Systematic Botany (1997), 22(3): pp. 529–542

Chloroplast DNA Evidence for Divergence in *Ferocactus* and its Relationships to North American Columnar Cacti (Cactaceae: Cactoideae)

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Communicating Editor: Anita F. Cholewa

ABSTRACT. An analysis of chloroplast DNA restriction site variation was undertaken to investigate the evolutionary divergence of *Ferocactus* and its possible relationship with North American columnar cacti of tribe Pachycereeae. Our chloroplast DNA study using parsimony-based phylogenetic reconstruction methods provides molecular synapomorphies to define major lineages within *Ferocactus* and columnar cacti of the tribe Pachycereeae. The issue of monophyly in *Ferocactus* remains problematic: it appears to be a paraphyletic assemblage derived from an *Echinocactus*-like ancestor from which three major lineages have evolved. Also, there is a lack of support for the hypothesized basal position of *F. flavovirens* and *F. robustus*, and no evidence was found to favor the phylogenetic relationship of *Ferocactus* with North American columnar cacti, in particular with *Escontria chiotilla*. For the columnar cacti, the study supports the monophyly of the tribe Pachycereeae as well as its two subtribes: Stenocereinae and Pachycereinae. In the Stenocereinae, *Stenocereus dumortieri* was found to be in a basal position, and forms a phylogenetically distinct lineage from *Stenocereus* s. str. and other columnar cacti such as *Escontria*, *Polaskia*, and *Myrtillocactus*. This supports the segregation of *S. dumortieri* from *Stenocereus*, and the resurrection of *Isolatocereus dumortieri* as a distinct genus.

Ferocactus Britton and Rose is distributed in the arid and semiarid regions of central and northern Mexico and southwestern United States, and is the fourth largest genus (25 to 30 species depending on the authority) within the tribe Cacteae (Barthlott and Hunt 1993). A number of species (11 to 13) are restricted to the Baja California Peninsula and surrounding islands. Taxa of *Ferocactus* exhibit a relatively wide variety of soil preferences and geographic ranges, and are associated with different plant communities. In general, the plants are globose, barrel-shaped or cylindric, branched or unbranched, and variable in height (Britton and Rose 1922; Bravo and Sánchez-Mejorada 1991). The species also exhibit variation in number, shape, color, length, and position of the spines. The flowers are actinomorphic, bee pollinated, with deltoid to orbicular scales, numerous stamens and several stigma lobes. The fruits are ovoid, fleshy or dry at maturity, some dispersing seeds by a basal pore (Lindsay 1955; Bravo and Sánchez-Mejorada 1991). The seeds are ovoid-globose, to elongate to reniform, variable in size, with black testa. *Ferocactus* has been characterized by the presence of reduced, gland-like spines [structures homologous to spines which have evolved in other genera, e.g., *Coryphantha* (Engelm.) Lem., *Hylocereus* (A. Berger) Britton and Rose and *Opuntia* Mill. (Buxbaum 1950), and *Stenocactus coptonogonus* (Lem.) A. Berger (Taylor 1980)] in the upper region of the areole (Bravo and Sánchez-Mejorada 1991; Barthlott and Hunt 1993), which is a character that distinguishes it from other morphologically similar genera such as *Echinocactus* Link and Otto and *Stenocactus* (K. Schum.) A. W. Hill. *Ferocactus* has a basic chromosome number of $x = 11$ (Pinkava et al. 1973) and all the species investigated so far are diploid (Cota et al. 1996).

The taxonomy of the genus has been discussed in several treatments. Nonetheless, some aspects regarding its origin and evolution remain unresolved. As for most groups within the Cactaceae, there has been a lack of extensive field research to document population-level variation. In addition, the relatively high level of morphological homoplasy and the lack of fossil records to determine character polarity make the establishment of species boundaries and assessment of phylogenetic relationships difficult. These factors, combined with the use of primarily vegetative morphological characters (which are likely to be environmentally influenced), has led to disagreements regarding the number of species recognized among authors. Britton and Rose (1922) proposed a classification in which they included 30 species. An ecological study of the genus by Lindsay (1955), proposed a total of 25 species with 10 varieties. More recently, in a taxonomic treatment of *Ferocactus* s. str., Taylor

(1984) recognized 23 species and 20 infraspecific taxa, while Bravo and Sánchez-Mejorada (1991) accepted 29 species. Finally, Unger (1992) provided a review of *Ferocactus* in which taxonomic descriptions and distributional data are discussed. Although his taxonomic delimitations were based on previous treatments, he presented a classification scheme which includes four sections and a list of naturally occurring hybrids.

