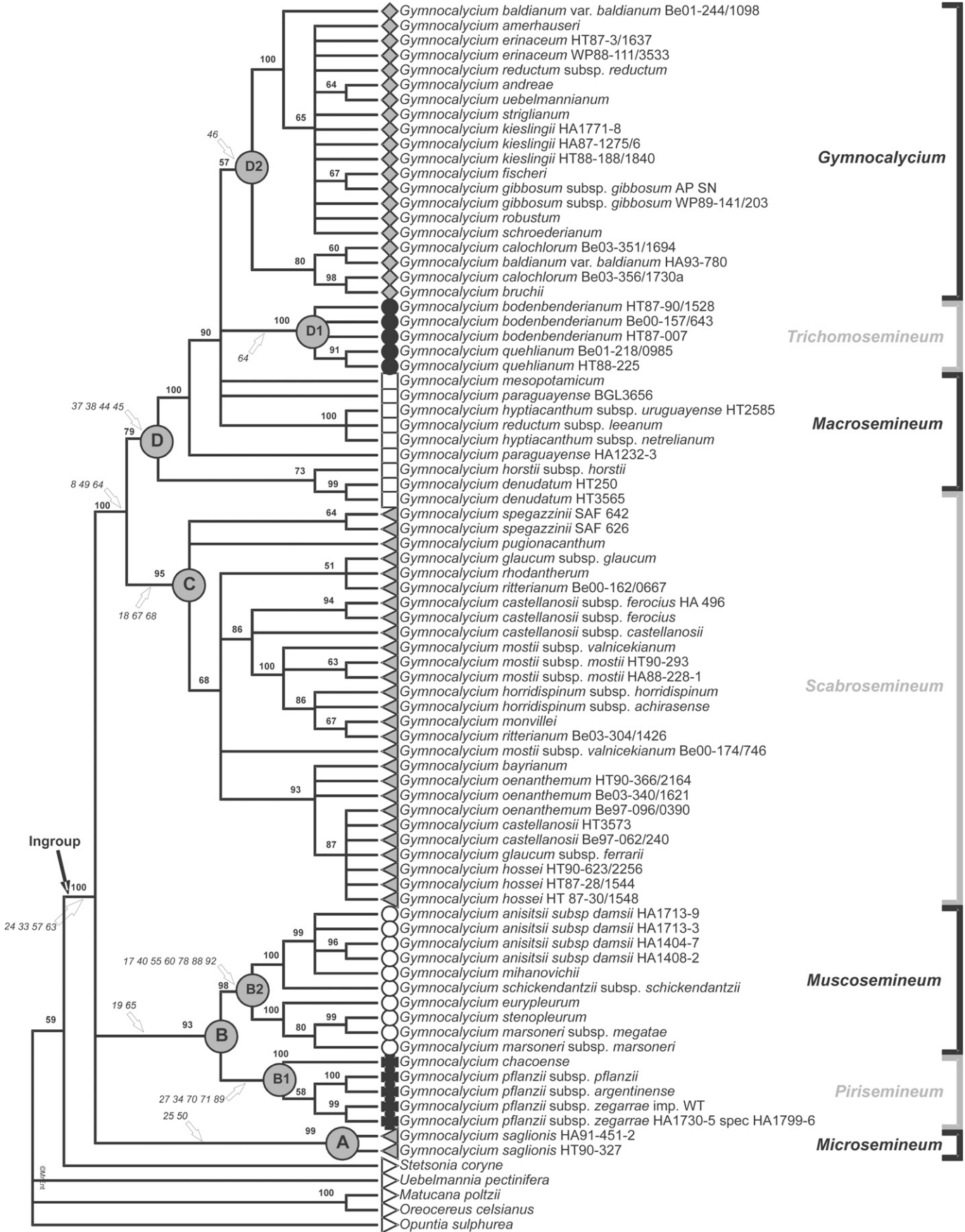
Conspicuously, *G. saglionis* does not appear to be closely related with species of clade C of our reconstruction, although in the systems of Buxbaum (1968), Schütz (1968), Till (2001), and Till et al. (2008), these species are closely related, based on seed and fruit characters (Tables 1, 3). The molecular study of Meregalli et al. (2010) also supported a close relationship between *G. saglionis* and species that make up most of clade C of our reconstruction. However, the relationship depicted in Meregalli et al. (2010) is based on a smaller sampling of two markers and is weakly supported (BS = 50%).

Gymnocalycium saglionis grows in northwestern mountain ranges of Argentina (Fig. 1A). The basal position of this species in our reconstruction might imply that ancestral forms of *Gymnocalycium* originated in these mountain regions of northwestern Argentina and southern Bolivia (Fig. 1). Ritz et al. (2007) proposed the same origin for Nyffeler's (2002) BCT clade, where *Gymnocalycium* belongs.

