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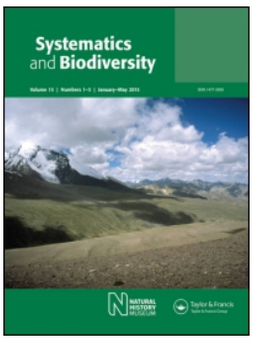

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Research Article

Phylogeny in *Echinocereus* (Cactaceae) based on combined morphological and molecular evidence: taxonomic implications

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Echinocereus is a morphologically diverse genus that includes 64 species grouped into eight taxonomic sections based on morphological traits. In previous molecular phylogenetic analyses, the relationships amongst *Echinocereus* species were not entirely revealed and useful characters to recognize clades were not provided. The inclusion of several sources of evidence in a phylogenetic analysis is likely to produce more supported hypotheses. Therefore, we performed a combined phylogenetic analysis with a set of 44 morphological characters and six chloroplast DNA sequences. Topologies from parsimony and Bayesian analyses were mostly congruent. However, the relationships of *E. poselgeri* were not consistent between analyses. A second Bayesian analysis using a long-branch extraction test resulted in a topology with the morphological position of *E. poselgeri* congruent with that in parsimony analysis. Parsimony and Bayesian analyses corroborated the monophyly of *Echinocereus*, which included eight monophyletic groups. The combined phylogeny integrated into different clades those taxa that were not determined in previous analyses and changed the relationships of some recognized clades. The clades did not recover the recent infrageneric classification. In the present study, a new sectional classification for *Echinocereus* is proposed based on the eight recovered clades, which is supported by a combination of morphological and molecular characters. An identification key for sections in the genus is included.

Key words: Bayesian inference, combined analyses, *Echinocereus poselgeri*, Echinocereeae, long-branch attraction, long-branch extraction, morphology, parsimony, taxonomy, *Wilcoxia*

Introduction

The Cactaceae are known for distinctive morphology and are characterized by the presence of areoles, extremely succulent stem and roots, several growth forms and great floral diversity (Bravo-Hollis, 1978; Gibson & Nobel, 1986). Previous research has provided detailed descriptions and considerations regarding the morphological diversity of stem, flower, fruit and seed in the cactus family (Buxbaum, 1951, 1953, 1955; Gibson & Nobel 1986), in addition to monographs with taxonomic classifications based on morphological variation (e.g., Anderson, 2001; Berger, 1926; Britton & Rose, 1919, 1920, 1922, 1923; Buxbaum, 1958; Endler & Buxbaum, 1974; Hunt, Taylor, & Charles, 2006; Schumann, 1899). In current analyses, the use of molecular characters has surpassed that of morphological characters. Morphological characters in Cactaceae show a tendency to be mapped on molecular phylogenies (*Echinopsis* Zucc., Schlumpberger & Renner, 2012; *Rebutia* K. Schum., Ritz, Martins, Mecklenburg, Goremykin, & Hellwig, 2007) and/or used in the reconstruction of putative ancestral states in the molecular phylogenies of some genera (*Copiapoa*, Larridon et al., 2015; *Gymnocalycium* Pfeiff., Demaio, Barfuss, Kiesling, Till, & Chiapella, 2011; *Pereskia* Mill., Edwards, Nyffeler, & Donoghue, 2005). Analyses of morphological features as part of phylogenetic reconstruction have been limited in Cactaceae (Albesiano & Terrazas, 2012; Arias & Terrazas, 2006; Guerrero, Arroyo, Bustamante, Hagemann, & Walter, 2011; Terrazas & Loza-Cornejo, 2002).

As demonstrated in recent decades, mapping morphological characters on a molecular phylogeny does not

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allow a test for congruence of characters and does not determine whether some morphological characters are synapomorphies (Assis, 2009, De Pinna, 1991, Patterson, 1982). However, the mapping of a few determinate morphological data (characters analysed rigorously and critically) on molecular phylogenies is proposed to represent a more robust approach to integrate the strengths of both data (Scotland, Olmstead, & Bennett, 2003). In particular, the argument to disallow the use of morphological characters into phylogenetic analyses in Cactaceae is based on the assumed high plasticity and parallelism within the characters (Wallace & Gibson, 2002); however, homologies or homoplasies should be recognized by a cladistic analysis and cannot be assumed a priori (De Pinna, 1991; Luna & Mishler, 1996). Nixon and Carpenter (1996) noted that analysis of combined data identifies the common phylogenetic signal, which results in phylogenetic relationships that were not detected in separate analyses. Although phylogenetic analysis using both molecular and morphological data has increased, studies in Cactaceae using combined morphological and molecular data have been conducted only in two South American lineages (Albesiano & Terrazas, 2012; Guerrero et al., 2011).

The genus *Echinocereus* (Cactoideae, Echinocereeae) has 64 species (Hunt et al., 2006) with short cylindrical stems (<50 cm), variable rib numbers, funnel-shaped flowers, fruits with spines and black and warty seeds (Engelmann, 1848). The distribution of *Echinocereus* extends from central Mexico to central USA, with the cacti inhabiting primarily desert scrub and conifer woods (Taylor, 1985). Recently, Sánchez, Arias, and Terrazas (2014) established that *Echinocereus* is a monophyletic group when *E. poselgeri* is included and *E. pensilis* (K. Brandegee) J. A. Purpus is excluded. These two species had been previously segregated into the genera *Wilcoxia* Britton & Rose (Britton & Rose, 1922) and *Morangaya* G. D. Rowley (Rowley, 1974), respectively. The characters green stigmas and erumpent buds have been proposed as synapomorphies for the genus (Sánchez et al., 2014; Sánchez, Grego-Valencia, Terrazas, & Arias, 2015), but these characters have not been tested in phylogenetic analyses. *Echinocereus* includes a wide diversity of both vegetative (root, stem, spines) and reproductive characters (flower, fruit, seed) (Blum, Lange, Rischer, & Rutow, 1998; Bravo-Hollis & Sánchez-Mejorada, 1991; Taylor, 1985). Considering the high number of species and the morphological diversity in both vegetative and reproductive characters in the genus, some authors have proposed infrageneric classification (Table 1). The infrageneric classifications in *Echinocereus* (Hunt et al., 2006) represent a hypothesis about the distribution of the characters that each taxon shares. This hypothesis can be evaluated in a combined phylogenetic analysis and its contribution to recovering lineages in the genus assessed.

In the phylogeny of *Echinocereus* based on chloroplast DNA sequences, nine clades are recovered (Sánchez et al., 2014); however, only one clade represents the section *Triglochidiati* (Hunt et al., 2006; Sánchez et al., 2014), whereas the remaining sections (Hunt et al., 2006) correspond to paraphyletic and polyphyletic groups. In particular, section *Wilcoxia* (Hunt et al., 2006) was not

Table 1. Historical summary of infrageneric classification in *Echinocereus*.

Engelmann, 1849	Engelmann, 1859	Schumann, 1899	Taylor, 1985
Two sections: *Costati* Engelm., *Sulcati* Engelm.	Four sections: *Pectinati* Salm-Dyck, *Decalophi* Salm-Dyck, *Pentalophi* Salm-Dyck, *Graciles* Engelm.	Four series: *Graciles*, *Subinermes* K. Schum., *Prostrati* K. Schum. *Erecti* K. Schum.	Eight sections: *Morangaya* (G.D.Rowley) N. P. Taylor, *Erecti* (K. Schum.) Bravo, *Echinocereus*, *Triglochidiati* Bravo, *Reichenbachii* N. P. Taylor, *Wilcoxia* (Britton & Rose) N. P. Taylor, *Pulchellus* N. P. Taylor
Bravo-Hollis & Sánchez-Mejorada, 1991	Taylor, 1993	Blum et al., 1998	Hunt et al., 2006
Six sections: *Subinermes*, *Scheera* Backeb., *Triglochidiati*, *Prostrati*, *Echinocereus*, *Erecti*	Eight sections: *Morangaya*, *Erecti*, *Costati*, *Echinocereus*, *Triglochidiati*, *Reichenbachii*, *Wilcoxia*, *Pulchellus*	Three subgenera: *Morangaya* (G. D. Rowley) Lange, *Triglochidiati* (Bravo) W.Blum, Mich.Lange & Rutow *Echinocereus* (with seven sections): *Erecti*, *Costati*, *Subinermes*, *Echinocereus*, *Reichenbachii*, *Wilcoxia*, *Pulchellus*	Eight sections: *Morangaya*, *Erecti*, *Triglochidiati*, *Costati*, *Echinocereus*, *Reichenbachii*, *Wilcoxia*, *Pulchellus*

recovered as a monophyletic group, although the morphological and anatomical characters of species in this section show a strong resemblance (Blum, Felix, & Waldeis, 2008; Loza-Cornejo & Terrazas, 1996; Taylor, 1985). This incongruence between morphological and molecular data may be due to parallel evolution (Wallace & Gibson, 2002) or an artefact, such as long-branch attraction (LBA; Bergsten, 2005). According to Bergsten (2005), the inclusion of certain morphological characters may change the topology in molecular phylogenies affected by LBA.

