American Journal of Botany

American Journal of Botany 99(8): 1335–1349. 2012.

Molecular phylogenetics of *Echinopsis* (Cactaceae): Polyphyly at all levels and convergent evolution of pollination modes and growth forms[1]

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- *Premise of the study:* In its current circumscription, *Echinopsis* with 100–150 species is one of the largest and morphologically most diverse genera of Cactaceae. This diversity and an absence of correlated characters have resulted in numerous attempts to subdivide *Echinopsis* into more homogeneous subgroups. To infer natural species groups in this alliance, we here provide a plastid phylogeny and use it to infer changes in growth form, pollination mode, and ploidy level.
- *Methods:* We sequenced 3800 nucleotides of chloroplast DNA from 162 plants representing 144 species and subspecies. The sample includes the type species of all genera close to, or included in, *Echinopsis* as well as a dense sample of other genera of the Trichocereeae and further outgroups. New and published chromosome counts were compiled and traced on the phylogeny, as were pollination modes and growth habits.
- *Key results:* A maximum likelihood phylogeny confirms that *Echinopsis* s.l. is not monophyletic nor are any of the previously recognized genera that have more than one species. Pollination mode and, to a lesser extent, growth habit are evolutionarily labile, and diploidy is the rule in *Echinopsis* s.l., with the few polyploids clustered in just a few clades.
- *Conclusions:* The use of evolutionary labile floral traits and growth habit has led to nonnatural classifications. Taxonomic realignments are required, but further study of less evolutionary labile traits suitable for circumscribing genera are needed. Surprisingly, polyploidy seems infrequent in the *Echinopsis* alliance and hybridization may thus be of minor relevance in the evolution of this clade.

Key words: Cactaceae; chromosome counts; Echinopsis; growth form evolution; phylogenetics; pollination modes; polyploidy; Trichocereeae.

Cactaceae comprise some 1870 species in 130 genera (Nyffeler and Eggli, 2010) and are estimated to have diversified since the mid-Tertiary, about 30 million years ago (Ma) (Hershkovitz and Zimmer, 1997). They are an important component of seasonally dry tropical and subtropical ecosystems, where their adaptations for water conservation and water storage permit them to survive even pronounced droughts. Different growth habits, such as columnar, globular, or barrel growth, may characterize larger species groups, but molecular-phylogenetic studies in Cactaceae have rarely addressed the evolution of growth forms (but see Hernández-Hernández et al., 2011). Similarly, floral characters and pollinator affiliation in cacti have rarely been studied in an evolutionary context, mostly for lack of large phylogenies and fieldwork on pollination biology. That polyploidy relates to diversification has been suggested for several clades of cacti (Remski, 1954; Pinkava et al., 1985; Cota and Philbrick, 1994; Cota and Wallace, 1995; Pinkava, 2002; Negrón-Ortiz, 2007), but again the evidence is limited due to a paucity of chromosome counts for species (or clades) of known relationships. Nevertheless, it is thought that polyploidy may be an important speciation mechanism in some Cactaceae (Negrón-Ortiz, 2007; Arakaki et al., 2007, and citations therein).

One of the least understood groups of cacti is the *Echinopsis* alliance, which includes 100–150 species that exhibit a great diversity in architecture and that variously are pollinated by bees, hummingbirds, or sphingids (Schlumpberger and Badano, 2005; Schlumpberger and Raguso, 2008; Schlumpberger et al., 2009; Walter, 2010; Ortega-Baes et al., 2011). The *Echinopsis* alliance belongs in the Trichocereeae (Anderson, 2001, 2005; Hunt, 2006) or Trichocereinae of a more broadly circumscribed Cereeae (Nyffeler and Eggli, 2010). The species occur from southeastern Brazil to the coast of Chile and north to northern Ecuador, with a center of species diversity in the eastern Andes of northern Argentina and Bolivia. With more than 500 species names, *Echinopsis* is taxonomically problematic and much in need of study of natural intraspecific variation as well as molecular phylogenetic work (Anderson, 2001; Hunt, 2006; Nyffeler and Eggli, 2010). In the broad circumscription of Hunt (2006), *Echinopsis* includes 18 genera, including the well-known and species-rich *Lobivia* Britton & Rose and *Trichocereus* Riccob. Because of the problematic generic boundaries, recent studies continue to lump these difficult genera "to be conservative" (Arakaki et al., 2011: p. 8383). The 18 genera included in *Echinopsis* by Hunt initially were created based on traits, such as diurnal vs. nocturnal flowering, flower color, and growth form, i.e., globular vs. columnar: Thus, the name *Lobivia*

[1] Manuscript received 22 June 2011; revision accepted 3 July 2012.

The authors thank Stephan Beck, Grischa Brokamp, Andrea Cocucci, Roberto Kiesling, Alicia Sérsic, and Sandra Siefken for help during fieldwork; Urs Eggli, Hans Frohning, Ralf Hillmann, Cyrill Hunkeler, Martin Lowry, Jörg Piltz, Eberhard Scholz, Daniel Schweich, and Matthias Uhlig for plant material; and Martina Silber for GenBank annotations and submission of all sequences. Verena Beyer and Kerstin Schuster produced some of the sequences as part of their research at the University of Munich. The project was supported by DFG grants SCHL 1820/1-1 and 1820/1-2 and the Alexander von Humboldt Foundation.

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doi:10.3732/ajb.1100288

applied to mostly globular cacti from the medium to high Andes with short, diurnal flowers of various colors; *Trichocereus* was used for mostly columnar, sometimes tree-like cacti with flowers that are usually large, white, and nocturnal; and *Echinopsis* in the original sense applied to globular plants with white, funnel-shaped flowers of predominantly nocturnal anthesis. Species combining these characters were placed in separate genera, such as *Helianthocereus*, created for species of columnar habit, but with diurnal flowers (see Table 1 for a short history of *Echinopsis* taxonomy).

Here we present a densely sampled molecular phylogeny for *Echinopsis* that includes 144 species and subspecies, including the type species of all relevant generic names, as well as representatives of all genera in recent years assigned to the tribe Trichocereeae, again including relevant generic type species. To assess the role of chromosome number changes, we generated new counts for 37 selected taxa and added these to previous counts for a phylogenetically distributed sampling. We use these data to address the following questions: (1) Is *Echinopsis* in the broad sense monophyletic? (2) What is the level of variation of growth habit and pollination mode, characters that have been used to define clades in *Echinopsis* sensu lato? And (3) How common are ploidy changes in the *Echinopsis* alliance, and are they clusterd in certain subclades, perhaps indicating a role for hybridization in the evolution of certain species groups.

MATERIALS AND METHODS

***Taxon sampling*—**Molecular phylogenies to date have included three to nine species of *Echinopsis* plus few of the potentially embedded related genera of Trichocereeae (Nyffeler, 2002; Ritz et al., 2007; Hernández-Hernández et al., 2011). We obtained tissue samples from 162 plants representing 31 genera, 129 species, 8 subspecies, and 2 varieties; 74 species belong to *Echinopsis* s.l., the remaining ones are species not previously assigned to *Echinopsis* (see Table 2 for taxonomic names and their authors, as well as information about generic type species). Our sample includes representatives of all nine infrageneric groups of *Echinopsis* defined by Friedrich and Glätzle (1983) and representatives of the 13 sections of *Lobivia* defined by Rausch (1985). We further included 54 species representing the 24 genera of the tribe Trichocereeae (Anderson, 2005), and part of the Browningieae-Cereeae-Trichocereeae (BCT) clade of Nyffeler (2002). For rooting, we chose *Neowerdermannia vorwerkii* from core Notocacteae, based on Nyffeler (2002).

Material was field-collected by the first author or obtained from documented material from public and private collections. All species identifications were made or confirmed by the first author. Vouchers are listed in Table 2.

TABLE 1. Major steps in the history of *Echinopsis* taxonomy.

Study	
Hunt, 2006	*Echinopsis* Zucc. (*Echinopsis* s.str., *Lobivia* and *Trichocereus* as artificial infrageneric groups)
Anderson, 2001, 2005	*Acanthocalycium* Backeb., *Echinopsis* Zucc. (2005: *Echinopsis* s.str., *Lobivia* and *Trichocereus* as artificial infrageneric groups)
Backeberg, 1958–1962	*Acanthocalycium* Backeb., *Acantholobivia* Backeb., *Chamaecereus* Britton & Rose, *Echinopsis* Zucc., *Helianthocereus* Backeb., *Leucostele* Backeb., *Lobivia* Britton & Rose, *Pseudolobivia* Backeb., *Soehrensia* Backeb., *Trichocereus* Riccob.
Britton and Rose, 1919–1923	*Chamaecereus* Britton & Rose, *Echinopsis* Zucc., *Lobivia* Britton & Rose, *Trichocereus* Riccob.
Schumann, 1899	*Cereus* Mill. (in part), *Echinopsis* Zucc.

***DNA extraction, amplification, sequencing, and alignments*—**Samples were collected from floral or vegetative tissue and silica-dried. Total genomic DNA was extracted using the NucleoSpin extraction kit (Macherey-Nagel, Düren, Germany), following the manufacturer's protocol, but with an elongated lysis step over night at 4°C.