Clade B: Pirisemineum (B1) and Muscosemineum (B2)—With the exception of *G. schickendantzii*, which extends its distribution to central Argentina, most of the species of this clade occur in the Grand Chaco forests (Pennington et al., 2000) of northern Argentina, southern Bolivia, and western Paraguay (Fig. 1B, C). Clade B is formed by two monophyletic subgroups, corresponding to subgenera *Pirisemineum* (B1) and *Muscosemineum* (B2) of the system of Schütz (1968) (Table 1). Meregalli et al. (2010) arrived at essentially the same topology for these species. Till and Hesse (1985) established the subgen. *Pirisemineum* when separating *G. pflanzii* from the rest of subgen. *Microsemineum*. Juicy fruits and very small mussel-shaped seeds characterize species of *Pirisemineum*. Till (2001) maintained the seed-based grouping when proposing sect. *Pirisemineum* of subgen. *Microsemineum* in the first version of his system. More recently, Till et al. (2008) classified *G. pflanzii* and their relatives together with *G. saglionis* in ser. *Saglionia* of subgen. *Microsemineum* (Table 3). Hunt (2006) and Charles (2009) treated *Pirisemineum* as a synonym of *Microsemineum*. Our molecular evidence suggests that *G. pflanzii* and its relatives should be kept as a well-differentiated group (Figs. 2, 3). Species of clade B2 were always considered as a well-supported group by different authors (sect. *Terminalia* in the system of Till; ser. *Schickendantzianae* in Buxbaum's system; Tables 2, 3), and the results presented here support this view. Its species are characterized by seeds of ca. 1 mm in diameter, with dull, light-brown testa, and a small hilum. Flowers are elongated with a spindle-shaped pericarpel, arising from lateral areoles; in the rest of the species, the flowers originate at the central areoles of the stems.

Clade C: Microsemineum s. l. (except G. saglionis)—All the species of this clade have been classified by Schütz (1968) in subgen. *Microsemineum*, including *G. saglionis* (Table 1). Buxbaum divided the subgenus into several series (Table 2) and Till et al. (2008) classified them with species of clades A, B, and D in subsect. *Saglionia* in their definition of subgen. *Microsemineum* (Table 3). Results presented here, however, indicate that clade C taxa form a well-separated group, and it is necessary to reconsider their classification (Figs. 2, 3). Most of the species of this group grow in the mountains of central-northwestern Argentina (Fig. 1D) in a temperate, subhumid climate with vegetation dominated by tall tussock grassland, interspersed throughout with rocky outcrops (Cabido et al., 1998).

Clade D: Macrosemineum, Trichomosemineum (clade D1), and Gymnocalycium (clade D2)—The species traditionally classified in subgen. *Macrosemineum* (subsect. *Macrosemineum* sensu Till), do not appear monophyletic in our reconstruction (Figs. 2, 3). *Gymnocalycium horstii* and *G. denudatum*, both from southern Brazil, form a well-supported clade, sister to the rest of clade D. *Gymnocalycium paraguayense*, which is not monophyletic according to this study, and *G. mesopotamicum*, are associated with the remaining species of clade D, showing different topologies in parsimony and Bayesian reconstructions (Figs. 2, 3). Accessions of *G. hyptiacanthum* are clustered in a clade sister to clades D1 and D2 in parsimony analysis (Fig. 2). In the Bayesian tree (Fig. 3), *G. hyptiacanthum* is nested to clade D2. Kiesling (1980) noted that the seeds of *G. mesopotamicum* show intermediate morphology between species of clade D1 and *G. hyptiacanthum*; the position of *G. mesopotamicum* in our reconstruction supports Kiesling's (1980) concept of a close relationship among



these species. Most species of subgen. *Macrosemineum* are morphologically similar and show a geographically restricted distribution (Fig. 1A), growing in rocky outcrops and similar habitats in Uruguay, south-central Paraguay, southern Brazil, and eastern Argentina.

Species of clade D1 inhabit the mountains and arid valleys of western-central Argentina (Fig. 1F). They were classified in subgen. *Trichosemineum* by Schütz (1968) (Table 1), in ser. *Quehliana* by Buxbaum (1968) (Table 2), and in subgen. *Microsemineum* sect. *Saglionia* subsect. *Pileisperma* by Till et al. (2008) (Table 3). The molecular evidence supports the grouping of these species in agreement with previous studies on morphology. The position of *Pileisperma* of the system of Till et al. (2008) is not in agreement with relationships found in the present study.

Species of clade D2 inhabit a disjunct area with most taxa in the mountain regions of central Argentina and with a few species in northern Patagonia, eastern Argentina, and western Uruguay (Fig. 1B). All species of clade D2 belong to subgen. *Gymnocalycium* as circumscribed in the systems of Schütz (1968), Till (2001), and Till et al. (2008). The further division of Till et al. (2008) into series and aggregates are presently not supported. Most of the new species of *Gymnocalycium* described in the last 10 years belong to this clade, and the species are morphologically very similar. Similarly, the molecular data gathered showed a remarkable homogeneity, since most sequences were identical (Figs. 2, 3). This lack of resolution, however, could also reflect a rapid and recent radiation, which could be difficult to resolve using conventional DNA sequence data (Hughes and Eastwood, 2006).

Assessment of infrageneric classification systems—The results of our study bring new evidence to support the system of Schütz (1968) (Tables 1, 3; Figs. 2, 3). Other infrageneric classification systems (Buxbaum, 1968; Till, 2001; Till et al., 2008) only partially agree with groups found in our reconstruction. The data presented here, however, imply changes to the original system of Schütz. (1) The status of subgen. *Macrosemineum* should be reconsidered because it is here supported as paraphyletic (Figs. 2, 3), but only when more species and additional DNA markers are included. (2) Clade C comprises most species of subgen. *Microsemineum* sensu Schütz (1968), but this name must be kept for clade A, which includes only *G. saglionis*, a species clearly different from clade C (Figs. 2, 3). Doweld (2005) recently proposed subgen. *Chiquicalycium* for *Microsemineum* s.l. excl. *G. saglionis*. Although *G. chiquitanum*, the type of subgen. *Chiquicalycium*, was not included in the present molecular study, its geographic and morphological separation supports it as a distinct evolutionary lineage (Meregalli et al., 2010). We here propose a new subgenus in Schütz's (1968) system for the species of clade C: *Gymnocalycium* Pfeiff. ex Mittler subgen. *Scabrosemineum* Demaio, Barfuss, R. Kiesling and Chiappella, described in the following synopsis of the subgenera.