As the philosophical basis of phylogenetic systematics, ancestry-descend relationships are reflected in a natural classification of organisms (Henning, 1966; Nelson, 1972); which contains information about the distribution of characters to diagnose each clade or taxon (Nixon & Carpenter 2000). With the inclusion of morphological characters in phylogenetic analyses, recovered taxa that store useful taxonomic characters are promoted. Therefore, in this work, we conducted a phylogenetic analysis of *Echinocereus* that included a set each of morphological and molecular characters to (i) evaluate the possibility of an LBA artefact in the phylogenetic position of *E. poselgeri*, (ii) obtain a set of morphological and molecular characters that supported the genus and internal clades, (iii) assess the recent infrageneric classification (Hunt et al., 2006) from the recovered monophyletic groups, and (iv) present a taxonomic treatment of *Echinocereus* and infrageneric taxa.

Materials and methods

Taxon sampling

The analysis included 59 species of *Echinocereus* that represented the morphological diversity of the genus and the eight sections recognized by Hunt et al. (2006). The sampling followed the species delimitation proposed by Hunt et al. (2006) and incorporated the recent taxonomic changes in the *Triglochidiati* (Baker, 2006a, 2006b; Sánchez, Arias, & Terrazas, 2013) and *Wilcoxia* (Blum et al., 2008) sections. Additionally, 10 species were included as a sister group of *Echinocereus*, according to recent findings (Bárcenas, Yesson, & Hawkins, 2011; Sánchez et al., 2014).

Morphological characters

A set of 44 morphological characters (including chromosome number) was generated in the present study (Appendix 1, see online supplemental material, which is available from the article's Taylor & Francis Online page at https://doi.org/10.1080/14772000.2017.1343260) by the examination of specimens collected in fieldwork and those in herbaria (ARIZ, ASU, BCMEX, CIIDIR, IBUG, MEXU, and UNM) and living collections (Botanic Gardens of Instituto de Biología, UNAM; El Charco del Ingenio, A. C.; and Regional de Cadereyta, CONCYTEQ). Additional characters were obtained from permanent slides prepared with a paraffin-embedding technique (Loza-Cornejo & Terrazas, 1996) and bibliographic reviews. A morphological characters matrix was coded with binary and multistate characters and was edited in Mesquite 3.02 (Maddison & Maddison, 2015). Details on morphological measures and anatomical work are described in Appendix 1 (see supplemental material online).

DNA sequences and alignment

We included six chloroplast DNA markers: the intergenic spacers *psbA-trnH* and *trnQ-rps16*; the *rpl16* intron; the region composed of the intron *trnL* and the IGS *trnL-trnF* (hereafter, *trnL-F*); the coding gene *matK*, flanked by the *trnK* intron (hereafter, *trnK/matK* marker); and the coding gene *rbcL* (see Sánchez et al., 2014 for details about primer sequences and thermal profiles in PCR amplification). The value of these markers in phylogenetic studies in Cactaceae has been demonstrated and discussed in several publications (Bárcenas, 2015; Bárcenas et al., 2011; Hernández-Ledesma & Bárcenas, 2017; Korotkova et al., 2011; Vázquez-Sánchez, Terrazas, Arias, & Ochoterena, 2013). DNA sequences stored in the GenBank database were compiled (Appendix 2, see supplemental material online) from the phylogenetic studies of Arias, Terrazas, Arreola-Nava, Vázquez-Sánchez, and Cameron (2005), Bárcenas et al. (2011), Hernández-Ledesma and Bárcenas (2017) and Sánchez et al. (2014), and DNA sequences were manually aligned and concatenated in a single matrix, and the extremes of sequences for each marker were deleted because of ambiguities. Highly variable regions that were difficult to align were not detected; only small regions of poly-A (in *rpl16* and *trnL-F*) and poly-T (in *psbA-trnH*, *rpl16*, *trnK/matK*, *trnL-F* and *trnQ-rps16*) of different lengths were observed. Additionally, we generated a binary matrix with DNA insertion and deletion events (indels) observed on the aligned sequences (Appendix 3, see supplemental material online); these indels were coded using a simple coding method (Ochoterena, 2009). Gaps generated by differences in lengths in the poly-A and poly-T regions were not coded. Sequence alignment and matrices editing were performed in Mesquite 3.02 (Maddison & Maddison, 2015).

Phylogenetic analyses

Four final matrices were built: the first was for the morphological data (morphology matrix); the second included only DNA sequences (DNA matrix); the third incorporated DNA sequences and indels data (molecular matrix), and the fourth incorporated morphological, DNA sequences,

Table 2. Numerical data of aligned sequences included.

	psbA-trnH	*rbcL*	*rpl16*	*trnK/matK*	*trnL-F*	*trnQ-rps16*	DNA matrix
Included taxa	68	66	66	69*	66	66	69
Sequence length	520	578	1238	2524	1158	630	6648
Non-informative sites	485	558	1181	2471	1105	603	6404
Informative sites	35	20	57	53	53	27	245
% informative sites	6.73	3.46	4.60	2.09	4.57	4.28	3.68
Informative indels	9	0	7	2	13	4	35
Model of nucl. subs.	F81+G	TPM1uF+I	TPM1uf+I+G	TVM+I	HKY+G	TPM1uf+I+G	

*14 taxa include only the coding region *matK*.

and indel data (combined matrix). All matrices were analysed under parsimony (MP), but DNA, molecular and combined matrices were also analysed using Bayesian inference (BI). The MP analysis was performed in TNT v. 1.1 (Goloboff, Farris, & Nixon, 2008) using parsimony informative characters only (Table 2). We performed a heuristic search of 10,000 random addition sequences using ratched, sectorial searches, drift and tree fusing algorithms (Goloboff et al., 2008), saving 10 trees per replica. Support values were calculated from 10,000 replicas, using the same parameters as the heuristic search. The standard bootstrap support (BS) shows the absolute frequencies. The jackknife support (JK) removed 36% of the characters and shows the absolute frequencies. A strict consensus tree was computed from the most parsimonious trees. A BI analysis of the DNA matrix was performed using the mixture model CAT-GTR implemented in PhyloBayes 4.01 (Lartillot & Philippe, 2004). The molecular and combined matrices were partitioned and analysed by BI using MrBayes 3.2.1 (Ronquist & Huelsenbeck, 2003), because categorical characters can be included. For those analyses, the morphological and indel partitions were analysed under the Mkv model and coded as variable; and for each DNA sequence, the nucleotide substitution was determined by the AIC using JModelTest 2 (Darriba, Taboada, Doallo, & Posada, 2012). The posterior probability values (BPP) were computed using two separate runs of Markov Monte Carlo chains (MCMC), each run with four chains and 5,000,000 generations. The Markov chains were sampled every 10,000 generations, the MCMC convergence was visually examined and 20% of the sampled trees were discarded. The remaining trees and BPP were summarized in a consensus majority rule tree.

Long-branch extraction test (LBE test). For cases of incongruence between the resulting topologies from the MP and BI analyses, a review of the tree graphic for long branches associated with problematic taxa was performed. The selected taxon was excluded and an LBE test using the same parameters as the previous analyses was performed. This test was proposed by Pol and Siddall (2001) to corroborate long-branch attraction (LBA), assuming that a long-branch is able to attract or be attracted by another long-branch in a phylogenetic analysis. Therefore, the exclusion of one of the long branches will allow the second long-branch to be grouped in the correct clade.

Character optimization

An unequivocal character optimization was conducted using Winclada (Nixon, 2002) on the strict consensus tree to understand the contribution of the characters in the phylogeny and to recognize the synapomorphies and homoplasies that defined each recovered clade. Delayed optimization (deltran) and fast optimization (acctran) were explored to recognize additional characters that supported certain clades (Agnarsson & Miller, 2008). Additionally, a character history of some characters on the strict consensus tree from the combined MP analysis was conducted in which character states in nodes were estimated using the parsimony model implemented in the "Trace character history" command in Mesquite 3.02 (Maddison & Maddison, 2015).