We amplified three noncoding chloroplast DNA regions using published standard primers: the *trnS-G* intergenic spacer (Hamilton, 1999), the *trnL* region (Taberlet et al., 1991), and the *rpl16* intron (Asmussen, 1999). PCR reactions were performed in 25 μL reactions (1.25 μL DNA, 0.1 μL *Taq* polymerase, 0.8 μL primer (10 μL), 0.5 μL 1.5 mmol/L $MgCl_2$, 4 μL 5× buffer, 4 μL 1.25 mmol/L dNTP, 025 μL BSA) as follows: initial denaturation was at 95°C for 5 min; followed by 35 cycles of 95°C for 30 s (denaturation), 48–55°C for 1 min (annealing) and 72°C for 2 min (elongation); and 72°C for 40 s (final extension). PCR reaction products were purified using the Wizard SV PCR clean-up kit (Promega GmbH, Mannheim, Germany). Sequencing reactions were performed with the Big Dye Terminator kit (Applied Biosystems, Warrington, UK), and cleaned with the Sephadex G-50 gel filtration system (Amersham, Uppsala, Sweden) using MultiScreen TM-HV membrane plates (Millipore, Bedford, Massachusetts, USA) according to the manufacturer's protocol. Reactions were run on an ABI 3100 Avant capillary sequencer. Sequences were edited and assembled using the program Sequencher 4.5 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were aligned using Prank (Löytynoja and Goldman, 2005) and corrected by eye in the program MacClade 4.08 (Maddison and Maddison, 2003).

***Phylogenetic analyses*—**Chloroplast partitions were first analyzed separately to check for possible contamination or mislabeling that might have led to incongruent topologies, and in the absence of statistically supported topological contradictions (defined as >70% maximum likelihood bootstrap support), all sequences were combined. Phylogenetic inference relied on maximum likelihood (ML) under the GTR + G model as implemented in the program RAxML BlackBox (Stamatakis et al., 2008, http://phylobench.vital-it.ch/raxml-bb/).

***Chromosome counts*—**Root tips were collected from plants cultivated at the Munich Botanical Garden and immediately transferred to 2 mmol/L 8-hydroxyquinoline to arrest metaphases, then fixed in 3c:c1 ethanol–acetic acid, and stored until use at −20°C. Chromosome spreads were obtained after hydrolysis in 0.5 N hydrochloric acid at 60°C for 10 min and staining with a saturated orcein-glacial acetic acid solution. Our sampling includes species from all groups (genera) embedded in *Echinopsis* or newly revealed as closely related by our molecular data.

***Pollination modes and growth forms*—**All species sequenced were assigned to one or several pollinator groups (bee, hummingbird, bat, or sphingid) on the basis of either our own field observations or inferred from combinations of floral traits, such as anthesis time, floral bauplan (i.e., mostly shape and length of the hypanthium), and nectar production. For growth form, we assigned species to one of two types, globular growth or columnar growth.

RESULTS

***Relative frequencies of changes in pollination mode, growth architecture, and chromosome number*—**We generated 457 new chloroplast sequences (Table 2). The concatenated aligned matrix comprised 3866 nucleotides. Maximum likelihood analysis of these data yielded the tree shown in Fig. 1A and B, which also shows growth form (categorized as globular or columnar), pollination mode, and chromosome number. Inspection of the tree reveals many more changes in pollination modes than in growth form, and even though chromosome counts are not available for all species, polyploidy (tetraploidy) appears common in the *Oreocereus* clade and in *Weberbauerocereus*, and rare elsewhere (Fig. 1, Table 3; more about chromosome numbers in the next section). Major clades were named using oldest available genus names.

A monophyletic *Echinopsis* would need to include *Acanthocalycium* Backeb., *Arthrocereus* A.Berger, *Cephalocleistocactus* F.Ritter, *Cleistocactus* Lem. (including *Borzicactus*

TABLE 2. Species and loci sequenced newly for this study, their sources and provenance, voucher informations, herbarium code, and GenBank accession numbers. Generic types are indicated (GT) and synonymous names are given where they are still widely used.

Species	DNA source	Geographic origin of sample or distribution	*trnS-trnG* intergenic spacer	*trnL-trnF* intergenic spacer	*rpl16* intron
Acanthocalycium ferrarii Rausch	B.O.Schlumpberger 482 (CORD)	Tucuman, Argentina*	JQ779438	JQ779600	JQ779744
Acanthocalycium glaucum F.Ritter	B.O.Schlumpberger x08 (M) J. & B.Piltz 394	Catamarca, Argentina**	JQ779439	JQ779601	JQ779745
Acanthocalycium spiniflorum (Schumann) Backeb., GT	B.O.Schlumpberger x07 (M) J. & B.Piltz 204	Córdoba, Argentina**	JQ779441	JQ779603	JQ779747
Acanthocalycium spiniflorum (Schumann) Backeb., GT	B.O.Schlumpberger 323 (CORD)	Córdoba, Argentina*	JQ779440	JQ779602	JQ779746
Acanthocalycium thionanthum (Spegazzini) Backeb.	B.O.Schlumpberger 523 (CORD)	Salta, Argentina*	JQ779437	JQ779599	JQ779743
Arthrocereus rondonianus Backeb. & Voll	B.O.Schlumpberger x102 (M) L.Horst 145	Minas Gerais, Brazil**	JQ779506	JQ779668	JQ779804
Arthrocereus spinosissimus (Buining & Brederoo) F.Ritter	B.O.Schlumpberger x103 (M) L.Horst 328	Mato Grosso, Brazil**	JQ779505	JQ779667	JQ779803
Browningia hertlingiana (Backeb.) Buxb.	B.O.Schlumpberger x101 (M)	Southern Peru	JQ779526	JQ779688	JQ779822
Cephalocleistocactus chrysocephalus F.Ritter, GT	B.O.Schlumpberger x111 (M)	La Paz, Bolivia	JQ779507	JQ779669	JQ779805
Cereus aethiops Haw.	B.O.Schlumpberger 330 (CORD)	San Luis, Argentina*	JQ779503	JQ779665	JQ779801
Cleistocactus baumannii Lem., GT	B.O.Schlumpberger 597 (M)	Córdoba, Argentina*	JQ779500	JQ779662	
Cleistocactus sepium (Kunth) F.A.C.Weber ex Rol.-Goss. (=*Borzicactus sepium* (Kunth) Britton & Rose)	B.O.Schlumpberger x117 (M)	North & central Ecuador	JQ779463	JQ779625	JQ779768
Cleistocactus sextonianus (Backeb.) D.R.Hunt (=*Borzicactus sextonianus* (Backeb.) Kimnach)	B.O.Schlumpberger x61 (M)	South & central Peru	JQ779462	JQ779624	JQ779767
Cleistocactus smaragdiflorus (F.A.C.Weber) Britton & Rose	B.O.Schlumpberger 424 (M)	Catamarca, Argentina*	JQ779499	JQ779661	JQ779798
Denmoza rhodacantha (Salm-Dyck) Britton & Rose, GT	B.O.Schlumpberger 366 (CORD)	La Rioja, Argentina*	JQ779446	JQ779608	JQ779752
Discocactus zehntneri subsp. *buenekeri* (W.R.Abraham) P.J.Braun & Esteves (incl. *D. zehntneri* subsp. *boomianus* Buining & Brederoo) N.P.Taylor & Zappi)	cult 08/2533 B.O.Schlumpberger x113 BGM	Bahia, Brazil	JQ779511	JQ779673	JQ779809
Echinopsis ancistrophora Speg.	B.O.Schlumpberger 152 (CORD)	Salta, Argentina*	JQ779367	JQ779529	JQ779691
Echinopsis ancistrophora Speg.	B.O.Schlumpberger 96 (CORD, M)	Jujuy, Argentina*	JQ779368	JQ779530	JQ779692
Echinopsis cf. *ancistrophora* Speg.	B.O.Schlumpberger x133 (M)	unknown	JQ779369	JQ779531	JQ779693
Echinopsis angelesiae (R.Kiesling) G.D.Rowley	B.O.Schlumpberger 383 (CORD)	Salta, Argentina*	JQ779413	JQ779575	JQ779725
Echinopsis arachnacantha (Buining & F.Ritter) H.Friedrich	B.O.Schlumpberger 656 (LPB)	Santa Cruz, Bolivia*	JQ779400	JQ779562	JQ779714
Echinopsis atacamensis subsp. *pasacana* (F.A.C.Weber) G.Navarro	B.O.Schlumpberger 518 (CORD)	Catamarca, Argentina*	JQ779486	JQ779648	
Echinopsis atacamensis subsp. *pasacana* (F.A.C.Weber) G.Navarro	B.O.Schlumpberger 593 (CORD)	Tucuman, Argentina*	JQ779487	JQ779649	JQ779787
Echinopsis aurea Britton & Rose	B.O.Schlumpberger 309 (CORD)	Córdoba, Argentina*	JQ779472	JQ779634	JQ779776
Echinopsis aurea Britton & Rose	B.O.Schlumpberger 313 (CORD)	Córdoba, Argentina*	JQ779471	JQ779633	JQ779775
Echinopsis aurea Britton & Rose	B.O.Schlumpberger x29 (M) W.Rausch 116 (*E. leucomalla* (Wessner) H.Friedrich)	San Luis, Argentina**	JQ779475	JQ779637	
Echinopsis aurea subsp. *fallax* (Oehme) M.Lowry	B.O.Schlumpberger x05 (M) J. & B.Piltz 137	La Rioja, Argentina**	JQ779474	JQ779636	
Echinopsis aurea subsp. *shaferi* (Britton & Rose) M.Lowry	B.O.Schlumpberger x100 (M) C.Hunkeler 300	Catamarca, Argentina**	JQ779473	JQ779635	JQ779777
E. aurea var. *callochrysea* (F.Ritter) J.Ullmann	B.O.Schlumpberger 384 (CORD)	Salta, Argentina*	JQ779476	JQ779638	JQ779778
Echinopsis backebergii Werderm.	B.O.Schlumpberger x33 (M) F.Ritter 1312 (*Lobivia winteriana* F.Ritter)	Huancavelica, Peru**	JQ779386	JQ779548	JQ779703
Echinopsis backebergii Werderm.	B.O.Schlumpberger x65 (M) W.Rausch 407 (*Lobivia zecheri* Rausch)	Ayacucho, Peru**	JQ779387	JQ779549	
Echinopsis bonnieae (Halda, Hogan & Janeba) Halda & Malina	B.O.Schlumpberger x127 (M)	Catamarca, Argentina	JQ779502	JQ779664	JQ779800
Echinopsis boyuibensis F.Ritter	B.O.Schlumpberger 301 (M)	Santa Cruz, Bolivia*	JQ779371	JQ779533	
Echinopsis breviflora (Backeb.) M.Lowry (= *Lobivia sanguiniflora* var. *breviflora* (Backeb.) Rausch)	B.O.Schlumpberger x80 (M)	Salta, Argentina	JQ779469	JQ779631	JQ779773

TABLE 2. Continued.