SYNOPSIS OF THE SUBGENERA OF *GYMNOCALYCIUM*

***Gymnocalycium* Pfeiff. ex Mittler.** Taschenb. Cactusliebhaber 2: 124. 1844. Type: *Cactus gibbosus* Haw., 1812.

***Gymnocalycium* subgen. *Gymnocalycium*.** Type: *Gymnocalycium gibbosum* (Haw.) Pfeiff. ex Mittler

Roots usually napiform, often fasciculate. Stems solitary or frequently offsetting at the base, globular, often depressed, rarely exceeding 10 cm diameter. Spines often slender and short, occasionally long or strong. Flowers campanulate, often equal or longer than stem height. Fruits fusiform, covered with wax, opening at maturity by a vertical split. Seeds ca. 1 mm diameter, spherical or nearly spherical, truncate. Testa black, dull, usually covered by a brownish film (cuticula). Hilum–micropyle region round, aril slightly protruding. Habitat: Argentina and Uruguay, mostly in grassland with rocky outcrops (Fig. 1G).

***Gymnocalycium* subgen. *Macrosemineum* Schütz ex Metzling,** *Gymnos* 9(17): 4, 1992. Type: *Gymnocalycium denudatum* (Link and Otto) Pfeiff. ex Mittler.

Roots usually fasciculate. Stems globular, offsetting at the base, often solitary. Spines often slender and short, occasionally long or strong. Flowers campanulate. Fruits oval or fusiform, green when ripe, opening at maturity by a vertical split. Seeds 1–3 mm diameter, subglobose, slightly compressed. Testa black. Hilum–micropyle region elongated and slightly depressed. Aril dark or pale around the hilum–micropyle region. Habitat: Uruguay, eastern Paraguay, southern Brazil, and northwestern Argentina, mostly in rocky hills (Fig. 1E).

***Gymnocalycium* subgen. *Microsemineum* Schütz,** *Friciana* 7(46): 8, 1968. Type: *Gymnocalycium saglionis* (Cels) Britton and Rose.

Roots fasciculate. Stems globose to shortly columnar, up to 30 cm in diameter. Spines strong and long. Flowers short, urn-shaped. Fruits large and round, red, opening at maturity by a horizontal split. Seeds very small, 0.6 mm diameter. Testa brownish, tuberculate, dull. Hilum–micropyle region with raised margin. Habitat: northwestern Argentina, in rocky outcrops in mountain ranges (Fig. 1A).

***Gymnocalycium* subgen. *Muscosemineum* Schütz,** *Friciana* 7(46): 10, 1968. Type: *Gymnocalycium mihanovichii* (Gürke) Britton and Rose.

Roots fasciculate. Stems globular to shortly columnar, small to medium size, up to 30 cm in diameter. Spines usually strong. Flowers campanulate, arising from lateral areoles, when all other subgenera are more or less central. Fruits oval or club-shaped, greenish blue to red, opening at maturity by a vertical split. Seeds 0.5–1 mm diameter, testa light brown, dull, with hollow testa cells. Hilum–micropyle region small. Habitat: southern Bolivia, western Paraguay, southwestern Brazil and northern Argentina, mostly in sandy plains, but also in rocky outcrops in mountain ranges (Fig. 1C).

***Gymnocalycium* subgen. *Pirisemineum* H. Till and M. Hesse,** *Pl. Syst. Evol.* 149(1–2): 151, 1985. Type: *Gymnocalycium pflanzii* (Vaupel) Werderm.

Fig. 2. Strict consensus tree of 217291 most parsimonious trees based on a combined data set of four plastid markers for subgenera of *Gymnocalycium*. Tree length = 700; consistency index = 0.773; retention index = 0.946; homoplasy index = 0.227. Bootstrap percentages are given above branches. Following current classification sensu Schütz: (◊) subgenus *Gymnocalycium*; (□) subgenus *Macrosemineum*; (●) subgenus *Trichosemineum*; (◁) subgenus *Microsemineum*; (○) subgenus *Muscosemineum*; (■) subgenus *Pirisemineum*. Bars in right indicate classification in subgenera here proposed. Informative indels for major clades are indicate with arrows (numbers in italics).

TABLE 6. Potentially informative indels for the total alignment.