Results

Data matrices

The morphological matrix with 44 characters included 31 characters of gross morphology, 10 stem and floral anatomical characters, two characters from seed micromorphology, and one corresponded to chromosome numbers. The DNA sequences matrix included 6648 sites of which 245 were parsimony informative, and each sequence presented a particular model of nucleotide evolution (Table 2). We coded 35 indels from the sequences of the six regions of which 12 were simple sequence repetitions, 16 were suppressions or gaps, four were insertions, and three were inversions (Appendix 3, see supplemental mate rial online). The molecular matrix included 280 parsimony informative characters for the MP analysis and 6683 characters for the BI analysis. The combined matrix incorporated 325 parsimony informative characters for the MP analysis and 6727 characters for the BI analyses.

Phylogenetic analyses

The MP analysis of the morphological matrix resulted in a consensus tree (not shown) that recovered *Echinocereus* as monophyletic group. However, topology displayed a polytomy in which most *Echinocereus* species were collapsed at the base of the genus and only a few clades were recovered (*sensu* Hunt et al., 2006: section *Triglochidiati* and species groups of sections *Costati*, *Erecti*, *Wilcoxia*, and *Reichenbachii*). Consensus trees (not shown) of the MP analyses of the DNA and molecular matrices showed a topology with many collapsed branches that did not recover the main clades observed in the previous molecular analysis (Sánchez et al., 2014). BI analysis of the same molecular matrices resulted in more resolved topology (not shown) that almost matched with the results of the previous molecular analysis (Sánchez et al., 2014), but the BPP of several clades was weak (Fig. S1). Moreover, MP and BI analyses of the combined matrix resulted in mostly congruent topologies. Both methods using the combined matrix recognized the genus *Echinocereus* as a monophyletic group (BS, JK and BPP = 100) with eight main clades with different support (Figs 1, 2). In the strict consensus tree from MP analyses, *E. poselgeri* was grouped into clade B, which was the sister group of clade C. Together, clades B and C were the sister group of the remaining major clades (D–H; Fig. 1). The majority consensus tree from BI analysis included *E. poselgeri* in clade C; clade B was recovered as a sister of the group that included clades C–H (Fig. 2.1).

Long-branch extraction test (LBE test). This analysis focused on the *E. poselgeri* and *E. mapimiensis* sister grouping of the topology from the BI analysis using the combined matrix in which both species with dissimilar morphology represented long branches (Fig. 2.1). The LBE test using the combined matrix and BI method that excluded *E. mapimiensis* resulted in the grouping of *E. poselgeri* into clade B with *E. leucanthus* and *E. waldeisii* together. The test also showed clades B and C as sister groups; both clades B and C represented the sister group of the clade that included the remaining major clades (D–H; Fig. 2.2). This result suggested an LBA effect in the first BI analysis. A second LBE test that excluded *E. poselgeri* from the analysis did not show any change in the topology; *E. mapimiensis* was grouped in clade C, as in the previous analysis (data not shown). LBE tests using the MP method and excluding the previous taxa also did not show changes in topology.

Taxonomic treatment

As a result of our study, the infrageneric classification of *Echinocereus* requires a new section name and species circumscription into the eight sections. Consequently, the sections and species are presented as follows:

Echinocereus Engelm., Wisliz. Tour North Mexico: 91 (1848). *Cereus* subgen. *Echinocereus* Engelm., *Proc. Amer. Acad. Arts* 3: 278 (1856). Lectotype (designed by Britton & Brown 1913): *Echinocereus viridiflorus* Engelm.

Section *Subinermes* (K. Schum.) Mich. Lange, *Echinocereenfreund* 8: 16 (1995). *Echinocereus* ser. *Subinermes* K. Schum., *Gesamtbeschr. Kakt*.: 246 (1899). Type species: *Echinocereus subinermis* Salm-Dyck ex Scheer.
Species included: *Echinocereus barthelowanus* Britton & Rose, *E. brandegeei* (J. M. Coult.) K. Schum., *E. ferreirianus* H. E. Gates, *E. knippelianus* Liebner, *E. laui* G. Frank, *E. maritimus* (M. E. Jones) K. Schum., *E. pentalophus* (DC.) Lem., *E. rigidissimus* (Engelm.) Haage, *E. stoloniferus* W. T. Marshall, *E. subinermis* Salm-Dyck ex Scheer.

Section *Wilcoxia* (Britton & Rose) N. P. Taylor, Gen. Echinocereus: 134 (1985). *Wilcoxia* Britton & Rose, *Contr. U.S. Natl. Herb*. 12: 434 (1909). Type species: *Echinocereus poselgeri* Lem.
Species included: *Echinocereus kroenleinii* (Mich. Lange) W. Blum & Waldeis, *E. leucanthus* N. P. Taylor, *E. poselgeri* Lem., *E. tamaulipensis* (Wenderm.) Mich. Lange, *E. waldeisii* Haugg.

Section *Costati* (Engelm.) N. P. Taylor, Piante Grasse 13 (4, Suppl.): 94 (1994). [1993 publ. 1994]). *Cereus* section *Costati* Engelm., *Mem. Amer. Acad. Arts. ser. 2*, 4: 50 (1849). Type species: *Echinocereus enneacanthus* Engelm.
Species included: *Echinocereus berlandieri* (Engelm.) Haage, *E. cinerascens* (DC.) Lem., *E. enneacanthus* Engelm., *E. longisetus* (Engelm.) Lem., *E. mapimiensis* Anderson, *E. nivosus* Glass & R. A. Foster, *E. parkeri* N. P. Taylor, *E. rayonesensis* N. P. Taylor, *E. schmollii* (Weing.) N. P. Taylor, *E. stramineus* (Engelm.) Engelm. ex F. Seitz, *E. viereckii* Werderm.

Section *Sciuri* Dan. Sánchez & S. Arias. **sect**. **nov**. Plants with cylindrical and simple stems (rarely branched), ribs 5 to 15, central spines 3 to 6, acicular, flowers 4 to 8.5 cm long, regular funnel-shaped receptacle tube, nectar chamber 3 to 8 mm long, basal nectarial tissue, trichomes > 1.5 mm long, fruit with juicy pulp, seed with convex periclinal cell wall, embryo with short cotyledons. Simple sequences repeat of four sites in the *psbA-trnH* cpDNA marker. This differs from section *Reichenbachii*, which has dry fruit pulp, seeds with a hemispherical periclinal cell wall and 54 site gaps in the *trnL-F* cpDNA marker. Type species: *Echinocereus sciurus* (K. Brandegee) Dams, Monatsschr. Kakteenk. 14: 130 (1904).
Species included: *Echinocereus adustus* Engelm., *E. bristolii* W. T. Marshall, *E. chisosensis* W. T. Marshall, *E.*