Species	DNA source	Geographic origin of sample or distribution	*trnS-trnG* intergenic spacer	*trnL-trnF* intergenic spacer	*rpl16* intron
Echinopsis bridgesii subsp. *vallegrandensis* (Cárdenas) M.Lowry	B.O.Schlumpberger 657 (LPB)	Cochabamba, Bolivia	JQ779403	JQ779565	JQ779717
Echinopsis bridgesii subsp. *vallegrandensis* (Cárdenas) M.Lowry	B.O.Schlumpberger 691 (LPB)	Santa Cruz, Bolivia*	JQ779404	JQ779566	JQ779718
Echinopsis bruchii (Britton & Rose) A.Cast. & H.V.Lelong	B.O.Schlumpberger x106 (M)	Northwestern Argentina	JQ779422	JQ779584	JQ779731
Echinopsis caineana (Cárdenas) D.R.Hunt	B.O.Schlumpberger x125 (M) W.Rausch 197	Cochabamba, Bolivia**	JQ779402	JQ779564	JQ779716
Echinopsis calliantholilacina Cárdenas	B.O.Schlumpberger x13 (M) W.Rausch 63	Chuquisaca, Bolivia**	JQ779381	JQ779543	
Echinopsis callichroma Cárdenas	B.O. Schlumpberger x24 (M) W.Rausch 461	Cochabamba, Bolivia**	JQ779380	JQ779542	
Echinopsis calochlora K.Schum.	B.O.Schlumpberger x10 (M)	Southwestern Brazil, east Bolivia	JQ779485	JQ779647	JQ779786
Echinopsis calorubra Cárdenas	B.O.Schlumpberger x90 (M)	South-central Bolivia	JQ779382	JQ779544	JQ779699
Echinopsis calorubra Cárdenas	B.O.Schlumpberger x128 (M) W.Rausch 463 (*Lobivia mizquensis* Rausch)	Cochabamba, Bolivia**	JQ779383	JQ779545	JQ779700
Echinopsis candicans (Gillies ex Salm-Dyck) D.R.Hunt	B.O.Schlumpberger 348C (CORD)	Córdoba, Argentina*	JQ779411	JQ779573	JQ779723
Echinopsis candicans (Gillies ex Salm-Dyck) D.R.Hunt	B.O.Schlumpberger 332 (CORD)	San Luis, Argentina*	JQ779412	JQ779574	JQ779724
Echinopsis cardenasiana (Rausch) H.Friedrich	B.O.Schlumpberger x116 (M) W.Rausch 498	Tarija, Bolivia**	JQ779370	JQ779532	
Echinopsis chamaecereus H.Friedrich & Glaetzle (= *Chamaecereus silvestrii* (Speg.) Britton & Rose)	B.O.Schlumpberger x37 (M)	Supposedly Salta/ Tucuman, Argentina	JQ779434	JQ779596	
Echinopsis chiloensis (Colla) H.Friedrich & G.D.Rowley	B.O.Schlumpberger x77 (M)	Chile*	JQ779489	JQ779651	JQ779789
Echinopsis chrysantha Werderm.	B.O.Schlumpberger x83 (M) W.Rausch 5	Salta, Argentina**	JQ779470	JQ779632	JQ779774
Echinopsis chrysochete Werderm.	B.O.Schlumpberger 576A (CORD)		JQ779394	JQ779556	
Echinopsis chrysochete Werderm.	B.O.Schlumpberger 577 (CORD) (*Lobivia chrysochete* var. *minutiflora* (Rausch))	Salta, Argentina*	JQ779395	JQ779557	JQ779709
Echinopsis cinnabarina (Hook.) Labor.	B.O.Schlumpberger 706 (LPB)	Santa Cruz, Bolivia*	JQ779390	JQ779552	JQ779706
Echinopsis coronata Cárdenas	V.Beyer & B.O.Schlumpberger x30 (M) W.Rausch 613	Santa Cruz, Bolivia**	JQ779372	JQ779534	
Echinopsis crassicaulis (Backeb. ex R.Kiesling) H.Friedrich & Glaetzle	B.O.Schlumpberger x23 (M)	Catamarca, Argentina	JQ779418	JQ779580	
Echinopsis densispina Werderm.	cult N/2956 B.O.Schlumpberger x107 BGM	Salta, Argentina**	JQ779482	JQ779644	JQ779784
Echinopsis famatimensis (Speg.) Werderm.	B.O.Schlumpberger x48 (M)	La Rioja, San Juan, Argentina	JQ779501	JQ779663	JQ779799
Echinopsis ferox (Britton & Rose) Backeb.	B.O.Schlumpberger 563 (CORD)	Jujuy, Argentina*	JQ779384	JQ779546	JQ779701
Echinopsis formosa (Pfeiff.) Jacobi ex Salm-Dyck subsp. *korethroides* (Werderm.) M.Lowry	B.O.Schlumpberger 390 (CORD)	Salta, Argentina*	JQ779420	JQ779582	
Echinopsis formosa (Pfeiff.) Jacobi ex Salm-Dyck	B.O.Schlumpberger 460 (CORD)	La Rioja, Argentina*	JQ779420	JQ779583	JQ779730
Echinopsis haematantha (Speg.) D.R.Hunt	B.O.Schlumpberger 175 (CORD)	Salta, Argentina*	JQ779480	JQ779642	JQ779782
Echinopsis haematantha (Speg.) D.R.Hunt	B.O.Schlumpberger 493 (CORD)	Salta, Argentina*	JQ779481	JQ779643	JQ779783
Echinopsis hahniana (Backeb.) R.S.Wallace	B.O.Schlumpberger x85 (M)	Northeast Paraguay	JQ779426	JQ779588	JQ779734
Echinopsis hertrichiana (Backeb.) D.R.Hunt	B.O.Schlumpberger x34 (M) W.Rausch 414	Cuzco, Peru**	JQ779396	JQ779558	JQ779710
Echinopsis huascha (F.A.C.Weber) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 374 (CORD)	La Rioja, Argentina*	JQ779409	JQ779571	JQ779721
Echinopsis huascha (F.A.C.Weber) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 351 (CORD)	La Rioja, Argentina*	JQ779408	JQ779570	
Echinopsis huascha subsp. *robusta* (Rausch) M.Lowry	B.O.Schlumpberger 519 (CORD)	Catamarca, Argentina*	JQ779410	JQ779572	JQ779722
Echinopsis jajoiana (Backeb.) Blossf.	B.O.Schlumpberger x88 (M) W.Rausch 213	Jujuy, Argentina**	JQ779467	JQ779629	JQ779771
Echinopsis jajoiana (Backeb.) Blossf.	B.O.Schlumpberger x79 (M) W.Rausch 815 (*Lobivia jajoiana* var. *fleischeriana* Backeb.)	Jujuy, Argentina**	JQ779468	JQ779630	JQ779772
Echinopsis lageniformis (C.F.Först.) H.Friedrich & G.D.Rowley	B.O.Schlumpberger x39 (M)	Bolivia	JQ779449	JQ779611	JQ779755

TABLE 2. Continued.