Indel	Position in the alignment matrix	Marker	Indel	Position in the alignment matrix	Marker
1	80–400	<i>atpI-atpH</i>	47	2972–2976	<i>trnT-trnL-trnF</i>
2	85–122	<i>atpI-atpH</i>	48	2985–2992	<i>trnT-trnL-trnF</i>
3	156–159	<i>atpI-atpH</i>	49	2994–3228	<i>trnT-trnL-trnF</i>
4	208–293	<i>atpI-atpH</i>	50	3001–3264	<i>trnT-trnL-trnF</i>
5	298–305	<i>atpI-atpH</i>	51	3003–3291	<i>trnT-trnL-trnF</i>
6	300–303	<i>atpI-atpH</i>	52	3020–3041	<i>trnT-trnL-trnF</i>
7	317–321	<i>atpI-atpH</i>	53	3075–3080	<i>trnT-trnL-trnF</i>
8	394–398	<i>atpI-atpH</i>	54	3083–3087	<i>trnT-trnL-trnF</i>
9	736–740	<i>atpI-atpH</i>	55	3187–3197	<i>trnT-trnL-trnF</i>
10	774–779	<i>atpI-atpH</i>	56	3210–3232	<i>trnT-trnL-trnF</i>
11	1247–1251	<i>trnT-trnL-trnF</i>	57	3287–3291	<i>trnT-trnL-trnF</i>
12	1246–1255	<i>trnT-trnL-trnF</i>	58	3302–3309	<i>trnT-trnL-trnF</i>
13	1275–1284	<i>trnT-trnL-trnF</i>	59	3346–3351	<i>trnT-trnL-trnF</i>
14	1311–1315	<i>trnT-trnL-trnF</i>	60	3688–3693	<i>trnK-matK</i>
15	1311–1321	<i>trnT-trnL-trnF</i>	61	4238–4243	<i>trnK-matK</i>
16	2064–2069	<i>trnT-trnL-trnF</i>	62	4550–4555	<i>trnK-matK</i>
17	2123–2127	<i>trnT-trnL-trnF</i>	63	4970–4975	<i>trnK-matK</i>
18	2122–2156	<i>trnT-trnL-trnF</i>	64	5031–5037	<i>trnK-matK</i>
19	2134–2144	<i>trnT-trnL-trnF</i>	65	5037	<i>trnK-matK</i>
20	2134–2139	<i>trnT-trnL-trnF</i>	66	5071–5077	<i>trnK-matK</i>
21	2148–2158	<i>trnT-trnL-trnF</i>	67	5095–5103	<i>trnK-matK</i>
22	2151–2156	<i>trnT-trnL-trnF</i>	68	5104–5112	<i>trnK-matK</i>
23	2170–2184	<i>trnT-trnL-trnF</i>	69	5142–5146	<i>trnK-matK</i>
24	2170–2179	<i>trnT-trnL-trnF</i>	70	5241–5245	<i>petL-psbE</i>
25	2203–2206	<i>trnT-trnL-trnF</i>	71	5262–5267	<i>petL-psbE</i>
26	2354–2361	<i>trnT-trnL-trnF</i>	72	5269–5273	<i>petL-psbE</i>
27	2359	<i>trnT-trnL-trnF</i>	73	5271–5333	<i>petL-psbE</i>
28	2371–2379	<i>trnT-trnL-trnF</i>	74	5291–5296	<i>petL-psbE</i>
29	2383–2386	<i>trnT-trnL-trnF</i>	75	5291–5292	<i>petL-psbE</i>
30	2399–2400	<i>trnT-trnL-trnF</i>	76	5301–5320	<i>petL-psbE</i>
31	2442–2451	<i>trnT-trnL-trnF</i>	77	5308–5320	<i>petL-psbE</i>
32	2540–2544	<i>trnT-trnL-trnF</i>	78	5335–5400	<i>petL-psbE</i>
33	2552–2564	<i>trnT-trnL-trnF</i>	79	5409–5438	<i>petL-psbE</i>
34	2645–2661	<i>trnT-trnL-trnF</i>	80	5403–5891	<i>petL-psbE</i>
35	2682–2691	<i>trnT-trnL-trnF</i>	81	5823–5852	<i>petL-psbE</i>
36	2692–2697	<i>trnT-trnL-trnF</i>	82	5843–5848	<i>petL-psbE</i>
37	2747–2839	<i>trnT-trnL-trnF</i>	83	5899–5908	<i>petL-psbE</i>
38	2748–2753	<i>trnT-trnL-trnF</i>	84	5904–5908	<i>petL-psbE</i>
39	2759–2768	<i>trnT-trnL-trnF</i>	85	5925–5930	<i>petL-psbE</i>
40	2769–2774	<i>trnT-trnL-trnF</i>	86	5937–5941	<i>petL-psbE</i>
41	2788–2814	<i>trnT-trnL-trnF</i>	87	5936–5945	<i>petL-psbE</i>
42	2812–2825	<i>trnT-trnL-trnF</i>	88	5954–5958	<i>petL-psbE</i>
43	2820–2824	<i>trnT-trnL-trnF</i>	89	5969–5973	<i>petL-psbE</i>
44	2831–2835	<i>trnT-trnL-trnF</i>	90	6048–6059	<i>petL-psbE</i>
45	2881–2885	<i>trnT-trnL-trnF</i>	91	6055–6059	<i>petL-psbE</i>
46	2969–2973	<i>trnT-trnL-trnF</i>	92	6052–6064	<i>petL-psbE</i>

Roots fasciculate. Stems globose, up to 30 cm in diameter. Spines strong, recurvate. Fruits globose, red or yellow at maturity, opening by a horizontal split. Seeds small, 0.5 mm diameter, pear-shaped, red-brown to reddish; testa smooth, shiny. Hilum–micropyle region dark brown. Habitat: southern Bolivia, southwestern Paraguay and northern Argentina, in sandy plains and rocky outcrops in mountain ranges (Fig. 1B).

Gymnocalycium* subgen. *Scabrosemineum Demaio, Barfuss, R. Kiesling and Chiapella, subgen. nov. Type: *Echinocactus monvillei* Lem., Cact. aliq. nov.: 14 (1838), as “*Echinocactus monvillii*”. Lectotype: original illustration. Epitype: *Kiesling et al.*, 4243 (SI). Synonym: *Gymnocalycium monvillei* (Lem.) Britton and Rose.

Diagnosis—Corpus saepe grandis, 10 usque ad 30 cm diameter, radices plerumque fasciculatae, vel napiformes, caulis saepe

solitarius, rariter subproliferans, globularis vel depressus, costae plerumque tuberculatae, rotundatae, areolae magnae, spinae plerumque longae validaeque. Flos magna, campanulata, ex vertice oriens. Fructus subglobosus, longitudinaliter aperiens. Semina parva, 0.6 usque ad 1 mm diametentia, brunnea vel atrobunnea, minute vel tuberculata, regio hili–micropyles parvior quam diameter seminis. Bolivia australis usque ad Argentina centralis, in locis saxosis montium.