Despite the substantial taxonomic work conducted during the past seven decades, a phylogeny for *Ferocactus* has not been presented. Moreover, the taxonomic uncertainty within the genus is evident: no classifications are similar yet the number of species are similar (e.g., 30, 29, 28, 25, 23). Neither Britton and Rose (1922) nor Lindsay (1955) used infrageneric taxonomic categories, whereas Bravo and Sánchez-Mejorada (1991), Taylor (1984), and Unger (1992) classified the genus using other taxonomic hierarchies, such as sections, groups and subgenera.

Among these taxonomic treatments, those proposed by Taylor and Clark (1983) and Taylor (1984) are of special importance because they integrated data from vegetative and reproductive characters, geography, and macro- and micromorphological seed coat characters. Furthermore, they provided a hypothesis for an evolutionary scenario regarding the origin and radiation of *Ferocactus*, as well as the presumably basal position of *F. flavovirens* (Scheidw.) Britton and Rose and *F. robustus* (Pfeiff.) Britton and Rose based on "unspecialized" vegetative characters. Because this information is essential to understanding the classification of the genus, these recent evolutionary hypotheses will be used to address aspects about the origin and phylogeny of *Ferocactus* in our discussion.

The significance of elucidating the phylogenetic position of *Ferocactus* within the tribe Cacteae (the predominant tribe in number of species within subfamily Cactoideae in North America), along with its patterns of speciation, is a necessary part of understanding the relationships between the tribes Pachycereeae and Cacteae. *Ferocactus* has been hypothesized to occupy a critical phylogenetic basal position from which other lineages possibly evolved, in both the Buxbaum (1951, 1958) and Barthlott and Hunt (1993) classifications for the Cacteae. On the other hand, the presence of morphologically similar floral scales have suggested some degree of relationship between *Ferocactus* and North American columnar cacti. Gibson (1988b, 1992) indicated that similarities in floral characters, such as sclerification in the bract tips or scales of the pericarpel and floral tube occur in both barrel cacti of tribe Cacteae (*F. flavovirens*) and Mexican columnar cacti of the tribe Pachycereeae, subtribe Pachycereinae [*Pachycereus* (A. Berger) Britton and Rose], and subtribe Stenocereinae [*Escontria chiotilla* (F. A. C. Weber) Rose, *Myrtillocactus cochal* (Orcutt) Britton and Rose, *Stenocereus stellatus* (Pfeiff.) Riccob.]. Thus, the development of a phylogenetic hypothesis for the genus will provide a sound basis for the subsequent determination of the evolutionary patterns with other genera of the Cacteae as well as potentially clarifying the relationships between the tribes Pachycereeae and Cacteae.

In this study, we examined chloroplast DNA (cpDNA) restriction site variation from selected taxa to investigate the monophyly of *Ferocactus* and to test the hypothesis of whether *F. flavovirens* and *F. robustus* are basal species within the genus. Also, we evaluated the phylogenetic and evolutionary relationships of *Ferocactus* with putatively allied North American columnar cacti of the tribe Pachycereeae.

Materials and Methods

Taxonomic Sampling. In this study, a total of 34 taxa were sampled (Table 1), including 15 species of *Ferocactus* representing the primary species groups and taxonomic sections as defined by Taylor (1984), with the exception of *F. recurvus* (Mill.) G. E. Linds. [specific epithet under *F. latispinus* (Haw.) Britton & Rose var. *spiralis* (Karw. ex Pfeiff.) N. P. Taylor, in Taylor (1984)], and four additional genera from tribe Cacteae. In addition to the outgroup (tribe Leptocereeae), 14 taxa from tribe Pachycereeae (sensu Gibson and Nobel 1986) were included. Living specimens for this study were obtained from various sources (Table 1) and were maintained under greenhouse conditions prior to DNA isolation. Institutions in which voucher specimens have been deposited are also listed in Table 1.