Species	DNA source	Geographic origin of sample or distribution	*trnS-trnG* intergenic spacer	*trnL-trnF* intergenic spacer	*rpl16* intron
Echinopsis lamprochlora (Lem.) F.A.C.Weber ex H.Friedrich & Glaetzle	B.O.Schlumpberger 318 (CORD)	Córdoba, Argentina*	JQ779416	JQ779578	JQ779728
Echinopsis lateritia Gürke	B.O.Schlumpberger x126 (M) W.Rausch 490	Chuquisaca, Bolivia**	JQ779398	JQ779560	JQ779712
Echinopsis leucantha (Gillies ex Salm-Dyck) Walp.	B.O.Schlumpberger 324 (CORD)	Córdoba, Argentina*	JQ779443	JQ779605	JQ779749
Echinopsis leucantha (Gillies ex Salm-Dyck) Walp.	B.O.Schlumpberger 329 (CORD)	San Luis, Argentina*	JQ779444	JQ779606	JQ779750
Echinopsis leucantha (Gillies ex Salm-Dyck) Walp.	B.O.Schlumpberger 382C (CORD)	Catamarca, Argentina*	JQ779442	JQ779604	JQ779748
Echinopsis lobivioides Backeb.	B.O.Schlumpberger 422 (CORD)	Catamarca, Argentina*	JQ779419	JQ779581	JQ779729
Echinopsis mamillosa Gürke	B.O.Schlumpberger x38 (M)	South Bolivia	JQ779401	JQ779563	JQ779715
Echinopsis marsoneri Werderm.	B.O.Schlumpberger x94 (M) D.Hardy 373	Jujuy, Argentina**	JQ779466	JQ779628	JQ779770
Echinopsis maximiliana Heyder ex A.Dietr.	B.O.Schlumpberger 621A (LPB)	La Paz, Bolivia*	JQ779391	JQ779553	JQ779707
Echinopsis mirabilis Speg.	V.Beyer & B.O.Schlumpberger x35 (M)	Dry Chaco, Argentina	JQ779445	JQ779607	JQ779751
Echinopsis obrepanda (Salm-Dyck) K.Schum.	B.O.Schlumpberger x132 (M)	Bolivia	JQ779376	JQ779538	JQ779695
Echinopsis obrepanda (Salm-Dyck) K.Schum.	B.O.Schlumpberger 629A (LPB)	Cochabamba, Bolivia*	JQ779377	JQ779539	JQ779696
Echinopsis obrepanda K.Schum.	B.O.Schlumpberger 686A (LPB)	Santa Cruz, Bolivia*	JQ779375	JQ779537	JQ779694
Echinopsis obrepanda K.Schum.	B.O.Schlumpberger 713A (LPB)	Santa Cruz, Bolivia*	JQ779379	JQ779541	JQ779698
Echinopsis oligotricha (Cárdenas) M.Lowry	B.O.Schlumpberger 638 (LPB)	Cochabamba, Bolivia*	JQ779397	JQ779559	JQ779711
Echinopsis oxygona (Link) Zucc.	B.O.Schlumpberger x01 (M) N.Gerloff 114	Rio Grande do Sul, Brazil**	JQ779483	JQ779645	
Echinopsis oxygona (Link) Zucc.	B.O.Schlumpberger x06 (M) L.Bercht 892	Rio Grande do Sul, Brazil**	JQ779484	JQ779646	JQ779785
Echinopsis cf. *pachanoi* (Britton & Rose) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 721 (LPB)	Santa Cruz, Bolivia*	JQ779451	JQ779613	JQ779757
Echinopsis pachanoi (Britton & Rose) H.Friedrich & G.D.Rowley	B.O.Schlumpberger x78 (M)	Ecuador, Peru	JQ779450	JQ779612	JQ779756
Echinopsis pentlandii Salm-Dyck	B.O.Schlumpberger 622 (LPB)	La Paz, Bolivia*	JQ779388	JQ779550	JQ779704
Echinopsis pereziensis Cárdenas	B.O.Schlumpberger x95 (M) K.Knize 933	Santa Cruz, Bolivia	JQ779406	JQ779568	JQ779719
Echinopsis pojoensis Cárdenas	B.O.Schlumpberger x32 (M)	Cochabamba, Bolivia	JQ779374	JQ779536	
Echinopsis pugionacantha Rose & Boed.	B.O.Schlumpberger 566 (CORD)	Jujuy, Argentina*	JQ779385	JQ779547	JQ779702
Echinopsis rojasii Cárdenas	B.O.Schlumpberger 647 (LPB)	Santa Cruz, Bolivia*	JQ779378	JQ779540	JQ779697
Echinopsis rowleyi H.Friedrich (= *Lobivia grandiflora* Britton & Rose)	B.O.Schlumpberger 391 (CORD)	Catamarca, Argentina*	JQ779417	JQ779579	
Echinopsis saltensis Speg.	B.O.Schlumpberger x50 (M)	Salta & Tucuman, Argentina	JQ779435	JQ779597	JQ779741
Echinopsis saltensis Speg.	B.O.Schlumpberger 163B (CORD)	Salta, Argentina*	JQ779436	JQ779598	JQ779742
Echinopsis schickendantzii F.A.C.Weber	B.O.Schlumpberger 538 (CORD)	Jujuy, Argentina*	JQ779423	JQ779585	
Echinopsis schieliana F.A.C.Weber	B.O.Schlumpberger x124 (M) W.Rausch 205 (*Lobivia schieliana* var. *quiabayensis* (Rausch) Rausch)	La Paz, Bolivia**	JQ779392	JQ779554	JQ779708
Echinopsis schreiteri (A.Cast.) Werderm.	B.O.Schlumpberger 227 (CORD)	Tucuman, Argentina*	JQ779433	JQ779595	JQ779740
Echinopsis smrziana Backeb.	B.O.Schlumpberger x02 (M) J. & B.Piltz 177A	Salta, Argentina**	JQ779432	JQ779594	JQ779739
Echinopsis sp.	B.O.Schlumpberger 652 (LPB)	Santa Cruz, Bolivia*	JQ779754	JQ779448	JQ779610
Echinopsis sp.	B.O.Schlumpberger 717 (LPB)	Cochabamba, Bolivia*	JQ779753	JQ779447	JQ779609
Echinopsis strigosa (Salm-Dyck) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 343 (CORD)	La Rioja, Argentina*	JQ779414	JQ779576	JQ779726
Echinopsis subdenudata Cárdenas	B.O.Schlumpberger x25 (M)	Tarija, Bolivia	JQ779373	JQ779535	
Echinopsis sucrensis Cárdenas	B.O.Schlumpberger x41 (M) W.Rausch 483	Potosí, Bolivia**	JQ779405	JQ779567	
Echinopsis tacaquirensis (Vaupel) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 118 (CORD, M)	Potosí, Bolivia*	JQ779407	JQ779569	JQ779720
Echinopsis tarijensis (Vaupel) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 562 (CORD)	Jujuy, Argentina*	JQ779430	JQ779592	JQ779738
Echinopsis tarijensis subsp. *bertramiana* (Backeb.) M.Lowry	B.O.Schlumpberger 631 (LPB)	Cochabamba, Bolivia*	JQ779428	JQ779590	JQ779736
Echinopsis tegeleriana (Backeb.) D.R.Hunt	B.O.Schlumpberger x66 (M) W.Rausch 443	Ayacucho, Peru**	JQ779393	JQ779555	
Echinopsis terscheckii (Parmentier ex Pfeiff.) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 354 (CORD)	La Rioja, Argentina*	JQ779488	JQ779650	JQ779788

TABLE 2. Continued.

Species	DNA source	Geographic origin of sample or distribution	*trnS-trnG* intergenic spacer	*trnL-trnF* intergenic spacer	*rpl16* intron
Echinopsis thelegona (F.A.C.Weber) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 208 (CORD, M)	Salta, Argentina*	JQ779415	JQ779577	JQ779727
Echinopsis thelegonoides (Speg.) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 543 (CORD)	Jujuy, Argentina*	JQ779427	JQ779589	JQ779735
Echinopsis tiegeliana (Wessner) D.R.Hunt	V.Beyer & B.O.Schlumpberger x82 (M) W.Rausch 323	Tarija, Bolivia**	JQ779389	JQ779551	JQ779705
Echinopsis tubiflora (Pfeiff.) Zucc. ex K.Schum.	B.O.Schlumpberger 106 (CORD)	Tucuman, Argentina*	JQ779479	JQ779641	JQ779781
Echinopsis tubiflora (Pfeiff.) Zucc. ex K.Schum.	B.O.Schlumpberger 386 (CORD)	Salta, Argentina*	JQ779477	JQ779639	JQ779779
Echinopsis tubiflora (Pfeiff.) Zucc. ex K.Schum.	B.O.Schlumpberger 387 (CORD)	Salta, Argentina*	JQ779478	JQ779640	JQ779780
Echinopsis vasquezii (Rausch) G.D.Rowley	B.O.Schlumpberger 722 (LPB)	Santa Cruz, Bolivia*	JQ779425	JQ779587	JQ779733
Echinopsis vasquezii (Rausch) G.D.Rowley	B.O.Schlumpberger x86 (M)	Cochabamba/ Santa Cruz, Bolivia	JQ779424	JQ779586	JQ779732
Echinopsis volliana (Backeb.) H.Friedrich & G.D.Rowley	B.O.Schlumpberger 635 (LPB)	Cochabamba, Bolivia*	JQ779429	JQ779591	JQ779737
Echinopsis walteri R.Kiesling (H.Friedrich & Glaetzle)	B.O.Schlumpberger x11 (M) J. & B.Piltz 177	Salta, Argentina**	JQ779431	JQ779593	
Echinopsis yuquina D.R.Hunt (incl. *Lobivia rauschii* Zecher)	B.O.Schlumpberger x81 (M) F.Ritter 807	Chuquisaca, Bolivia**	JQ779399	JQ779561	JQ779713
Espostoa guentheri (Kupper) Buxb. ex Eggli (= *Vatricania guentheri* (Kupper) Backeb., GT)	B.O.Schlumpberger x70 (M)	Central Bolivia	JQ779495	JQ779657	JQ779795
Espostoa lanata (Kunth) Britton & Rose, GT	B.O.Schlumpberger x54 (M)	North Peru, south Ecuador	JQ779454	JQ779616	JQ779760
Espostoopsis dybowskii (Rol.-Goss.) Buxb., GT	B.O.Schlumpberger x112 (M)	Bahia, Brazil	JQ779509	JQ779671	JQ779807
Facheiroa ulei (Gürke) Werderm., GT	B.O.Schlumpberger x109 (M)	Bahia, Brazil	JQ779527	JQ779689	
Gymnocalycium andreae (Boed.) Backeb.	B.O.Schlumpberger x84 (M)	Córdoba & San Luis, Argentina	JQ779512	JQ779674	JQ779810
Gymnocalycium mostii (Gürke) Britton & Rose	B.O.Schlumpberger 305 (CORD)	Córdoba, Argentina*	JQ779513	JQ779675	JQ779811
Haageocereus pseudomelanostele (Werderm. & Backeb.) Backeb., GT	B.O.Schlumpberger x56 (M)	Central Peru	JQ779456	JQ779618	JQ779762
Harrisia gracilis (Miller) Britton, GT	B.O.Schlumpberger x120 (M)	Jamaica	JQ779492	JQ779654	JQ779792
Harrisia martinii (Labor.) Britton & Rose	B.O.Schlumpberger x58 (M)	Argentina, Paraguay	JQ779493	JQ779655	JQ779793
Harrisia pomanensis (F.A.C.Weber) Britton & Rose	B.O.Schlumpberger 119 (CORD)	Salta, Argentina*	JQ779491	JQ779653	JQ779791
Harrisia tetracantha (Labor.) D.R.Hunt	B.O.Schlumpberger 643 (LPB)	Cochabamba, Bolivia*	JQ779490	JQ779652	JQ779790
Harrisia tortuosa (J.Forbes ex Otto & A.Dietr.) Britton & Rose	B.O.Schlumpberger x53 (M)	Argentina, Uruguay, Paraguay; Bolivia?	JQ779494	JQ779656	JQ779794
Lasiocereus fulvus F.Ritter	B.O.Schlumpberger x59 (M)	Amazonas, Peru	JQ779525	JQ779687	JQ779821
Leocereus bahiensis Britton & Rose, GT	Eggli 1283 (ZSS)	Bahia, Brazil*	JQ779510	JQ779672	JQ779808
Matucana haynei (Otto ex Salm-Dyck) Britton & Rose, GT	B.O.Schlumpberger x118 (M)	Peru	JQ779460	JQ779622	JQ779765
Matucana haynei subsp. *hystrix* (Rauh & Backeb.) Mottram (incl. *Matucana multicolor* Rauh & Backeb.)	B.O.Schlumpberger x119 (M)	Peru	JQ779459	JQ779621	
Matucana intertexta F.Ritter	B.O.Schlumpberger x60 (M)	Peru	JQ779458	JQ779620	JQ779764
Micranthocereus densiflorus Buining & Brederoo	B.O.Schlumpberger x69 (M)	Bahia, Brazil	JQ779524	JQ779686	JQ779820
Mila caespitosa Britton & Rose, GT	B.O.Schlumpberger x47 (M)	Central Peru	JQ779457	JQ779619	JQ779763
Neowerdermannia vorwerkii Frič, GT	B.O.Schlumpberger x74 (M)	Northern Argentina & Bolivia	JQ779528	JQ779690	JQ779823
Oreocereus celsianus (Lem. ex Salm-Dyck) Riccob., GT	B.O.Schlumpberger 569 (CORD)	Jujuy, Argentina*	JQ779464	JQ779626	JQ779769
Oreocereus trollii (Kupper) Backeb.	B.O.Schlumpberger 559 (CORD)	Jujuy, Argentina*	JQ779465	JQ779627	
Oroya peruviana (K.Schum.) Britton & Rose, GT	B.O.Schlumpberger x99 (M)	Central Peru	JQ779461	JQ779623	JQ779766
Praecereus euchlorus F.A.C.Weber (N.P.Taylor)	B.O.Schlumpberger 700 (LPB)	Santa Cruz, Bolivia*	JQ779504	JQ779666	JQ779802
Pygmaeocereus bieblii var. *kuehhasii* Diers	B.O.Schlumpberger x46 (M)	Central Peru	JQ779452	JQ779614	JQ779758
Pygmaeocereus bylesianus Andreae & Backeb., GT	B.O.Schlumpberger x115 (M)	Southern Peru	JQ779453	JQ779615	JQ779759
Rauhocereus riosaniensis Backeb., GT	B.O.Schlumpberger x110 (M)	Northern Peru	JQ779455	JQ779617	JQ779761