Diagnosis—Plants often large (10–30 cm diameter), roots usually fasciculate, also napiform. Stems frequently solitary or scarcely proliferous, globular, or depressed, ribs mostly tuberculate, rounded, areoles large, spines mostly long, frequently strong. Flowers large, campanulate, growing near the apex. Fruit nearly globose, longitudinally splitting. Seeds small, 0.6–1 mm, brown to dark brown, finely or roughly tuberculate, hilum–micropylar region smaller than the seed diameter. Southern Bolivia to

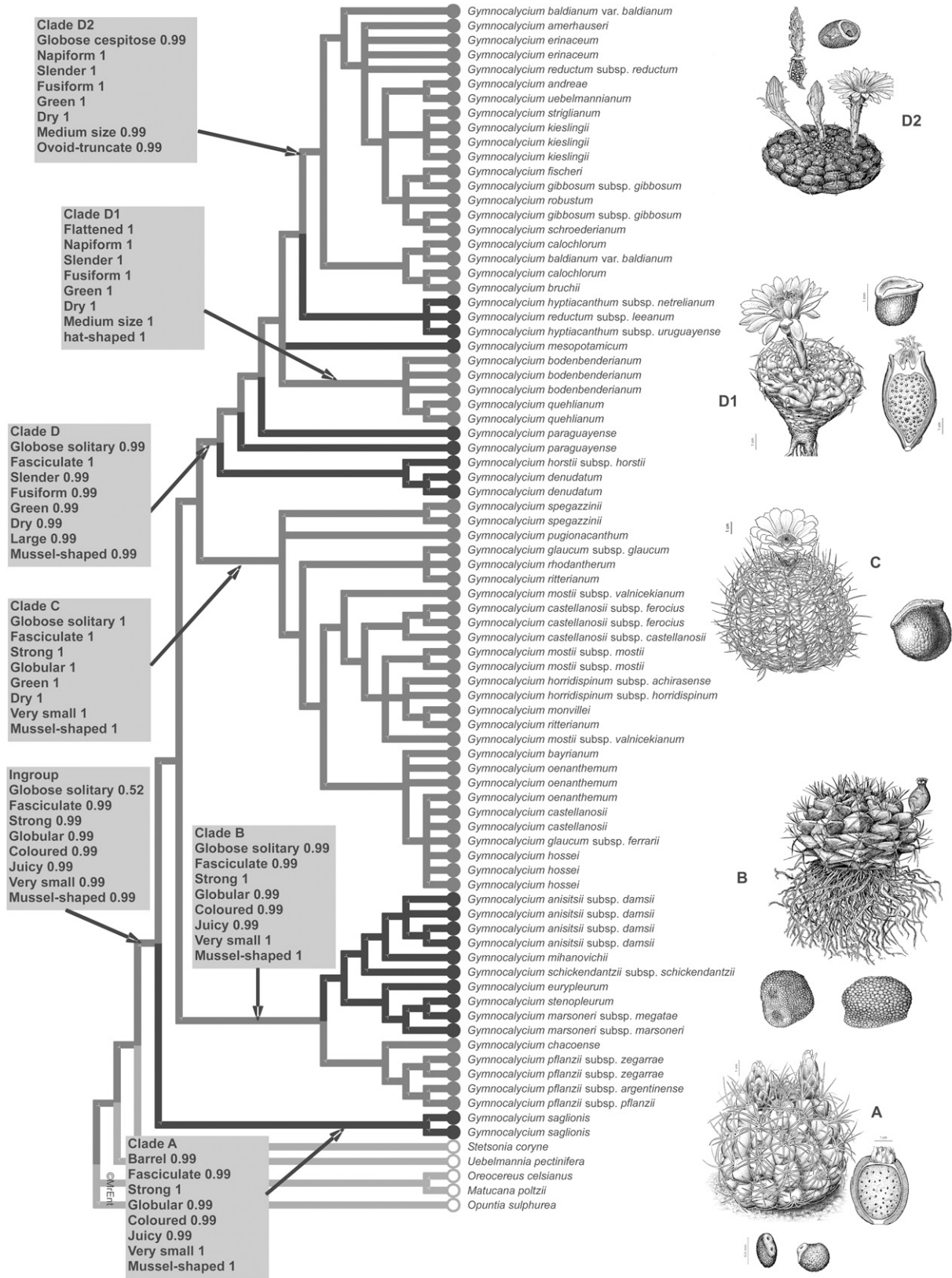


Fig. 4. Maximum likelihood reconstruction of ancestral morphological characters within genus *Gymnocalycium*. Text box shows character states found to have the highest probability and probabilities for major clades. Different colors in branches reflect major groups as in Fig. 2. (A) *G. saglionis*; (B) *G. schickendantzii*; (C) *G. castellanosii*; (D1) *G. bodenbenderianum*; (D2) *G. schroederianum*.

northern and central Argentina, in rocky outcrops in mountain ranges (Fig. 1D).

Gymnocalycium* subgen. *Trichomosemineum Schütz, *Friciana* 46: 10, 1968. Type: *Gymnocalycium quehlianum* (F. Haage ex Quehl) Vaupel ex Hosseus.

Roots napiform. Stems flat, mostly solitary, up to 15 cm in diameter, usually with brownish epidermis. Spines small and slender, often adpressed. Flowers campanulate. Fruits oval, vertically splitting at maturity. Seeds up to 1 mm, helmet-shaped, light to dark brown, very shiny. Aril large, lighter colored. Hilum—micropylar region basal, elliptical. Habitat: central Argentina, in sandy plains and rocky outcrops in mountain ranges (Fig. 1F).