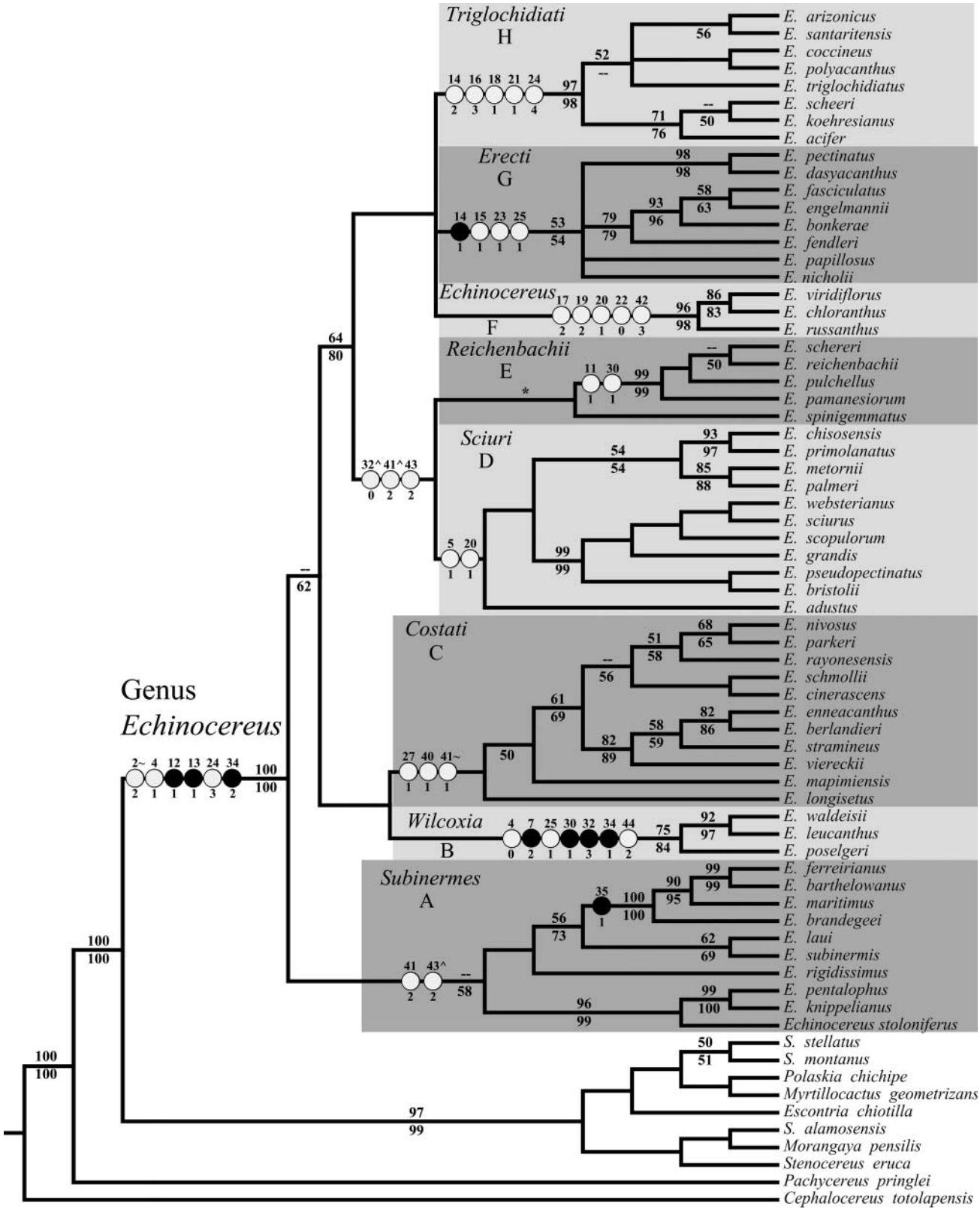

Fig. 1. Strict consensus tree from six most parsimonious trees from MP analysis (combined matrix). Length = 898 steps, Consistency Index = 0.44 and Retention Index = 0.73. Numbers above/below branches represent bootstrap/jackknife values. Unambiguous character optimization are represented by circles on branches, black circle = synapomorphy; white circle = homoplasy. Numbers above/below circles indicate character/state (see character list, Appendix 1, see supplemental material online); ^ specifies deltran optimization; ~ specifies acctran optimization. * indicates that clade was supported by molecular characters rather morphological characters.

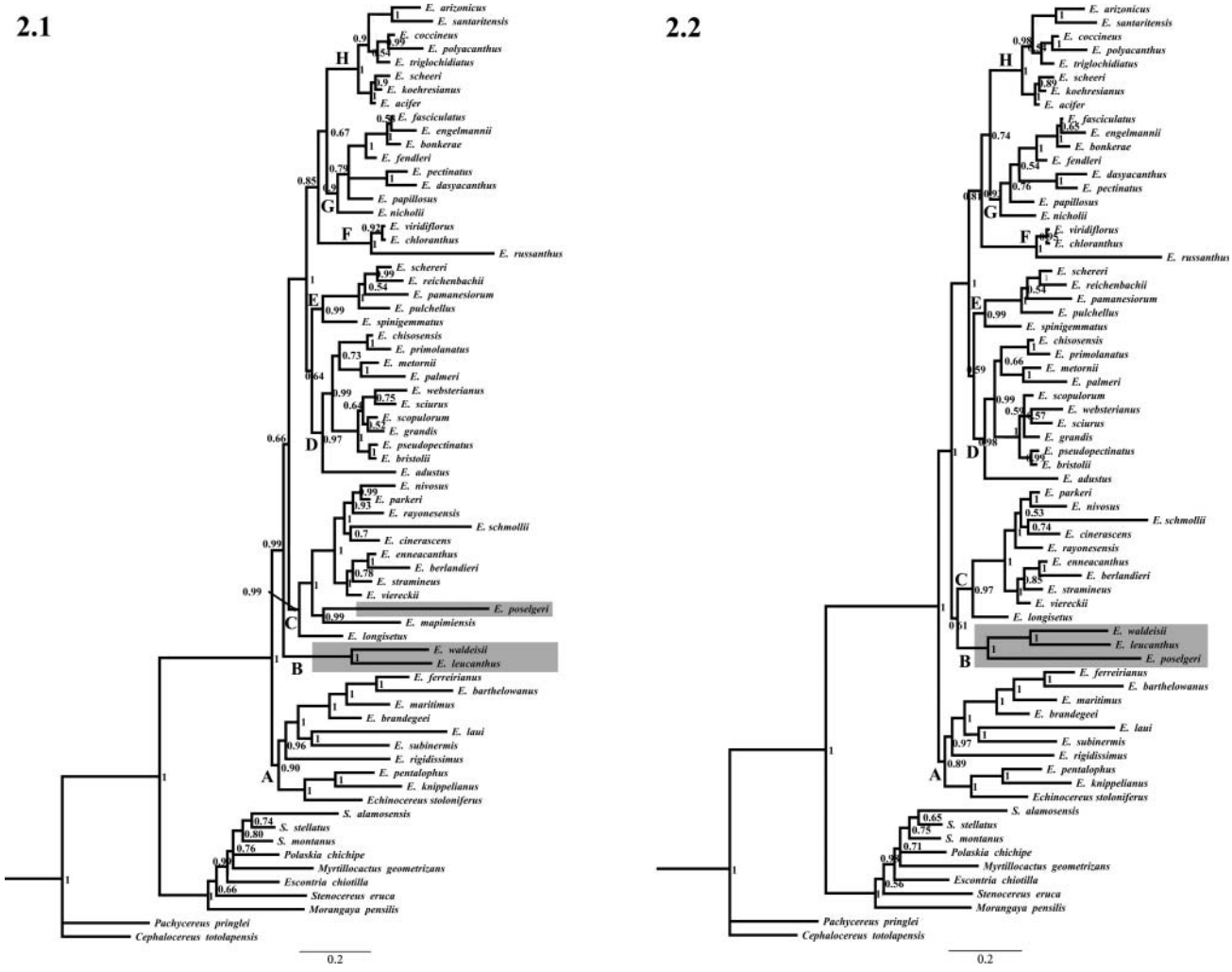

Fig. 2. Majority consensus trees shown as phylograms from the BI analyses (combined matrix), the grey shadow shows *E. poselgeri* and sister species (Hunt et al., 2006). **2.1**. Phylogram from the IB analysis including all taxa. **2.2**. Phylogram from the LBE test excluding *E. mapimiensis*.

grandis Britton & Rose*, *E. metornii* G. Frank, *E. palmeri* Britton & Rose, *E. primolanatus* Fritz Shwarz ex N. P. Taylor, *E. pseudopectinatus* (N. P. Taylor) N. P. Taylor*, *E. sciurus* (K. Brandegee) Dams*, *E. scopulorum* Britton & Rose*, *E. websterianus* G. E. Linds*. * Included in the informal species group *Sciurus* according to Blum et al. (1998).

Section *Reichenbachii* N. P. Taylor, *Gen. Echinocereus*: 105 (1985). Type species: *Echinocereus reichenbachii* (Terscheck ex Walp.) Haage.
Echinocereus section *Pulchellus* N. P. Taylor, *Gen. Echinocereus*: 140 (1985).
Species included: *Echinocereus pamanesiorum* A. B. Lau, *E. pulchellus* (Mart.) C. F. Först ex F. Seitz, *E. reichenbachii* (Terscheck ex Walp.) Haage, *E. schereri* G. Frank, *E. spinigemmatus* A. B. Lau.

Section *Echinocereus*. Engelm. *Cereus* sección *Sulcati* Engelm., *Mem. Amer. Acad. Arts. ser. 2*, 4: 50 (1849). Type species: *Echinocereus viridiflorus* Engelm.
Species included*: *Echinocereus chloranthus* (Engelm.) Haage, *E. russanthus* Weniger, *E. viridiflorus* Engelm.
*See discussion about other recognized species (above).