TABLE 2. Continued.

Species	DNA source	Geographic origin of sample or distribution	*trnS-trnG* intergenic spacer	*trnL-trnF* intergenic spacer	*rpl16* intron
Rebutia arenacea Cárdenas (= *Sulcorebutia arenacea* (Cárdenas) F.Ritter)	B.O.Schlumpberger x76 (M)	Cochabamba, Bolivia	JQ779522	JQ779684	
Rebutia atrovirens (Backeb.) Pilbeam (= *Mediolobivia atrovirens* (Backeb.) Backeb., incl. *Rebutia pygmaea* (R.E.Fr.) Britton & Rose)	B.O.Schlumpberger x71 (M) H.Joschko 75	Jujuy, Argentina**	JQ779517	JQ779679	JQ779815
Rebutia fabrisii Rausch	B.O.Schlumpberger x104 (M) W.Rausch 688	Salta, Argentina**	JQ779516	JQ779678	JQ779814
Rebutia haagei Fric & Schelle (incl. *Mediolobivia pygmaea* (R.E.Fr.) Krainz, *Rebutia pygmaea* (R.E.Fr.) Britton & Rose)	B.O.Schlumpberger x75 (M) W.Rausch 333	Jujuy, Argentina**	JQ779519	JQ779681	JQ779817
Rebutia heliosa Rausch	B.O.Schlumpberger x97 (M) A.B.Lau 405	Tarija, Bolivia**	JQ779520	JQ779682	JQ779818
Rebutia margarethae Rausch (*R. padcayensis* Rausch)	B.O.Schlumpberger x73 (M) A.B.Lau 550	Jujuy/Salta, Argentina**	JQ779514	JQ779676	JQ779812
Rebutia minuscula K.Schum., GT	B.O.Schlumpberger x108 (M)	Northern Argentina	JQ779515	JQ779677	JQ779813
Rebutia muscula F.Ritter & Thiele ex F.Ritter (= *Aylostera muscula* (F.Ritter & Thiele ex F.Ritter) Backeb.; incl. *Rebutia fiebrigii* (Gürke) Britton & Rose)	B.O.Schlumpberger x98 (M) F.Ritter 753	Tarija, Bolivia**	JQ779521	JQ779683	JQ779819
Rebutia neocumingii (Backeb.) D.R.Hunt (*Weingartia neocumingii* Backeb.)	B.O.Schlumpberger x72 (M)	Bolivia, south-central Andes	JQ779523	JQ779685	
Rebutia pygmaea (R.E.Fr.) Britton & Rose (= *Mediolobivia pygmaea* (R.E.Fr.) Krainz)	B.O.Schlumpberger & G.Brokamp 670 (M)	Oruro, Bolivia*	JQ779518	JQ779680	JQ779816
Samaipaticereus corroanus Cárdenas, GT	B.O.Schlumpberger 720 (LPB)	Santa Cruz, Bolivia*	JQ779498	JQ779660	
Stetsonia coryne (Salm-Dyck) Britton & Rose, GT	B.O.Schlumpberger x57 (M)	Chaco of Argentina, Bolivia and Paraguay	JQ779508	JQ779670	JQ779806
Weberbauerocereus weberbaueri (K.Schum. ex Vaupel) Backeb., GT	B.O.Schlumpberger x55 (M)	Southwestern Peru	JQ779496	JQ779658	JQ779796
Yungasocereus inquisivensis (Cárdenas) F.Ritter ex Eggli, GT	B.O.Schlumpberger x121 (M)	Bolivia	JQ779497	JQ779659	JQ779797

Notes: * wild-collected, ** samples from cultivated plants of documented origin

Riccob.), *Denmoza* Britton & Rose, *Espostoa* Britton & Rose (including *Vatricania* Backeb.), *Haageocereus* Backeb., *Harrisia* Britton, *Matucana* Britton & Rose, *Mila* Britton & Rose, *Oreocereus* (A.Berger) Riccob., *Oroya* Britton & Rose, *Pygmaeocereus* H.Johnson & Backeb., *Rauhocereus* Backeb., *Samaipaticereus* Cárdenas, *Weberbauerocereus* Backeb., and *Yungasocereus* F.Ritter, all of which are part of a well-supported clade (100% bootstrap support) interspersed with species of *Echinopsis* (Fig. 1A, B). Species previously assigned to *Lobivia*, i.e., species with diurnal flowers, *Trichocereus*, i.e., columnar species, or *Echinopsis* s.str., i.e., globular species with funnel-shaped flowers of nocturnal anthesis, do not form clades. The genera *Espostoa*, *Cleistocactus* s.l. (i.e., sensu Hunt, 1999, 2006) and *Matucana* are polyphyletic (Fig. 1), and *Acanthocalycium* is paraphyletic since *Echinopsis leucantha* is embedded among its species. Our sampling also reveals a few taxonomic problems at the species level: thus, *E. aurea* is polyphyletic, with the isolated northernmost form (*E. aurea* var. *callochrysea*) close to *E. tubiflora*.

Chromosome numbers— Table 3 lists new chromosome counts for numerous *Echinopsis* (sub) species or varieties included in the phylogeny as well as for 11 outgroup species from eight genera. The basic chromosome number is $2x = 22$. We discovered six cases of tetraploidy of which two involve intraspecific variation between diploidy and tetraploidy. In the *Helianthocereus* clade, tetraploids occurred in *E. thelegonoides* and *E. candicans* (Fig. 1B). In the *Lobivia* clade, we found diploids and tetraploids in *E. tiegeliana*, and in the *Oreocereus* clade we found tetraploids in *Espostoa* and *Oreocereus* itself (Fig. 1, Table 3). In *Rebutia* K.Schum., we found tetraploidy in a species previously assigned to *Mediolobivia* Backeb.

DISCUSSION

We now address the main questions posed in this study, namely (1) Is *Echinopsis* in the broad sense monophyletic? (2) What is the level of plasticity of growth habit and pollination mode, characters that have been used to define clades in *Echinopsis* sensu lato? And (3) How common are ploidy changes in the *Echinopsis* alliance and are they clustered in certain subclades?