Analysis of cpDNA Restriction Site Variation. Genomic DNA was obtained by initially isolating plastids and/or total organelles in a modified organelle pellet method suitable for mucilaginous cactus tissues (Wallace 1995). With this method, living tissue was homogenized in a buffer containing 0.35 M sorbitol, 50 mM Tris-HCl (pH 8), 5 mM

TABLE 1. List of taxa used for cpDNA restriction site analysis. BCMEX = Universidad Autónoma de Baja California, CANTE = CANTE Botanic Garden, CONN = University of Connecticut, DES = Desert Botanical Garden, ISC = Ada Hayden Herbarium, HNT = Huntington Botanic Garden, and HUMO = Universidad Autónoma del Estado de Morelos. * = specific epithet according to Bravo and Sánchez-Mejorada (1991) and listed as *F. latispinus* var. *spiralis* (Karw. ex Pfeiff.) N. P. Taylor in Taylor (1984).

Tribe	Subtribe	Taxon	Sample number	Source/voucher
Leptocereeae		*Leptocereus quadricostatus* (Bello) Britton and Rose	1	*R. Ross s.n.*—ISC
Pachycereeae	Pachycereinae	*Bergerocactus emoryi* (Engelm.) Britton and Rose	2	HNT 16514A—HNT
		Carnegiea gigantea (Engelm.) Britton and Rose	3	*DES s.n.*—DES
		Lemaireocereus hollianus (F. A. C. Weber) Britton and Rose	4	HNT Bed 2N—HNT
		Lophocereus schottii (Engelm.) Britton and Rose	5	HNT 43975—HNT
		Neobuxbaumia euphorbioides (Haw.) Buxb.	6	HNT Bed 58–59—HNT
		Pachycereus marginatus (DC.) Britton and Rose	7	HNT Bed 2N—HNT
	Stenocereinae	*Escontria chiotilla* (F. A. C. Weber) Rose	8	*H. Cota 8041*—HUMO
		Myrtillocactus schenckii (Purpus) Britton and Rose	9	HNT 55789—HNT
		Polaskia chende (Gosselin) A. C. Gibson and E. Horak	10	HNT 630—HNT
		Stenocereus alamosensis (Coult.) A. C. Gibson and E. Horak	11	HNT Old 2, SE path—HNT
		S. dumortieri (Scheidw.) Buxb.	12	HNT 9550—HNT
		S. griseus (Haw.) Buxb.	13	DES 1953–4041-101—DES
		S. stellatus (Pfeiff.) Riccob.	14	HNT, BED 2N—HNT
		S. thurberi (Engelm.) Buxb.	15	HNT 20446—HNT
Cacteae	Echinocactinae	*Ferocactus cylindraceus* (Engelm.) Orcutt var. *Cylindraceus*	16	*L. Slauson 110*—DES
		F. pottsii (Salm-Dyck) Backeb. var. *alamosanus* (Britton and Rose) G. Unger	17	HNT 39309—HNT
		F. flavovirens (Scheidw.) Britton and Rose	18	*H. Cota 8051*—HUMO
		F. glaucescens (DC.) Britton and Rose	19	*C. Glass 6815*—CANTE
		F. gracilis H. E. Gates	20	*H. Cota 8034*—BCMEX, ISC
		F. hamatacanthus (Muehlenpf.) Britton and Rose	21	*C. Glass 6879*—CANTE
		F. histrix (DC.) G. E. Linds.	22	*H. Cota 8037*—CANTE
		F. latispinus (Haw.) Britton and Rose	23	*H. Cota 8039*—CANTE
		F. lindsayi Bravo	24	*M. Mendez 222*—CANTE
		F. macrodiscus (Mart.) Britton and Rose	25	*C. Glass 6234*—CANTE
		F. pilosus (Galeotti) Werderm.	26	HNT 28036—HNT
		F. echidne (DC.) Britton and Rose	27	HNT 6291—HNT
		F. recurvus (Mill.) G. E. Linds.*	28	*H. Cota 8047*—HUMO
		F. robustus (Pfeiff.) Britton and Rose	29	*H. Cota 8045*—HUMO
		F. wislizeni (Engelm.) Britton and Rose	30	*L. Slauson 112*—DES
		Echinocactus grusonii Hildm.	31	*R. S. Wallace s.n.*—CONN
		Stenocactus lloydii (Britton and Rose) A. Berger	32	*R. S. Wallace s.n.*—CONN
	Cactinae	*Coryphantha pallida* Britton and Rose	33	*H. Cota 8050*—HUMO
		Sclerocactus spinosior (Engelm.) Woodruff and L. D. Benson	34	*Hughes 2*—ISC