Section *Erecti* (K. Schum.) Bravo, *Cact. Suc. Mex*. 27: 16 (1982). *Echinocereus* serie *Erecti* K. Schum., Gesamtb. Kakt. 247 (1987). *Cereus* subsection *Erecti* (K. Schum.) Berger, *Rep. (Annual) Missouri. Bot. Gard.* 16: 80 (1905). Type species: *Echinocereus engelmannii* (Parry ex Engelm.) Lem.
Species included: *Echinocereus bonkerae* Thornber & Bonker, *E. dasyacanthus* Engelm., *E. engelmannii* (Parry ex Engelm.) Lem., *E. fasciculatus* (Engelm. ex S. Watson) L. D. Benson, *E. fendleri* (Engelm.) Rümpler, *E. nicholii*

(L. D. Benson) B. D. Parfitt, *E. papillosus* A. Linke ex Rümpler, *E. pectinatus* (Scheidw.) Engelm.

Section *Triglochidiati* Bravo, *Cact. Suc. Mex.* 28: 109 (1973). *Echinocereus* subgenus *Triglochidiatus* (Bravo) W. Blum, Mich. Lange & Rutow 1998: 357. Type species: *Echinocereus triglochidiatus* Engelm.

Species included: *Echinocereus acifer* (Otto ex Salm-Dyck) Jacobi, *E. arizonicus* Rose ex Orcutt, *E. coccineus* Engelm., *E. koehresianus* (G. Frank) W. Rischer, *E. polyacanthus* Engelm., *E. santaritensis* W. Blum & Rutow, *E. scheeri* (Salm-Dyck) Scheer, *E. triglochidiatus* Engelm., *E. yavapaiensis* M. A. Baker*. *See discussion.

Key to *Echinocereus* sections

This is based on morphological characters used at present study and geographic distribution.

1a. Stem up to 2.2 cm diameter; roots thickened. . . . . *Wilcoxia*
1b. Stem 2.3–20 cm diameter; roots diffuse, or main root thickened 2
2a. Stem with fibrous cortical bundles; widespread in Baja California Peninsula . . . . . *Subinermes* (part)
2b. Stem without fibrous cortical bundles; widespread in N, central Mexico and S USA 3
3a. Flowers displaying a predominantly red (scarlet/carmine) perianth; larger inner stamens than outer ones; anthers purple (exceptionally yellowish) . . . . . *Triglochidiati*
3b. Flowers displaying white, yellow to brownish or pink perianth (exceptionally carmine); similar length between inner and outer stamens; anthers yellow 4
4a. Flowers yellow, yellowish-green, green to brown . . 5
4b. Flowers pink (including various shades of pink), purple or white. 7
5a. Stem mostly solitary (unbranched); flower <5 cm long *Echinocereus*
5b. Stem solitary or branched; flower >5 cm long 6
6a. Receptacular tube funnel-shaped; widespread in NW Mexico . . . . . *Subinermes* (part)
6b. Receptacular tube broadly funnel-shaped; widespread in N & NE Mexico and adjacent regions of S USA . . . . . *Erecti* (part)
7a. Flowers pink to purple; receptacular tube broadly funnel-shaped and > 4 mm thickness . . . . . *Erecti* (part)
7b. Flower pink; receptacular tube funnel-shaped and <4 mm 8
8a. Stem mostly solitary; nectarial tissue in basal position; trichomes on receptacular tube >1.5 mm long; tepals base <2mm thickness 9
8b. Stems mostly branched; nectarial tissue in basal or lateral position; trichomes on receptacular tube > or <1.5 mm long 11
9a. Central spines present . . . . . *Sciuri*
9b. Central spines none 10
10a. Stem spines bright pink or pink and white, forming alternate colour bands around stem; fruit pulp juicy . . . . . *Subinermes*
10b. Stem spines grey or reddish, not forming alternate colour bands around stem; fruit pulp semi-dry . . . . . *Reichenbachii* (part)
11a. Trichomes on receptacular tube ≤1.5 mm long 12
11b. Trichomes on receptacular tube >1.5 mm long 13
12a. Tepals with tannins into epidermal cells (turns brown in conservation fluid); seeds 1.3–1.7 mm long . . . . . *Costati* (part)
12b. Tepals without tannins into epidermal cells, seeds 0.9–1.2 mm long . . . . . *Reichenbachii*
13a. Stem 4–8 ribs; spines yellowish . . . . . *Subinermes* (part)
13b. Stem > 9 ribs; spines white 14
14a. Stem up to 2.5 cm diameter; spines setous; flower >5 cm long . . . . . *Costati*
14b. Stem >2.6 cm diameter, spines rigid; flower <5 cm long. . . . . *Subinermes*

Discussion

Long-branch attraction effect in *Echinocereus*

Different phylogenetic reconstruction methods (MP, BI, or ML) using the same data set commonly result in topologies with minor differences or differences in support values (Rindal & Brower, 2011). The same pattern is observed in phylogenetic analyses on Cactaceae; however, the causes of these differences in topologies have not been discussed (e.g., Demaio et al., 2011; Vázquez-Sánchez et al., 2013). Our results of the MP and BI analyses showed a strong inconsistency in the phylogenetic position of *E. poselgeri*, a very distinctive taxon within *Echinocereus* (see discussion of section *Wilcoxia* below). In the MP analysis, *E. poselgeri* was recovered as sister of *E. leucanthus* and *E. waldeisii*, forming clade B with moderate support (BS 75%, JK 84%; Fig. 1). In the BI analysis, *E. poselgeri* was grouped in clade C with high support (BPP 0.99) as a sister to *E. mapimiensis* (Fig. 2.1). However, the LBE test using the same BI parameters and excluding *E. mapimiensis* recovered *E. poselgeri* within clade B as a sister species to *E. leucanthus* and *E. waldeisii* (BPP = 1; Fig. 2.2), as it was grouped in the MP analysis (Fig. 1).

Lartillot, Brinkmann, and Philippe (2007) proposed that the use of a site heterogeneous model (e.g., CAT-GTR) in a phylogenetic analysis suppresses long-branch artefacts; however, the BI analyses of the DNA matrix using the CAT-GTR model did not show any change in the position

of *E. poselgeri* (Fig. S1). Other authors suggest adding morphological characters to the analyses (Bergsten, 2005) or using MP analysis (Pol & Sidal, 2001) as a strategy to obtain more accurate topologies and avoid LBA problems. Our results showed that with the inclusion of morphological characters in the MP analysis, the LBA effect on *E. poselgeri* relationships could be avoided. This result is consistent with the conclusions of Kolaczkowsky and Thornton (2009) who suggest that LBA bias can affect BI analyses. MP analysis was not the most susceptible method to improperly group taxa through the LBA effect. We surmised that the results of the MP analysis were better because all taxa sampled were included and the analysis was not affected by LBA. Therefore, based on the principles of ontological and epistemological congruency in phylogenetic analyses (Assis & Rieppel, 2011), the strict consensus tree from the combined MP analysis was used to describe the phylogenetic relationships in *Echinocereus* and to optimize the characters to recognize synapomorphies and homoplasies that supported the main clades. Bayesian posterior probabilities (BPP) from the LBE test were added for comparison in the support data of each clade.

Combined analyses of *Echinocereus*

The combined analyses of morphological and molecular characters corroborated that the genus *Echinocereus* was a monophyletic group with high support (Figs 1, 2), as was proposed previously (Sánchez et al., 2014). Wortley and Scotland (2006) suggested that a combined analysis positively affects topology resolution, but does not necessarily elevate the support values, which was observed in our results. The strict consensus tree showed a decrease in the support values in some clades (e.g., clade A; Fig. 1) and an increase in those values in some other clades (e.g., clade H, Fig. 1), compared with previous studies (Sánchez et al., 2014). The decrease in certain support values was due to several vegetative characters (i.e., stem diameter, number of ribs and number of central spines; Appendix 1, see supplemental material online) that were revealed as homoplasies but have been useful in species group delimitations (Baker, 2006a, 2006b; Sánchez et al., 2013). However, according to de Carvalho (1996), although several clades had low support values, an analysis resulting in few parsimonious trees (6 in our analysis) is evidence of congruence amongst data. Incorporation of a set of morphological characters and the *trnK/matK* marker allowed the inclusion of *E. chloranthus*, *E. russanthus* and *E. papillosus* and recovered a more resolved relationship of the main clades and grouped *E. poselgeri* in a morphologically congruent clade.