Monophyly of Echinopsis sensu lato—*Echinopsis* as circumscribed by Anderson (2001) or Hunt (2006) would become monophyletic with the inclusion of *Acanthocalycium* (part of *Echinopsis* in Hunt, 2006, but not in Anderson, 2001 and 2005), *Arthrocereus*, *Cephalocleistocactus*, *Cleistocactus* (including *Borzicactus*), *Denmoza*, *Espostoa* (including *Vatricania*), *Haageocereus*, *Harrisia*, *Matucana*, *Mila*, *Oreocereus*,

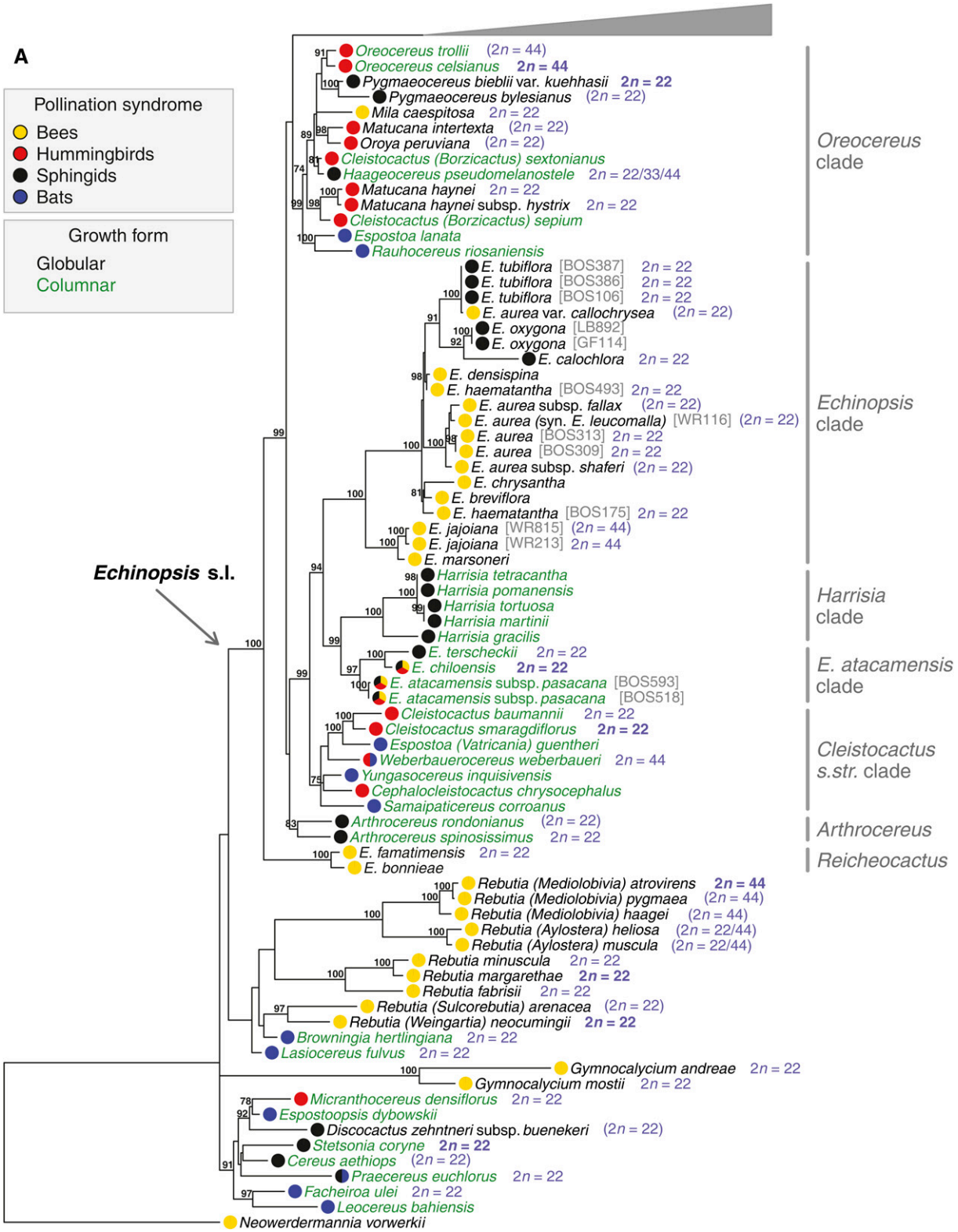

Fig. 1. (A, B). Maximum likelihood (ML) phylogram for 144 taxa of *Echinopsis* and relatives obtained from 3866 aligned nucleotides of chloroplast DNA. Numbers at nodes indicate ML bootstrap support >70% from 100 replicates. Known or putative pollinators are indicated by pie diagrams, with the color codes explained in the inset; sphingid-adapted taxa with bees as backup pollinators are treated as sphingid-pollinated. Growth form is indicated by different taxon name colors as explained in the inset. New or previously published chromosome counts are shown as follows: Bold numbers indicate counts that were made from the same plant as was sequenced; numbers in parentheses refer to counts made from a closely related taxon; the remaining numbers refer to counts from the same taxon but not the sequenced individual (see Table 2 for sources). Collection numbers behind plant names refer to vouchers cited in Table 1; they are given only for species represented by multiple accessions.

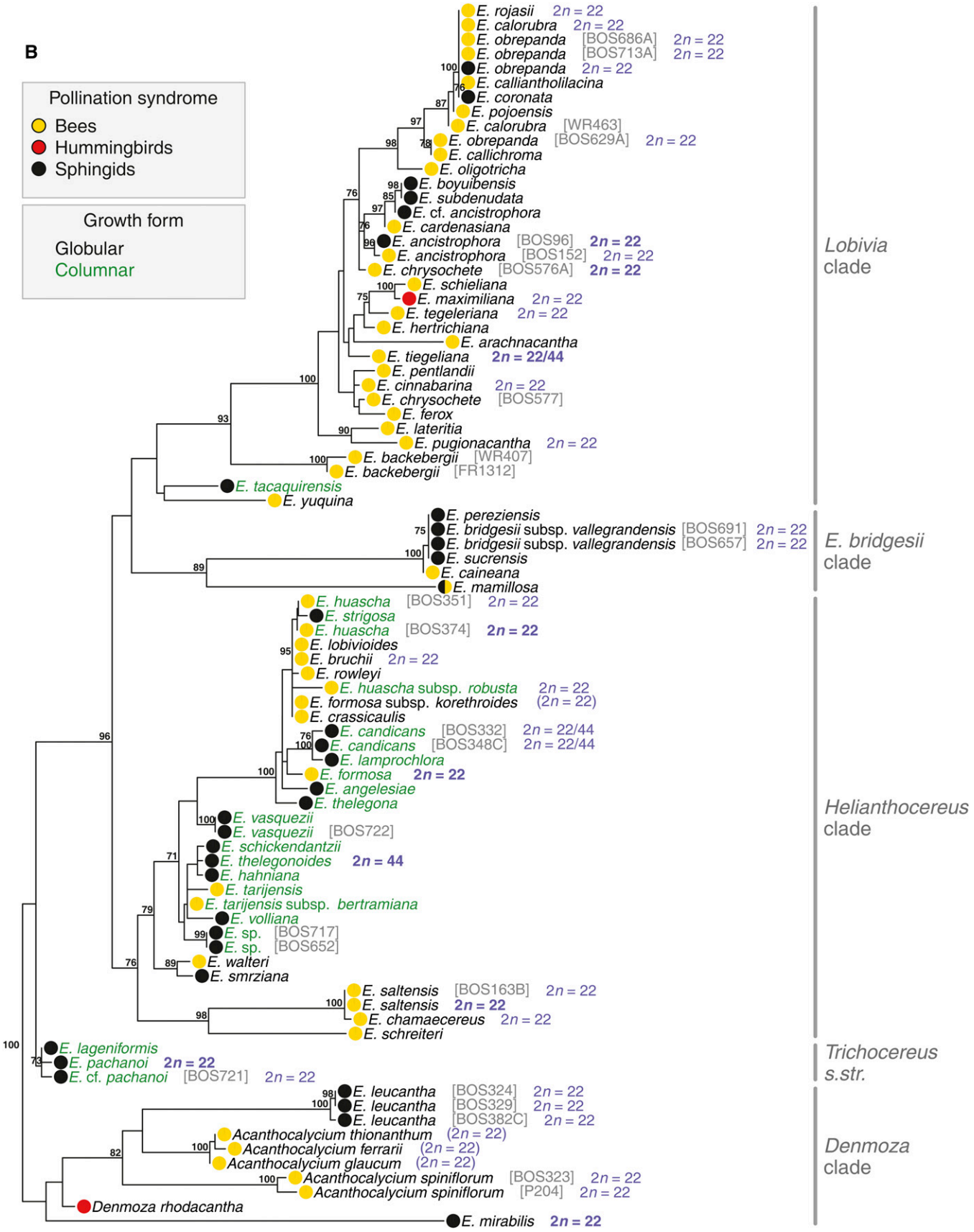

Fig. 1. Continued.

TABLE 3. Chromosome numbers in *Echinopsis* and related Trichocereeae. Authors for taxon names can be found in Table 1, except for taxa not sequenced for which they are listed here. Abbreviations: BGM—Botanical Garden Munich. Collectors' acronyms are BOS: B. O. Schlumpberger, DH: D. Herzog, ES: E. Scholz, FR: F. Ritter, GF: N. Gerloff, HJW: H. J. Wittau, HUN: C. Hunkeler, KK: K. Kniže, LB: L. Bercht, LK: L. Kunte, MN: M. Winberg, P: J. & B. Piltz, UE: U. Eggli, WR: W. Rausch, and many individual collections are also cited in Table 1. Details for those not cited there are available online under http://ralph.cs.cf.ac.uk/cacti/fieldno.html.