EDTA, 1% bovine serum albumin, and 5 mM 2-mercaptoethanol. The homogenate was filtered through a fine cloth (Miracloth, Calbiochem) and pelleted at 2,000 rpm at 4°C for 45 min.; the resultant organelle pellet was resuspended in 2X CTAB buffer (Doyle and Doyle 1987) and incubated at 60°C for 1 hr. The aqueous samples were then partitioned against 24:1 $CHCl_3$:octanol, precipitated with 2/3 volume 2-propanol (-20°C), and further purified with isopycnic ultracentrifugation in CsCl/ethidium bromide gradients. All samples were cut with a battery of 12 restriction endonucleases (*Ava*I, *Bam*HI, *Ban*II, *Bgl*II, *Bst*NI, *Cla*I, *Dra*I, *Eco*O109, *Eco*RI, *Eco*RV, *Hinc*II, and *Hind*III). The DNA fragments were separated in 1.0-1.5% agarose gels (TAE buffer system), bidirectionally transferred (Smith and Summers 1980) to nylon membranes (Zetabind, AMF-CUNO), and used for DNA hybridization experiments. Hybridization with nick-translated [^{32}P] plasmid probes followed conditions described by Jansen and Palmer (1987) and were conducted for 16–20 hr at 61°C in 4X SSC, 0.5% SDS, and 2.5X Denhart's solution with 25 µg/ml carrier DNA. Recombinant plasmid subclones for the entire chloroplast genome of *Nicotiana tabacum* L. (Shinozaki et al. 1986) obtained from J. Palmer (Indiana University) were used to assess restriction site variation, following standard methods (Palmer 1986).

Data Analysis. Restriction site variants were identified relative to the condition observed in the outgroup taxon *Leptocereus quadricostatus* (Bello) Britton and Rose and were scored for cladistic analysis as either absent (0) or present (1). The data matrix was analyzed using parsimony methods; no cells of the data matrix were scored as missing. The complete data matrix is available from the authors upon request. Phylogenetic reconstruction was conducted on a Power Macintosh 8500/120 Microcomputer using PAUP software version 3.1.1 (Swofford 1993). The cladistic analysis included the heuristic search option with closest addition sequence, MULPARS on, and tree-bisection reconnection (TBR) branch swapping. Bootstrap sampling analysis (Felsenstein 1985) was performed using two-hundred replications, and a strict consensus tree was computed. In addition, a decay analysis (Bremer 1988) for trees up to four steps longer than maximum parsimony was also performed to determine the robustness of the clades in the trees obtained. A strict consensus of each set of longer trees was computed.

RESULTS

Restriction Site Variants. The 12 restriction endonucleases used in this study provided a total of 247 variable restriction sites. Of these, 168 (68%) were shared by more than one taxon and were potentially phylogenetically informative; 79 (32%) of the remaining restriction sites were autapomorphic. The distribution of site changes scored throughout the chloroplast genome were 182 for single copy regions and 65 within the inverted repeat. All scored restriction site variants, their approximate location in the chloroplast genome, and the taxa that they characterize are included in Appendix I. Although no mutational "hotspots" were detected within the chloroplast genome, the large and small single copy regions were observed to have a greater number of mutations relative to the inverted repeats.