Morphological trends in *Gymnocalycium*—*Growth form and spines*—Ancestral taxa of the BCT clade of Nyffeler (2002) were probably barrel cacti (Hernández-Hernández et al., 2011). Our analysis suggests that the ancestors of *Gymnocalycium*, which belongs to the BCT clade, probably had a barrel growth form or a globular, solitary growth form (Fig. 4). *Gymnocalycium saglionis* (clade A in our reconstruction) is the only species of the genus with a barrel growth form and probably looks like ancestral members of the group. The remaining species show a trend to a gradual reduction in stem size, expressed as a change in growth form toward globose, solitary and globose, cespitose plants (Fig. 4). The distribution of columnar and barrel cacti appears to be severely constrained by low temperatures, while globose cacti are more tolerant to cold (Mourelle and Ezcurra, 1996). Stem size reduction could reflect the adaptation of *Gymnocalycium* evolving species from warm habitats to cooler conditions during radiation or climatic changes (see Fig. 1 for actual distribution of subgenera). Globular cacti diversity is also correlated with summer rainfall and microsites and soil rockiness (Mourelle and Ezcurra, 1996), and the same trend is noted in *Gymnocalycium*, with most of the species growing in the environmentally heterogeneous and relatively humid central ranges of Argentina (Fig. 1).

Although there is no definitive evidence, strong spines showed by most *Cactaceae* are usually described as an adaptation to herbivory (Gibson and Nobel, 1986). Spines, however, also carry some costs, e.g., energy expended in their growth and the reduction of photosynthetically active radiation received by the stem surface (Gibson and Nobel, 1986). The presence of spines must therefore be regarded as a trade-off between the benefits of protection and the losses in the carbon uptake (Gibson and Nobel, 1986). The trend in *Gymnocalycium* ranges from strong and abundant spines, like in *G. saglionis* (clade A, Fig. 2) to tiny, scarce and harmless spines, as in *G. quehlianum* (clade D1, Fig. 2).

Roots—Fascicular and superficial roots were described in *Cactaceae* as an adaptation to shallow rains permeating only the uppermost soil layer and to absorb water that drips off the plant after condensation over the epidermis (Gibson and Nobel, 1986). These features were maintained in basal lineages of *Gymnocalycium* (Fig. 4). Napiform roots were associated with water and starch storage (Nobel, 2002). Water storage tissues in these kinds of roots have the ability to withstand a high degree of dehydration without irreversible damage and may also help to prevent water loss and decrease root shrinkage during drought (Nobel, 2002). Terminal clades of *Gymnocalycium* show a trend to develop a unique and succulent root, especially in species belonging to subgen. *Trichomosemineum* and *Gymnocalycium* (Fig. 4).

Fruits and seeds—Species of basal clades A and B (Fig. 2) have small seeds and juicy, colorful fruits (Fig. 4). These features are related with endozoochory (Rojas-Aréchiga and Vázquez-Yanes, 2000). Many cactus species produce fleshy fruits (berries) with bright colors that constitute an attraction mechanism, encouraging consumption by many frugivorous animals (birds, small mammals, rodents, reptiles, and bats). All these animals can disperse seeds away from the parent plant by means of regurgitation or defecation, sometimes to safe sites for germination and establishment. The trend in *Gymnocalycium* is to develop dry and green fruits, in clade D (Fig. 4), related with myrmecochory. Seed dispersal by ants provides an additional advantage for the plant, in that a suitable site for the seed to germinate is created and the seed is protected from predation (Rojas-Aréchiga and Vázquez-Yanes, 2000). Clade D also contains taxa with increased seed size. Stebbins (1971) mentioned that large seeds, although less easily dispersed, produce the most vigorous seedlings under many different ecological conditions.

Conclusions—The present study helps to clarify conflicting classification systems of *Gymnocalycium* (Buxbaum, 1968; Schütz, 1968; Till, 2001; Till et al., 2008). The scheme of Schütz (1968), slightly modified (Till and Hesse, 1985; Metzging, 1992; and the new subgenus proposed here), mostly based on seed morphology, seems to best represent a natural classification system for the genus, assuming that classification should reflect phylogenetic patterns (Wiley et al., 1991) and that the plastid data used here reflect this phylogeny.

Analysis of morphological features of distinct lineages of *Gymnocalycium* shows a tendency toward reduced plant size, a change from fascicular roots to napiform, and the development of a myrmecochory syndrome in fruits and seeds (Fig. 4). These evolutionary trends in morphology are probably related with environmental changes that occurred during the diversification of the genus and also enabled range expansions into temperate and Andean regions. This radiation would be reflected by the numerous phylogenetically young taxa (suggested by the unresolved tip branches) in these regions. The present results clearly show the need for the inclusion of more morphological and molecular data to resolve the relationships within clades C and D, especially clade D2, and inclusion of detailed and reliable biogeographic analysis.

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APPENDIX 1. EMBL accession for taxa used in this study.

Taxon — Voucher number — EMBL accessions: *atpI-atpH*, *petL-psbE*, *trnK-matK*, *trnT-trnL-trnF*.