Species	Infraspecific taxa and synonyms	2*n*	No. of counts	Origin of material studied or reference
Eechinopsis ancistrophora		22	8	BGM: BOS96, P231, MN125, ES80
E. aurea		22	3	BGM: BOS313
E. aurea	*E. aurea* Britton & Rose var. *albiflora* (Rausch) J.Ullmann	22	5	BGM: BOS596
E. aurea	*Lobivia cylindracea* Backeb.	22	?	Diers, 2008a
E. backebergii Werderm. subsp. *wrightiana* (Backeb.) M.Lowry		44	?	Diers, 2008a
E. bridgesii subsp. *vallegrandensis*	*E. cotacajesii* Cárdenas	22	5	BGM: WR604
E. bridgesii subsp. *vallegrandensis*	*E. cochabambensis* Backeb.	22?	?	Diers, 2008a
E. bruchii		22	?	Diers, 2008a
E. calliantholilacina		22	3	BGM: WR63
E. callichroma		22	3	BGM: WR461
E. calochlora		22	?	Diers, 2008a
E. calorubra	*E. rauschii* Friedrich var. *megalocephala* Rausch	22	5	BGM: WR272
E. candicans		22	5	BGM: BOS404
E. candicans		22/44	17	BGM: BOS380
E. candicans		44	27	BGM: BOS328
E. candicans		44	?	Las Peñas et al., 2009
E. chamaecereus	*Chamaecereus silvestrii*	22	?	Ross, 1981
E. chiloensis		22	3	BGM: BOSx77
E. chiloensis		22	?	Katagiri, 1952
E. chrysochete		22	5	BGM: BOS576
E. chrysochete	*Lobivia minutiflora*	22	4	BGM: BOS577
E. cinnabarina	*Lobivia pseudocinnabarina* Backeb.	22	?	Diers, 2008a
E. coronata		22	?	Diers, 2008a
E. crassicaulis		22	9	BGM: BOSx23
E. densispina		22	3	BGM: ES31, ES37
E. deserticola	*Trichocereus fulvilanus* F.Ritter	22	?	Diers, 2008a
E. famatimensis	*Lobivia pectinifera* Wessner	22	?	Diers, 2008a
E. formosa	*E. formosa* subsp. *formosa*	22	2	BGM: BOS460
E. formosa	*E. formosa* (Pfeiff.) Jacobi ex Salm-Dyck subsp. *rosarioana* (Rausch) M.Lowry	22	3	BGM: WR528
E. haematantha		22	5	BGM: ES66
E. haematantha	*Lobivia amblayensis* Rausch	22	?	Diers, 2008a
E. haematantha	*Lobivia amblayensis* Rausch var. *albispina* Rausch	22	?	Diers, 2008a
E. huascha		22	10	BGM: BOS368, BOS374
E. huascha		22	?	Diers, 2008a
E. huascha subsp. *robusta*		22	16	BGM: BOS380
E. jajoiana		44	?	Diers, 2008a
E. leucantha		22	5	BGM: BOS329
E. maximiliana		22	3	BGM: HJW189
E. maximiliana		22	?	Diers, 2008a
E. maximiliana	*Lobivia corbula* Britton & Rose	22	9	BGM: ES133
E. maximiliana	*Lobivia intermedia* Rausch	22	1	BGM: LK37
E. maximiliana	*Lobivia miniatiflora* F.Ritter	22	8	BGM: BOS676 (LPB)
E. mirabilis		22	3	BGM: BOSx35
E. mirabilis		22	?	Diers, 2008a
E. obrepanda		22	?	Diers, 2008a
E. oxygona	*E. eyriesii* Pfeiff. & Otto	22	?	Sato, 1958; Katagiri, 1952; Diers, 2008a
E. oxygona	*eyriesii*	22	2–10	Arakaki et al., 2007
E. oxygona	*E. multiplex* (Pfeiff.) Zucc. ex Pfeiff. & Otto	22	?	Sato, 1958
E. oxygona		22	?	Katagiri, 1952
E. oxygona	*E. turbinata* Pfeiff. & Otto	22	?	Diers, 2008a
E. pachanoi		22	3	BGM: BOSx78
E. pereziensis		22?	?	Diers, 2008a
E. pugionacantha		22	5	BGM: BOS566
E. rhodotricha K. Schum.	*E. klingleriana* Cárdenas	22	?	Diers, 2008a
E. rojasii		22	?	Diers, 2008a
E. rowleyi	*Lobivia grandiflora* Britton & Rose	22	?	Diers, 2008a
E. saltensis		22	5	BGM: BOSx50
E. saltensis	*Lobivia nealeana* Backeb.	22	?	Diers, 2008a
E. spachiana (Lem.) H. Friedrich & G. D. Rowley		22?	?	Diers, 2008a

TABLE 3. Continued.

Species	Infraspecific taxa and synonyms	2*n*	No. of counts	Origin of material studied or reference
E. spachiana		44	?	Katagiri, 1952
E. tegeleriana		22	?	Diers, 2008a
E. terscheckii		22	?	Diers, 2008a
E. thelegona		22	1	BGM: BOS208
E. thelegonoides		44	4	BGM: BOS543
E. tiegeliana		22/44	?	BGM: R323
E. tiegeliana		22	5	BGM: BOSx82
E. tiegeliana		22	14	BGM: WR84a
E. tubiflora		22	?	Katagiri, 1952; Sato, 1958; Diers, 2008a; Las Peñas et al., 2009
Other genera				
Acanthocalycium spiniflorum	*Acanthocalycium violaceum* (Werderm.) Backeb.	22	?	Diers, 2008a
Acanthocalycium spiniflorum		22	3	BGM: BOS401
Acanthocalycium thionanthum		22	6	BGM: ES9
Arthrocereus A. Berger, 2 taxa		22	?	Diers, 2008a
Browningia candelaris (Meyen) Britton & Rose		22	?	Diers, 2008a
Cereus Mill., 3 taxa		22	?	Sato, 1958
Cereus 5 taxa		22	?	Diers, 2008a
Cereus 4 taxa		22	?	Katagiri, 1952
Cleistocactus baumannii	*Cleistocactus* s.str.	22	?	Ross, 1981
Cleistocactus icosagonus F.A.C.Weber	*Borzicactus aurivillus* Britton & Rose	22	?	Katagiri, 1952; Sato, 1958
Cleistocactus samaipatanus (Cárdenas) D.R.Hunt	*Bolivicereus samaipatanus* Cárdenas	22	?	Diers, 2008a
Cleistocactus smaragdiflorus	*Cleistocactus* s.str.	22	5	BGM: BOS424
Cleistocactus Lem., 5 taxa	*Borzicactus*, *Loxanthocereus*	22/44	2–10	Arakaki et al., 2007
Cleistocactus 3 taxa	*Borzicactus*	22	?	Baker, 2002
Cleistocactus 3 taxa	*Cleistocactus* s.str.	22/44	?	Diers, 2008a
Discocactus Pfeiff., 2 taxa		22	?	Diers, 2008a
Espostoa blossfeldiorum (Werderm.) Buxb.		22	2–10	Arakaki et al., 2007
Espostoa lanata		22/44/66	2–10	Arakaki et al., 2007
Espostoa Britton & Rose, 2 taxa		22/44?	?	Diers, 2008a
Espostoa melanostele (Vaupel) Borg subsp. *nana* (F.Ritter) G.J.Charles		22	4	Cultivated at BGM, no voucher
Espostoa senilis (F.Ritter) N.P.Taylor		22	2–10	Arakaki et al., 2007
Espostoa sp.		44	4	Cultivated at BGM, no voucher
Facheiroa ulei		22	?	Diers, 2008a
Gymnocalycium Pfeiff., 9 taxa		22	?	Diers, 2008a
Gymnocalycium 4 taxa		22	?	Katagiri, 1952
Gymnocalycium 30 taxa		22/44/66	?	Lambrou and Till, 1993
Gymnocalycium 2 taxa		22/44	?	Ross, 1981
Haageocereus Backeb., 11 taxa	17 taxa fide Arakaki et al., 2007	22/33/44	2–10	Arakaki et al., 2007
Lasiocereus fulvus		22	2–10	Arakaki et al., 2007
Lasiocereus rupicola F.Ritter		22	2–10	Arakaki et al., 2007
Matucana haynei		22	2–10	Arakaki et al., 2007
Matucana Britton & Rose, 4 taxa		22	?	Diers, 2008a
Micranthocereus densiflorus		22	2	BGM: BOSx69
Micranthocereus Backeb., 3 taxa		22	?	Diers, 2008b
Mila caespitosa		22	2-10	Arakaki et al., 2007
Oreocereus celsianus		44	12	BGM: BOS569
Oreocereus pseudofossulatus D.R.Hunt	*Oreocereus. fossulatus* var. *gracilior* (K.Schum.) Backeb.	44	?	Diers, 2008b
Oroya borchersii (Boed.) Backeb.		22	?	Diers, 2008b
Praecereus euchlorus		22	?	Diers, 2008c
Pygmaeocereus bieblii var. *kuehhasii*		22	6	BGM: BOSx46
Rebutia K. Schum., 3 taxa	*Aylostera*	22/44	?	Diers, 2008c
Rebutia atrovirens		44	6	BGM: J75
Rebutia glomeriseta Cárdenas	*Sulcorebutia menesesii* (Cárdenas) Backeb.	22	?	Diers, 2008c
Rebutia minuscula	*Rebutia marsoneri* Werderm. & *R. senilis* Backeb.	22?	?	Diers, 2008c
Rebutia padcayensis Rausch	*R. margarethae* Rausch	22	4	BGM: L550
Rebutia neocumingii	*Weingartia*	22	1	BGM: BOSx72
Rebutia 4 taxa	*Rebutia*, *Sulcorebutia*	22	?	Ross, 1981
Stetsonia coryne		22	5	BGM: BOSx57
Weberbauerocereus Backeb. 3 taxa		44/ca. 88	?	Diers, 2008c
Weberbauerocereus weberbaueri		44	2–10	Arakaki et al., 2007
Weberbauerocereus 2 taxa		44/88	2–10	Arakaki et al., 2007

Fig. 2. Examples for convergent evolution in *Echinopsis*. Floral adaptation to hawkmoth pollination in *E. ancistrophora* (A, *Lobivia* clade) and *E. tubiflora* (B, *Echinopsis* s.str. clade) and columnar growth in *E. tarijensis* (C, *Helianthocereus* clade) and *E. atacamensis* subsp. *pasacana* (D, *E. atacamensis* clade).

Oroya, *Pygmaeocereus*, *Rauhocereus*, *Samaipaticereus*, *Weberbauerocereus*, and *Yungasocereus.* Alternatively, *Echinopsis* will need to be divided into smaller, more homogeneous units, requiring the resurrection of generic names and transfers of species epithets. We now briefly discuss the main clades found in this study.