Phylogenetic Analysis. Cladistic analysis of the restriction site data including all characters yielded four equally most parsimonious trees with length of 317 steps, a Consistency Index (CI) of 0.779, and a Retention Index (RI) of 0.939. Excluding non-informative characters the CI was 0.703. Both the strict and 50% majority rule consensus trees exhibited the same topology; the strict consensus tree of this analysis including decay values, bootstrap values >50%, and number of restriction site changes is shown in Fig. 1. For the decay analysis, one step length increments up to four steps longer than the most parsimonious trees yielded 54, 434, 2,716, and 14,198 trees. When a much smaller outgroup sample was included in the analysis to evaluate the stability of the ingroup, the choice of outgroup had no effect on phylogenetic inferences of the ingroup and the overall topology of the cladogram.

The results from our study of cpDNA restriction site variation provide preliminary phylogenetic resolution within *Ferocactus*, and between *Ferocactus* and members of the tribe Pachycereeae. *Echinocactus grusonii* Hildm. is included as a basal lineage and sister to *F. glaucescens* (DC.) Britton and Rose and *F. histrix* (DC.) G. E. Linds.; this clade is supported by 74% bootstrap value and by decay analysis one step longer, suggesting paraphyly in *Ferocactus* (Fig. 1). Neither *F. flavovirens* nor *F. robustus* were found to be in a basal position and it appears that at least three primary lineages have evolved in *Ferocactus*.