Although synapomorphies are preferred as evidence of monophyly, homoplasies are also important because they can support many of the nodes in a phylogeny; thus, they are fundamental in a group diagnosis (Assis, 2009; Assis & Riepel, 2011; de Carvalho, 1996; Nixon & Ochoterena, 2000). The character optimization on the MP strict consensus tree showed that morphological and molecular characters (including indels) were important in the definition of the clades recovered in *Echinocereus*. Therefore, the genus and the main clades were defined by synapomorphies (when present) and/or a combination of homoplasies, as determined for other angiosperm lineages (Hughes, Lewis, Yomona, & Reynel, 2004; Norup et al., 2006).

Echinocereus and its infrageneric classification. *Echinocereus* (Figs 1, 2) was defined by a combination of six morphological characters: erumpent buds, green stigmas, non-fibrous secondary xylem, cylindrical growth form, and stem diameter from 3 to 15 cm, with three of them as synapomorphies (Fig. 1; Appendix 1, see supplemental material online). Erumpent buds are reported for all *Echinocereus* species and described in detail by Sánchez et al. (2015) who suggest that this trait protects buds from extremely low winter temperatures and favoured lineage diversification in the temperate and semiarid regions of northern Mexico and the south-west USA. Green stigmas are reported in *Opuntia robusta* H. L. Wendl. (Bravo-Hollis, 1978) and *Mammillaria dioica* K. Brandegee (Bravo-Hollis & Sánchez-Mejorada, 1991), and non-fibrous secondary xylem is described for several species in the tribe Cacteae (Vázquez-Sánchez & Terrazas, 2011). Cylindrical growth form is a distinctive character in *Echinocereus* because it is related to other lineages within Echinocereeae that have a tendency to show a tree-like or scrub-like columnar growth form. DNA sequences defined *Echinocereus* by 15 synapomorphic sites (two in *psbA-trnH*, three in *rbcL*, three in *rpl16*, four in *trnK/matK*, one in *trnL-F*, and two in *trnQ-rps16*) and two homoplasic sites (one in *psbA-trnH* and one in *trnQ-rps16*), in addition to the absence of two indel events in *psbA-trnH* and *trnL-F*. The genus is currently divided into eight sections (Hunt et al., 2006; Table 1); however, this classification of the genus is not supported by the phylogenetic relationships of the genus. Therefore, we propose an infrageneric classification of *Echinocereus* based on phylogenetic information that includes a brief discussion of each section.

Section *Subinermes* (Clade A, Figs 1, 3.1, 3.2)

This group of 10 species with heterogeneous morphology included taxa previously classified in sections *Erecti*, *Pulchellus*, and *Reichenbachii* (*sensu* Hunt et al., 2006). This section was supported by two morphological characters, a hemispheric periclinal cell wall in the lateral region of testa seed and the cotyledon size (Figs 1, 3.2; Appendix 1,

see supplemental material online), plus one synapomorphic site of DNA in the *rpl16* marker. Sánchez et al. (2014) also recovered this group and with better support values. Within this clade, a first group formed by *Echinocereus stoloniferus*, *E. pentalophus*, and *E. knippelianus* was primarily supported by several synapomorphies in DNA sequences (one site in the *rbcL*, *trnK/matK*, and *trnL-F* markers and two sites in the *trnQ-rps16* marker); however, the group can be recognized by the rhizomes. A distinctive subgroup with high support was composed of four endemic species from Baja California and the Gulf of California, which were included as part of section *Erecti* (Hunt et al., 2006; Taylor, 1985). This group had cortical bundles with phloic fibres and six DNA sites as synapomorphies (two in each marker: *rpl16*, *trnK/matK*, and *trnL-F*). Taylor (1985) claimed that these species do not show erumpent buds, but a recent anatomical study corroborated the development of erumpent buds in the group (Sánchez et al., 2015).

Section *Wilcoxia* (clade B, Figs 1, 3.3, 3.4)

This clade was composed of three species classified in section *Wilcoxia* (Blum et al., 2008; Hunt et al., 2006) but did not include *Echinocereus schmollii*, which was grouped into the sister clade (clade C, section *Costati*). *Echinocereus poselgeri* was grouped with *E. leucanthus*, which represented its sister species (Blum et al., 2008; Taylor, 1985). In previous phylogenies (Arias et al., 2005; Sánchez et al., 2014), based on the LBA effect (discussed above), *E. poselgeri* and *E. leucanthus* were not determined as sister species. Based on our current results, this section was characterized by tuberous roots (Fig. 3.4), the elliptic form of fruit, fibrous rayless wood and non-collenchymatic hypoderm in the stem; in addition to a columnar growth form, a stem diameter less than 2.2 cm and rugose ornamentation in the lateral region of testa seed (Appendix 1, see supplemental material online). DNA sequences also supported this clade with four homoplasic sites in the *trnK/matK* marker. Section *Wilcoxia* represents a lineage with high specialization in stem and root (Taylor, 1985) because the aforementioned traits allow it to clamber over surrounding bushes. The fibrous, rayless wood provides better support to the long and thin stem (Loza-Cornejo & Terrazas, 1996).

Section *Costati* (clade C; Figs 1, 3.5, 3.6)

This clade included 11 species of which nine were previously classified in section *Costati* (*sensu* Hunt et al., 2006); hence, this section must be expanded to integrate *Echinocereus mapimiensis* and *E. schmollii*. Unlike in the previous molecular phylogeny (Sánchez et al., 2014), *E. longisetus* was grouped in this clade. Short trichomes on the areolas of the receptacular tube, transparent spines on the receptacular tube in fixing solution, an embryo with large cotyledons and tannins in the epidermis of tepals supported this section (Fig. 1; Appendix 1, see supplemental material online). Additionally, acctran optimization recognized one homoplasy of the *trnL-F* marker for this section. Tannins in the tepal epidermis are observed in several members of the sister group of *Echinocereus* (e.g., *Escontria chiotilla* and *Myrtillocactus geometrizans*; Fuentes, 2004) and several members of this clade (Sánchez, unpubl. data). The tannin character is easily recognized because flowers turn brown when they are fixed in formalin (Taylor, 1993; Fig. 3.6). The inclusion of *E. schmollii* in *Costati* remains controversial because it does not share any of the diagnostic characters; in this study, *E. schmollii* was grouped in this clade because of molecular characters (several homoplasic sites in *trnQ-rps16*).

Section *Sciuri* (clade D; Figs 1, 3.7, 3.8)

This group included 11 species from sections *Reichenbachii* and *Pulchellus sensu* Hunt et al. (2006). Commonly unbranched stems and a nectary with basal nectarial tissue supported the clade (Fig. 3.8; Appendix 1, see supplemental material online). Although *Echinocereus adustus* has a large nectary, flower anatomy showed basal nectarial tissue (Sánchez, unpubl. data). Furthermore, DNA sequences showed one homoplasic site in the *rpl16* marker and one synapomorphic indel in the *psbA-trnH* marker (simple sequence repetition of four bases).

Section *Reichenbachii* (clade E; Figs 1, 3.9, 3.10)

This clade recovered five species previously included in sections *Reichenbachii* and *Pulchellus* (*sensu* Hunt et al., 2006) and was also recognized in the molecular phylogeny of Sánchez et al. (2014). DNA sequences defined this group with one synapomorphic site in the *rpl16* marker and one homoplasic site and one homoplasic indel in the *trnL-F* marker (deletion of 54 sites). *Echinocereus spinigemmatus* was the earliest diversified taxon in this clade; the remaining species formed a clade with strong support (BS = 99%, JK = 99%; Fig. 1) and shared two morphological characters: fruits with semidry pulp (Fig. 3.10) and stem areoles without central spines (Fig. 1; Appendix 1, see supplemental material online).