Echinopsis s.s. is well supported (100% bootstrap support; the type species is *E. oxygona*) and homogeneous in growth form. Its sister group consists of the likewise well-supported *E. atacamensis* clade and the genus *Harrisia* (100% and 97% bootstrap, respectively, Fig. 1A), refuting suggestions that *Harrisia* might be part of the Hylocereeae (A. Areces-Mallea, personal communication, cited in Anderson, 2001) or the Echinocereeae (Barthlott and Hunt, 1993). Of the ca. 20 species of *Harrisia*, we sampled *H. gracilis* from the Carribean and four species from central South America.

The *Cleistocactus* s.str. clade (Fig. 1A, 100% bootstrap) comprises the columnar cacti *Espostoa guentheri* (syn. *Vatricania*), *Samaipaticereus*, *Weberbauerocereus*, *Yungasocereus*, *Cephalocleistocactus*, and *Cleistocactus*. Species formerly placed in *Borzicactus* but transferred to *Cleistocactus* (Hunt, 2006) turn out not to belong there but instead to the *Oreocereus* clade (Fig. 1A), and future studies will have to investigate if other species transferred to *Cleistocactus* from *Winterocereus* (syn. *Winteria* and *Hildewintera*) and *Bolivicereus* also instead belong to *Borzicactus*. Most species in the *Cleistocactus* clade are adapted to pollination by bats (*Espostoa*, *Samaipaticereus*, and *Yungasocereus*) or hummingbirds (*Cephalocleistocactus* and *Cleistocactus*), and *Weberbauerocereus* species are visited, and apparently pollinated, by both bats and hummingbirds (Sahley, 1996). That *Espostoa* (*Vatricania*) *guentheri* is not grouping with *E. lanata*, the type species of *Espostoa*, fits with an anatomical study that found it to have "the greatest number of unusual features" among six species of *Espostoa* studied (Mauseth, 1999: p. 36). One of these unusual features, a two-layered hypodermis with clusters of columnar cells, resembles the hypodermis of *Cleistocactus strausii* and *Weberbauerocereus albus*, which are related to species that group with *Espostoa* (*Vatricania*) *guentheri* in our study.

Next closest is the *Reicheocactus* clade (Fig. 1A). The relatively early-diverging species *E. famatimensis* and *E. bonnieae* have long troubled Cactaceae taxonomists. First described as *Echinocactus famatimensis* (Spegazzini, 1921), the former was subsequently tranferred to *Rebutia* (Spegazzini, 1923) and *Lobivia* (Britton and Rose, 1919–1923), and in 1942 to the new genus *Reicheocactus* Backeb. (as *Reicheocactus pseudoreicheanus*). In recent years, it was usually included in *Echinopsis* (Anderson, 2001; Hunt 2006; but see Kiesling et al., 2001, who returned it to *Lobivia*). *Echinopsis bonnieae* likewise has been moved between *Lobivia* (as *Lobivia bonnieae* Halda & Horácek, 1999) and *Echinopsis*.

Another well-supported group is the *Oreocereus* clade (Fig. 1A, 99% bootstrap), which includes the type species of *Oreocereus* plus the eight genera *Borzicactus* (here referred to *Cleistocactus*, according to Anderson, 2001 and Hunt, 2006), *Espostoa*, *Haageocereus*, *Matucana*, *Mila*, *Oroya*, *Pygmaeocereus*, and *Rauhocereus*, with a total of about 60 species (Hunt, 2006).

Next in the tree comes the *Denmoza* clade (Fig. 1B), which contains the monotypic *Denmoza rhodacantha*, *E. mirabilis* (syn. *Setiechinopsis*, monotypic as well), *E. leucantha*, and *Acanthocalycium*. *Acanthocalycium* was synonymized under *Echinopsis* s.l. by Hunt (2006), but was kept separate by Anderson (2001, 2005). All these taxa are endemic to northwestern Argentina, and their flowers are adapted to pollination by hummingbirds (*Denmoza*), bees (*Acanthocalycium*), or moths (*E. leucantha* and *E. mirabilis*, the latter unique in its short life-cycle, flowers closing before dawn, and autogamy; Kiesling and Ferrari, 2005).

The *Trichocereus* s.str. clade (Fig. 1B) is here represented by *Echinopsis pachanoi* and *E. lageniformis*, both columnar species characterized by few, blunt ribs, poor spination, and a moderate stem diameter (usually <20 cm). The type species of *Trichocereus*, *E. macrogona* [*Cereus macrogonus* Salm-Dyck; *Trichocereus macrogonus* (Salm-Dyck) Riccob.], is based on a cultivated specimen of unknown origin and appears related to *E. pachanoi* (Anderson, 2005; R. Kiesling, CONICET, Mendoza, Argentina, personal communication). The taxonomy is further complicated by *E. pachanoi*, a species long cultivated and hence widely distributed ("San Pedro cactus").

The *Helianthocereus* clade (Fig. 1B) consists of species of mostly columnar to rarely globular growth with flowers adapted to pollination by bees or hawkmoths. Friedrich (1974) and Friedrich and Glätzle (1983), in a study of seed morphology for which they sampled ca. 20 of the species from this clade, found that all had seeds with an obliquely placed oval hilum surrounded by flat testa cells. Next in the tree comes the *E. bridgesii* clade (89% bootstrap support), which consists of five species from central Bolivia and northern Argentina with globular to short-columnar growth and flowers suggesting either bee or moth pollination. The recently described *Lobivia krahn-juckeri* (Diers, 2009) may also belong here. A final group worth discussing is the *Lobivia* clade (Fig. 1B), with a well-supported core clade (93% bootstrap support) and an unresolved group of early-branching species. Typical "lobivioid" species are small, mostly globular plants with short diurnal flowers. However, the *Lobivia* clade also includes species with long (>20 cm) white flowers adapted to pollination by sphingids (Schlumpberger and Raguso, 2008; Schlumpberger et al., 2009; Fig. 2A). Examples for those are *E. ancistrophora*, *E. obrepanda*, and *E. rojasii*.

In sum, the molecular data presented here conflict in large parts with previous taxonomic classifications of *Echinopsis* and its relatives (Anderson, 2001, 2005; Hunt, 2006). Morphological characters other than those traditionally used will have to be found to circumscribe natural taxonomic groups in the Trichocereeae, but large morphological differences may be difficult to find since the entire Trichocereinae/Cereinae clade is only 7.5–6.5 Myr old (Arakaki et al., 2011).

Relative phylogenetic plasticity of growth habit and pollination syndromes— In most of the clades revealed by our molecular data, growth form (globular vs. columnar) is phylogenetically conserved. The *Helianthocereus* clade (Fig. 1B), however, comprises globular species (*E. crassicaulis*, *E. lobivioides*), barrel cacti (*E. formosa* subsp. *korethroides*), species with thin, creeping or upright columns (*E. huascha*, *E. candicans*, *E. thelegona*), and large, robust columnar cacti (*E. tarijensis*, *E. formosa* subsp. *formosa* BOS460; Fig. 1B, 2C, 2D).

Pollination modes are evolutionary less conserved. Switches from bee to hawkmoth pollination or vice versa appear to be especially common. Examples are found in the *Lobivia*, *Helianthocereus* and *Echinopsis* s.s. clades (Fig. 1A, B). The finding of closely related species having different pollinators fits with population-level variation between bee and sphingid pollination, and nocturnal or diurnal flower opening (Schlumpberger and Raguso, 2008; Schlumpberger et al., 2009). A review of inferred evolutionary shifts between pollinators in genera of Cactaceae more generally is provided in Schlumpberger (2012).

The role of polyploidy in the evolution of the Echinopsis clade— The 37 new chromosome counts reported here support the basic chromosome number of $2x = 22$ inferred for Cactaceae (Remski, 1954; Pinkava et al., 1985). Tetraploidy is present in the *Lobivia* and *Helianthocereus* clades (Fig. 1A, B) and has also been reported from a few species not included in our tree, such as *E. spachiana* (likely part of our *Helianthocereus* clade) and *E. backebergii* subsp. *wrightiana* (*Lobivia* clade; Diers, 2008a; Table 3). Overall, the distribution of diploid and tetraploid (or higher) numbers on the phylogeny does not point to particularly frequent polyploidy events, compared for example with similarly sized clades in *Aristolochia*, *Passiflora*, *Carex*, and *Helianthus* (Ohi-toma et al., 2006; Hansen et al.,

2006; Hipp et al., 2007; Timme et al., 2007). It has been suggested that hybridization plays a major role in the evolution of Cactaceae (Rowley, 1994; Machado, 2008) and has also been hypothesized for *Echinopsis* (Friedrich, 1974; Font and Picca, 2001; Anderson, 2005) and related genera (Rowley, 1994). Observations in the field also suggest occasional hybridization, for example, between *E. atacamensis* subsp. *pasacana* × *E. huascha* subsp. *robusta*, *E. terscheckii* × *E. candicans*, *E. terscheckii* × *E. huascha*, and *E. ancistrophora* × *E. saltensis* (B. Schlumpberger, unpublished data). However, at least the first cross in this list involves homoploidy (BS, unpublished data), and the relative scarcity of tetraploidy in the phylogeny (Fig. 1) thus does not necessarily reflect a rarity of hybridization.

***Conclusions*—**The chloroplast DNA phylogeny presented here for 144 species and subspecies representing all major groups of *Echinopsis* sensu lato as well as all genera of Trichocereeae demonstrates the polyphyly of this genus as currently circumscribed. The phylogeny further reveals much convergent evolution in flower traits that relate to pollination modes (see Fig. 2A, B) and less convergent evolution in growth forms (but see Fig. 2C, D). There is no conspicuous role of genome doubling in the evolution of *Echinopsis* sensu lato. A new generic classification of the Trichocereeae now requires finding morphological characters sufficiently conservative for distinguishing larger groups of species. Seed morphology and growth form, perhaps in combination, seem promising starting points.

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