- Gymnocalycium amerhauseri* H. Till — HT229 — FN822037, FR848285, FR821475, FR667113. *Gymnocalycium andreae* (Bödeker) Backeberg — HA192 — FN822074, FR848322, FR821512, FR667150. *Gymnocalycium anisitsii* (Schumann) Britton & Rose subsp. *damsii* (Schumann) Charles — HA1404-7 — FN822033, FR848281, FR821471, FR667109. *Gymnocalycium anisitsii* (Schumann) Britton & Rose subsp. *damsii* (Schumann) Charles — HA1713-3 — FN822073, FR848321, FR821511, FR667149. *Gymnocalycium anisitsii* (Schumann) Britton & Rose subsp. *damsii* (Schumann) Charles — HA1713-9 — FN822059, FR848307, FR821497, FR667135. *Gymnocalycium anisitsii* (Schumann) Britton & Rose subsp. *damsii* (Schumann) Charles — HA1408-2 — FN822065, FR848313, FR821503, FR667141.
- Gymnocalycium baldianum* var. *baldianum* (Spegazzini) Spegazzini — Be01-244/1098 — FN822023, FR848271, FR821461, FR667099. *Gymnocalycium baldianum* var. *baldianum* (Spegazzini) Spegazzini — HA93-780 — FN822058, FR848306, FR821496, FR667134. *Gymnocalycium bayrianum* H. Till — Be01-242/1091 — FN822040, FR848288, FR821478, FR667116. *Gymnocalycium bodenbenderianum* (Hosseus ex Berger) A. W. Hill — Be00-157/643 — FN822014, FR848262, FR821452, FR667090. *Gymnocalycium bodenbenderianum* (Hosseus ex Berger) A. W. Hill — HT87-90/1528 — FN822009, FR848257, FR821447, FR667085. *Gymnocalycium bodenbenderianum* (Hosseus ex Berger) A. W. Hill — HT87-007 — FN822062, FR848310, FR821500, FR667138. *Gymnocalycium bruchii* (Spegazzini) Hosseus — GN 91-328 — FN822003, FR848251, FR821441, FR667079.
- Gymnocalycium calochlorum* (Bödeker) Ito — Be03-351/1694 — FN822041, FR848289, FR821479, FR667117. *Gymnocalycium calochlorum* (Bödeker) Ito — Be03-356/1730a — FN822054, FR848302, FR821492, FR667130. *Gymnocalycium capillaense* (Schick) Hosseus — HT 337 — FN822066, FR848314, FR821504, FR667142. *Gymnocalycium capillaense* (Schick) Hosseus — HA 1799-6 — FN822060, FR848308, FR821498, FR667136. *Gymnocalycium castellanosii* Backeberg — Be97-062/240 — FN822070, FR848318, FR821508, FR667146. *Gymnocalycium castellanosii* Backeberg — HT3573 — FN822051, FR848299, FR821489, FR667127. *Gymnocalycium castellanosii* subsp. *castellanosii* Backeberg — HT90-332/1991 — FN822017, FR848265, FR821455, FR667093. *Gymnocalycium castellanosii* Backeberg subsp. *ferocius* (H. Till & Amerhauser) Charles — HA 496 — FN822067, FR848315, FR821505, FR667143. *Gymnocalycium castellanosii* Backeberg subsp. *ferocius* (H. Till & Amerhauser) Charles — HA 92-495-4 — FN822053, FR848301, FR821491, FR667129. *Gymnocalycium chacoense* Amerhauser — HA95-990-4a — FN822036, FR848284, FR821474, FR667112.
- Gymnocalycium denudatum* (Link & Otto) Pfeiffer ex Mittler — PR437 = HT3565 — FN822011, FR848259, FR821449, FR667087. *Gymnocalycium denudatum* (Link & Otto) Pfeiffer ex Mittler — HT250 — FN822046, FR848294, FR821484, FR667122.
- Gymnocalycium erinaceum* Lambert — HT87-3/1637 — FN822020, FR848268, FR821458, FR667096. *Gymnocalycium erinaceum* Lambert — WP88-111/3533 — FN822034, FR848282, FR821472, FR667110. *Gymnocalycium eurypleurum* Plesnik ex Ritter — HA1650-2 — FN822024, FR848272, FR821462, FR667100.
- Gymnocalycium fischeri* Halda, Kupcák, Lukasik & Sladkovsky — Be01-281/1298 — FN822035, FR848283, FR821473, FR667111.
- Gymnocalycium gibbosum* subsp. *gibbosum* (Haworth) Pfeiffer ex Mittler — WP89-141/203 — FN822005, FR848253, FR821443, FR667081. *Gymnocalycium glaucum* Ritter subsp. *ferrarii* (Rausch) Charles — Be01-255/1150 — FN822064, FR848312, FR821502, FR667140. *Gymnocalycium glaucum* subsp. *glaucum* Ritter — HA 1767 3 — FN822031, FR848279, FR821469, FR667107.
- Gymnocalycium horridispinum* subsp. *horridispinum* Frank ex H. Till — HT47 — FN822022, FR848270, FR821460, FR667098. *Gymnocalycium horstii* subsp. *horstii* Buining — HT 3538 — FN821999, FR848247, FR821437, FR667075. *Gymnocalycium hossei* F. Haage — HT87-28/1544 — FN822013, FR848261, FR821451, FR667089. *Gymnocalycium hossei* F. Haage — HT90-623/2256 — FN822072, FR848320, FR821510, FR667148. *Gymnocalycium hossei* F. Haage — HT 87-30/1548 — FN822006, FR848254, FR821444, FR667082. *Gymnocalycium hyptiacanthum* (Lemaire) Britton & Rose — HT451 — FN822039, FR848287, FR821477, FR667115. *Gymnocalycium hyptiacanthum*