Section *Echinocereus* (clade F; Figs 1, 3.11, 3.12)

This clade included three species that had been previously classified into section *Echinocereus sensu* Hunt et al. (2006). This section was recognized by flower length less

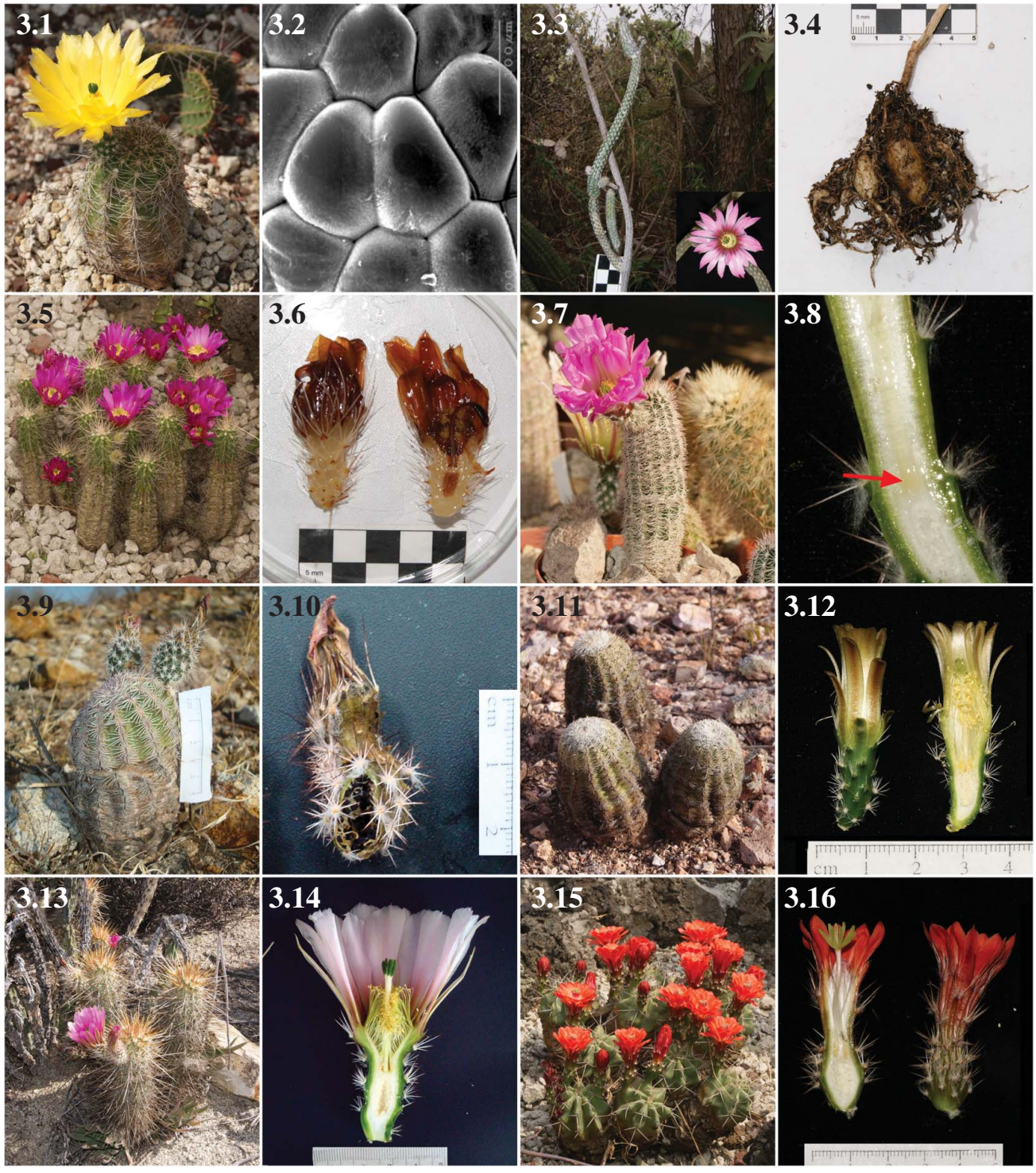

Fig. 3. Members of the recognized sections in *Echinocereus* and their distinctive characters. **3.1**. *E. stoloniferus* (*D. Sánchez 32*, MEXU). **3.2**. Periclinal wall of the lateral side of seed in *E. pentalophus* (*S. Arias 1746*, MEXU). **3.3**. *E. poselgeri* (*S. Arias 2129*, MEXU). **3.4**. Tuberous roots in *E. poselgeri* (*S. Arias 1492*, MEXU). **3.5**. *E. viereckii* (*S. Arias 1996*, MEXU). **3.6**. Tannins into tepals epidermis of *E. berlandieri* (*S. Arias 1454*, MEXU). **3.7**. *E. metornii* (*D. Sánchez 83*, MEXU). **3.8**. Floral nectary of *E. adustus* (*D. Sánchez 23*, MEXU). **3.9**. *E. schereri* (*D. Sánchez 72*, MEXU). **3.10**. Fruit with semi-dry pulp in *E. schereri* (*D. Sánchez 72*, MEXU). **3.11**. *E. viridiflorus* (*D. Sánchez 80*, MEXU). **3.12**. Flower morphology in *E. viridiflorus* (*D. Sánchez 80*, MEXU). **3.13**. *E. engelmannii* (*D. Sánchez* s. n.). **3.14**. Flower morphology in *E. fendleri* (*S. Arias 2023*, MEXU). **3.15**. *E. coccineus* (*D. Sánchez 79*, MEXU). **3.16**. Flower morphology in *E. polyacanthus* (*D. Sánchez 24*, MEXU).

than 4 cm, nectary length 1–2 mm (Fig. 3.12), basal nectarial tissue and small seeds (Fig. 1; Appendix 1, see supplemental material online). DNA sequences defined the clade with four synapomorphic sites in the *trnK/matK* marker. Additionally, this section was easily identified by the combination of both flower length and flower colour (yellow and/or brown). Recently, Blum, Felix, and Bauer (2012) described new taxa and recognized some infraspecific taxa as species of this section (e.g., *E. blumii* and *E. canus*), although the taxonomic status of those taxa should be corroborated with more systematic studies.

Section *Erecti* (clade G; Figs 1, 3.13, 3.14)

This clade recovered eight species of the section *Erecti* (*sensu* Hunt et al., 2006) and was characterized by a wide, funnel-shaped receptacular tube, a thickness of the receptacular tube more than 4 mm, a thickness of the base of tepals more than 2 mm, and dark colour in the flower throat (Fig. 3.14; Appendix 1, see supplemental material online). DNA sequences supported this group with one synapomorphic site, one homoplasic site and one synapomorphic indel (14 sites) in the *rpl16* marker. Unlike the previous molecular phylogeny (Sánchez et al., 2014), *E. nicholii* was grouped in this clade with the addition of morphological characters.

Section *Triglochidiati* (clade H; Figs 1, 2, 3.15, 3.16)

This lineage has been largely recognized based on its distinctive floral morphology (Taylor, 1985) and has even been proposed as a subgenus of *Echinocereus* (Blum et al., 1998). All species of this clade shared a narrow, funnel-shaped receptacular tube, a receptacular tube 1.5-fold larger than the perianth, larger inner stamens than outer ones, purple anthers, a predominantly red perianth and an embryo with large cotyledons (Fig. 3.16; Appendix 1, see supplemental material online). Those floral traits are cited as adaptations to hummingbird pollination syndrome (Cota, 1993; Taylor, 1985). Moreover, one synapomorphic site (in the *trnK/matK* marker) and four homoplasic sites (one in *psbA-trnH* and *rpl16* and two in the *trnL-F* markers) in DNA sequences supported the section. Although *E. yavapaiensis* was not included in the analysis because of a lack of available molecular data, floral morphology suggested its relationship with members of this section.

Adaptive significance of distinctive traits in *Echinocereus*

Growth form. Because of the diversity in succulence and stem form, the evolution of growth form (Buxbaum, 1951) and its conceptualization (Vázquez-Sánchez, Terrazas, & Arias, 2012) have been of particular interest in Cactaceae. In tribe Echinocereeae, growth form is extraordinarily diverse and more character states were proposed to cover the diversity (Appendix 1, see supplemental material online); however, the erect columnar growth form dominated in shrubs and trees of the tribe. In *Echinocereus*, ancestral growth form was ambiguous under unequivocal optimization (erect cylindrical/erect columnar; Fig. 4); although erect cylindrical growth form was ancestral under ACCTRAN optimization (Fig. 1), and according to Sánchez et al. (2014) is the most likely ancestral state for the genus. From erect cylindrical growth form, three additional states were independently derived in (Fig. 4): depressed globose (e.g., *E. subinermis*), erect columnar (e.g., *E. poselgeri*), and decumbent cylindrical (e.g., *E. scheeri*). The ancestral growth form with short stems probably represents a paedomorphic change, which resulted in the retention of juvenile traits in derived species (Box & Glover, 2010). Therefore, a decrease in stem length in *Echinocereus* allowed it to reach the reproductive stage in a few years of growth, resulting in short generations. According to Smith and Donoghue (2008), the rate of molecular evolution (promoting diversification) is higher in lineages with short generations than that in lineages with longer generations. Growth form can be related to certain anatomical modifications in the stem; species with a columnar growth form have fibrous wood (Loza-Cornejo & Terrazas, 1996), whereas other species with a decumbent cylindrical growth form have only fibrous patches in the non-fibrous wood matrix (Sánchez, unpublished data). The relationship between growth form and anatomical characters can be evaluated using allometric analyses (Vázquez-Sánchez & Terrazas, 2011).