At the generic level, molecular characters indi-

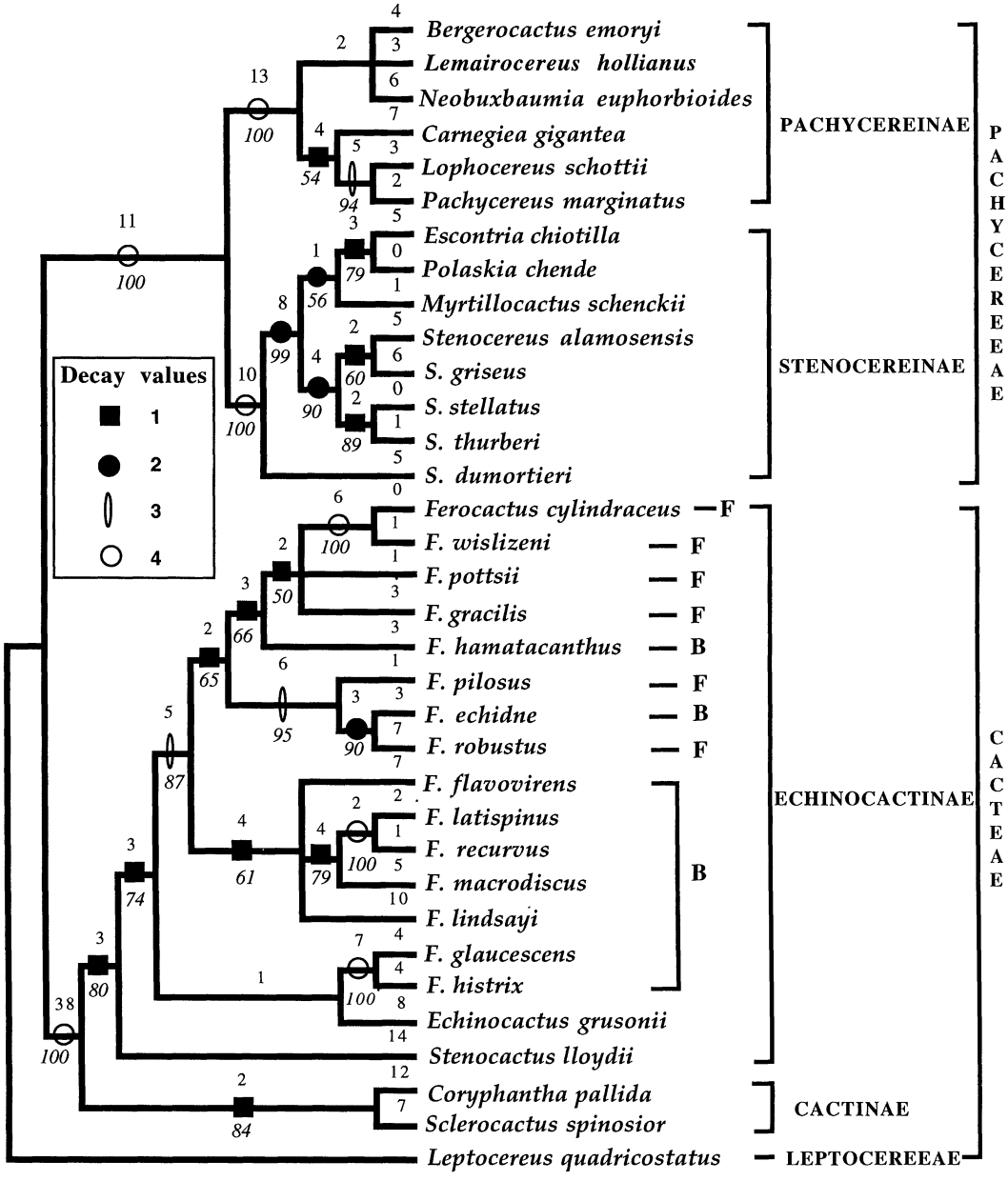

FIG. 1. Strict consensus tree of four most parsimonious trees of length 317, CI = 0.779 and RI = 0.939 including all characters. Numbers above branches indicate synapomorphic restriction site variants observed; italic numbers below the branches represent bootstrap percentages > 50% obtained from 200 replicates; symbols on branches are decay values for each node examined and represent the number of steps longer at which the clade is still supported. **F** = section *Ferocactus*, **B** = section *Bisnaga* as defined by Taylor (1984).

cate that within tribe Cacteae (subtribe Echinocactinae) *Stenocactus* is basal to *Ferocactus* and *Echinocactus*; three synapomorphies, 80% bootstrap value, and decay analysis one step longer support the basal position of *Stenocactus* in the phylogeny of the strict consensus (Fig. 1).

The analysis showed no direct phylogenetic relationship of *Ferocactus* (in particular *F. flavovi-*

rens), with *Escontria chiotilla* and other columnar members of subtribe Stenocereinae (Fig. 1). For the columnar cacti, the cpDNA-based phylogeny supports the monophyly of the tribe Pachycereeae (100% bootstrap and decay value four steps longer) and each of its two subtribes (Pachycereinae and Stenocereinae), and confirms the taxonomic distinction between *Stenocereus* (A. Berger) Riccob. and the group *Myrtillocactus* Console - *Escontria* Rose - *Polaskia* Backeb. within the Stenocereinae as evidenced by high bootstrap and decay values (Fig. 1). Finally, a high bootstrap value (100%) also supports *S. dumortieri* (Scheidw.) Buxb. as a basal lineage within the Stenocereinae, which suggests the taxonomic resurrection of *Isolatocereus dumortieri* (Scheidw.) Backeb.

Discussion

***Phylogeny of* Ferocactus.** Early phylogenetic studies of the North American Echinocacti have considered *Echinocactus* as the "most primitive" member of tribe Euechinocactinae, from which *Ferocactus*, *Sclerocactus* Britton and Rose and *Coryphantha* possibly evolved as independent lineages (Buxbaum 1951). Similarly, a basal position for *Echinocactus* is indicated by Buxbaum (1958) and Barthlott and Hunt (1993), however, none of these studies is based on cladistic analysis. In addition to the putative origin of *Ferocactus* from an *Echinocactus*-like ancestor, hypotheses regarding the paraphyletic and polyphyletic origin of *Ferocactus* have been postulated. Lindsay (1965) referred to the genus as a "non-natural group," which today is interpreted as being polyphyletic. Taylor (1980) proposed that *Ferocactus* and *Stenocactus* should be united based on morphological affinities. Similarities in rib pattern and presence of glandular spines in *S. coptonogonus* and *Ferocactus* suggested a common origin, and thus a close relationship between the two genera. Consequently, four species previously placed in *Stenocactus* were transferred to the newly established subg. *Stenocactus* of *Ferocactus*. Similarly, Buxbaum's (1951) phylogeny suggested that *Stenocactus* (*Echinofossulocactus*) derived from *Ferocactus*. Our cpDNA phylogeny argues in favor of both of these hypotheses: a possible paraphyletic origin of *Ferocactus* from a *Echinocactus*-like ancestor. The inclusion of *E. grusonii* within *Ferocactus* (Fig. 1) indicates that the latter may form a paraphyletic assemblage possibly derived from within *Echinocactus*.