- (Lemaire) Britton & Rose subsp. *netrelianum* (Monville ex Labouret) Meregalli — HT2565 — FN822047, FR848295, FR821485, FR667123. *Gymnocalycium hyptiacanthum* (Lemaire) Britton & Rose subsp. *uruguayense* (Arechavaleta) Meregalli — HT2585 — FN822025, FR848273, FR821463, FR667101.
- Gymnocalycium kieslingii* Ferrari — HA87-1275-6 — FN822002, FR848250, FR821440, FR667078. *Gymnocalycium kieslingii* Ferrari — HT88-188/1840 — FN822032, FR848280, FR821470, FR667108. *Gymnocalycium kieslingii* Ferrari — HA1771-8 — FN822012, FR848260, FR821450, FR667088.
- Gymnocalycium marsoneri* subsp. *marsoneri* Fric ex Ito — HA 1851-1 — FN822004, FR848252, FR821442, FR667080. *Gymnocalycium marsoneri* Fric ex Ito subsp. *megatae* (Ito) Charles — HA998-5 — FN822063, FR848311, FR821501, FR667139. *Gymnocalycium mesopotamicum* Kiesling — Kiesling 4. 11. 87 — FN822016, FR848264, FR821454, FR667092. *Gymnocalycium mihanovichii* (Fric & Gürke) Britton & Rose — HA1208-6 — FN822021, FR848269, FR821459, FR667097. *Gymnocalycium monvillei* (Lemaire) Britton & Rose — HT2541 — FN822061, FR848309, FR821499, FR667137. *Gymnocalycium mostii* subsp. *mostii* (Gürke) Britton & Rose — HA88-228-1 — FN822052, FR848300, FR821490, FR667128. *Gymnocalycium mostii* subsp. *mostii* (Gürke) Britton & Rose — HT90-293 — FN822019, FR848267, FR821457, FR667095. *Gymnocalycium mostii* (Gürke) Britton & Rose subsp. *valnicekianum* (Jajó) Meregalli & Charles — HT87-102 — FN822000, FR848248, FR821438, FR667076. *Gymnocalycium mostii* (Gürke) Britton & Rose subsp. *valnicekianum* (Jajó) Meregalli & Charles — Be00-174/746 — FN822055, FR848303, FR821493, FR667131.
- Gymnocalycium oenanthemum* Backeberg — Be03-340/1621 — FN822008, FR848256, FR821446, FR667084. *Gymnocalycium oenanthemum* Backeberg — HT90-366/2164 — FN822029, FR848277, FR821467, FR667105. *Gymnocalycium oenanthemum* Backeberg — Be97-096/0390 — FN822043, FR848291, FR821481, FR667119.
- Gymnocalycium paraguayense* (Schumann) Hosseus — HA1232-3 — FN822044, FR848292, FR821482, FR667120. *Gymnocalycium paraguayense* (Schumann) Hosseus — BGL3656 — FN822010, FR848258, FR821448, FR821448. *Gymnocalycium pflanzii* subsp. *argentinense* — HA91-447 — FN822018, FR848266, FR821456, FR667094. *Gymnocalycium pflanzii* subsp. *pflanzii* (Vaupel) Werdermann — HA1617-3 — FN822045, FR848293, FR821483, FR667121. *Gymnocalycium pflanzii* (Vaupel) Werdermann subsp. *zegarrae* (Cárdenas) Charles — HA1730-5 spec. HA1799-6 — FN822056, FR848304, FR821494, FR667132. *Gymnocalycium pflanzii* (Vaupel) Werdermann subsp. *zegarrae* (Cárdenas) Charles — imp. W.T. — FN822049, FR848297, FR821487, FR667125. *Gymnocalycium pugionacanthum* Backeberg ex H. Till — HT88-45/1823 — FN822048, FR848296, FR821486, FR667124.
- Gymnocalycium quehlianum* (F. Haage ex Quehl) Vaupel ex Hosseus — Be01-218/0985 — FN822071, FR848319, FR821509, FR667147. *Gymnocalycium quehlianum* (F. Haage ex Quehl) Vaupel ex Hosseus — HT88-225 — FN822015, FR848263, FR821453, FR667091.
- Gymnocalycium reductum* (Link) Pfeiffer ex Mittler subsp. *leeaanum* (Hooker) Papsch — HT3567 — FN822042, FR848290, FR821480, FR667118. *Gymnocalycium reductum* subsp. *reductum* (Link) Pfeiffer ex Mittler — HAT s. n. Sierra de la Ventana — FN822007, FR848255, FR821445, FR667083. *Gymnocalycium rhodantherum* (Bödeker) Backeberg — HT87-75/1605 — FN822026, FR848274, FR821464, FR667102. *Gymnocalycium ritterianum* Rausch — Be00-162/0667 — FN822057, FR848305, FR821495, FR667133. *Gymnocalycium ritterianum* Rausch — Be03-304/1426 — FN822027, FR848275, FR821465, FR667103. *Gymnocalycium robustum* Kiesling, Ferrari & Metzging — HA 93-817 — FN821998, FR848246, FR821436, FR667074.
- Gymnocalycium saglionis* (Cels) Britton & Rose — HT90-327 — FN821997, FR848245, FR821435, FR667073. *Gymnocalycium saglionis* (Cels) Britton & Rose — HA91-451-2 — FN822038, FR848286, FR821476, FR667114. *Gymnocalycium schickendantzii* subsp. *schickendantzii* (F. A. C. Weber) Britton & Rose — HA88-123-1 — FN822001, FR848249, FR821439, FR667077. *Gymnocalycium stenopleurum* Ritter — HA1720-3 — FN822050, FR848298, FR821488, FR667126. *Gymnocalycium striglianum* Jeggle ex H. Till — HT96-1019 — FN822030, FR848278, FR821468, FR667106.
- Gymnocalycium uebelmannianum* Rausch — Rausch 141 — FN822028, FR848276, FR821466, FR667104.
- Matucana polzii* L. Diers, Donald & E. Zecher — EZ 762 — FN822068, FR848316, FR821506, FR667144.
- Oreocereus celsianus* (Lemaire ex Salm-Dyck) Riccobono — VG 04-139 — FN822069, FR848317, FR821507, FR667145.