Storage roots

A simple taproot system is common in Cactaceae, but some taxa can store water in their roots by thickening their parenchymatic tissue (Buxbaum, 1951). Storage roots in *Echinocereus* are absent in ancestors, but a simple taproot system developed into two new states during evolution of the genus, depending on the allocation of the storage tissue (Fig. 4). A thickened main root (e.g., *E. palmeri*) appeared in parallel in six species, whereas thickened lateral roots (e.g., *E. leucanthus*) evolved once and defined section *Wilcoxia*. Most likely, thickened roots (main or lateral) originated as a result of the loss of storage tissue due to decreases in length and diameter; thus, root thickening replaced the role of a water storage organ. This strategy is also observed in other species with thin or short stems (e.g., *Ariocarpus agavoides*, Bravo-Hollis, 1978; *Peniocereus* spp., Arias et al., 2005).

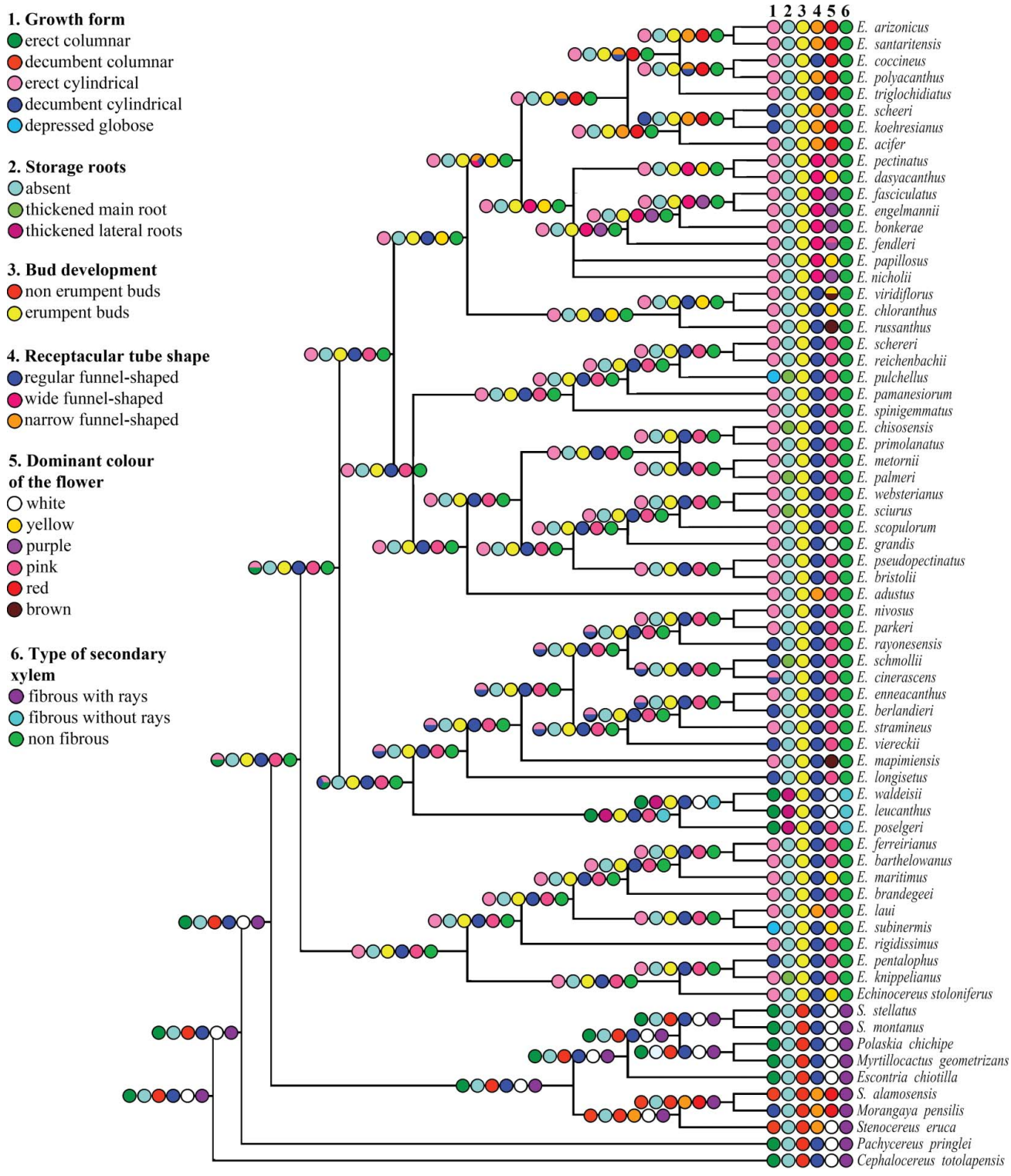

Fig. 4. Summary of character state history for six morphological characters selected of *Echinocereus* and outgroup, on the strict consensus tree from the MP analysis of the combined matrix. For details about character definition and character states see Appendix 1 (see supplemental material online).

Receptacular tube shape

According to Arias and Terrazas (2006), flower shape in Cactaceae is a complex trait. In this study, flower shape was primarily defined by receptacular tube shape, although other characters, such as receptacular tube length and perianth length or colour, are also responsible for the wide floral diversity. In *Echinocereus*, the plesiomorphic state of the receptacular tube was a regular funnel shape, which transformed into two derived states (Fig. 4). The wide funnel-shaped state originated in two different lineages (section *Erecti*; e.g., *E. engelmannii*; and section *Costati*; e.g., *E. enneacanthus*), whereas the narrow funnel-shaped state originated in three different groups (section *Subinermes*, *E. laui*; section *Sciuri*, *E. adustus*; and section *Triglochidiati*; e.g., *E. acifer*). A regular funnel-shaped or a wide funnel-shaped receptacular tube indicates species that rely on diurnal pollination by Hymenoptera (Cota, 1993). Differences in receptacular tube length and perianth morphology promote a more restricted vector, excluding other visitors. A narrow funnel-shaped receptacular tube was typical in section *Triglochidiati*, although it was also found in other *Echinocereus* species (e.g., *E. adustus* and *E. laui*) and sister lineages (e.g., *Morangaya pensilis* and *Stenocereus alamosensis*). The narrow funnel-shaped receptacular tube acts as an exclusion trait because it does not allow entrance to any visitors (Cronk & Ojeda, 2008). In *Echinocereus*, for species with a diurnal bloom, the pollinator specificity is due to the perianth colour; red flowers attract hummingbirds (*Triglochidiati*, Taylor, 1985; Sánchez, pers. obs.), whereas pale pink flowers favour moth visits (e.g., *E. adustus*, Sánchez, pers. obs.).

We concluded that the approach followed in the phylogenetic study of *Echinocereus* represented an effective scheme to explore the systematics of diverse plant lineages. A first phylogeny base on cpDNA (Sánchez et al., 2014) allowed us to understand the genus limits, in addition to the relationships within the genus, and enabled a preliminary evaluation of the infrageneric classification. Although the use of nuclear markers is desirable, these markers have scarcely been probed in Cactaceae (Calvente, Zappi, Forest, & Lohmann, 2011; Edwards et al., 2005; Majure et al., 2012; Perez, Carstens, Rodrigues, & Moraes, 2016; Ritz et al., 2012). Additionally, the ITS marker has paralogues with a high degree of intra-individual polymorphism (Harpke & Peterson, 2006), which is not suitable for phylogenetic analyses. Therefore, the inclusion of a set of morphological characters represented the logical complement to corroborate and strengthen the molecular phylogeny. In summary, this work demonstrated the importance of combining morphological and molecular evidence because morphology allowed secondary signals to arise when interacting with molecular markers. Additionally, the combination of evidence avoided long-branch attraction and established the set of characters to diagnose the genus and its sections and to propose a formal classification for *Echinocereus*.

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Supplemental data

Supplemental data for this article can be accessed here: https://doi.org/10.1080/14772000.2017.1343260

Disclosure statement

No potential conflict of interest was reported by the authors.

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