Two lines of evidence support the affinities between *Ferocactus* and *Echinocactus*. First, decay (one step longer) and bootstrap (74%) values and three molecular synapormophies confirm the inclusion of *E. grusonii* as a basal lineage within the *Ferocactus* clade together with *F. glaucescens* and *F. histrix* (Fig. 1). Furthermore, when *Ferocactus* was forced to be monophyletic following Taylor's (1984) taxonomic treatment, i.e., excluding *E. grusonii*, the most parsimonious tree was seven steps longer (L = 324 versus L = 317), suggesting that this monophyletic group is not supported by the analysis. Second, on morphological grounds, both genera have features in common such as shape of the plant with many-ribbed stems, and the presence of areolar hair in some species [*F. hamatacanthus* (Muehlenpf.) Britton and Rose]. Moreover, early classifications of *Ferocactus* agree with the morphological relationships between *F. histrix* and *F. glaucescens*, which share some vegetative features with species of *Echinocactus*, particularly *E. grusonii* and forms of *E. platyacanthus* Link and Otto (sensu Bravo and Sánchez-Mejorada 1991). Although a morphological cladistic analysis which allows the identification of possible synapomorphies has not been performed, all of these taxa are relatively similar in general stem morphology (globose with a woolly apex) and have numerous ribs (20–30) arranged in a vertical or straight pattern as opposed to fewer ribs and spiral arrangement in some species of *Ferocactus*. Unlike the typical central spines of *Ferocactus* (hooked and flat in cross section), *E. grusonii*, *F. glaucescens*, and *F. histrix* lack the flat hooked central spine, and have straight or slightly curved spines that are nearly circular in cross section. Thus, the morphological affinities of *Echinocactus* with *F. histrix* and *F. glaucescens* argue in favor of the close phylogenetic relationship between *Ferocactus* and *Echinocactus* as shown in Fig. 1. Since *Echinocactus* contains six species in two subgenera (Bravo and Sánchez-Mejorada 1991), we consider it premature to conclude that *Ferocactus* is indeed a paraphyletic or polyphyletic unit until further studies are conducted with wider taxonomic sampling from within *Echinocactus* (in particular the type species *E. platyacanthus*) and *Stenocactus*.

In spite of the morphological resemblance of *Ferocactus* with *Echinocactus* and *Stenocactus* and recent taxonomic transfers, the phylogenetic relationships among them have remained unclear. This is in part due to difficulties in establishing directionality in character evolution. Contrary to previous hypotheses about the basal position of

Echinocactus suggested by Buxbaum (1951, 1958) and Barthlott and Hunt (1993), it seems that *Stenocactus lloydii* (Britton and Rose) A. Berger is basal within the Echinocactinae, at least for the taxa investigated herein (Fig. 1). Preliminary DNA sequence analyses of non-coding regions of the intergenic spacer between chloroplast genes *trn*L-*trn*F and the *rpl*16 intron also place *Stenocactus* spp. basal relative to these two genera (Cota and Wallace, unpubl. data).

***Putatively Basal Species and Major Lines of Evolution Within* Ferocactus.** The hypothesis that *F. flavovirens* (sect. *Bisnaga*) and *F. robustus* (sect. *Ferocactus*), two endemic species from the Tehuacán Valley in central Mexico, are the most ancestral species within the genus, is based on the assumption that these species are the least "specialized" within *Ferocactus* in both taxonomic sections (Taylor 1984; Taylor and Clark 1983). "Specialization" assumes that the shrubby semi-succulent habit found in *Pereskia* Mill. represents the ancestral type for the Cactaceae (reviewed in Gibson and Horak 1978; Taylor and Clark 1983). According to this hypothesis, plants with many-branched and narrow stems with few ribs should be considered least derived, whereas plants with unbranched stems and many ribs are more derived.

The presumably basal species *F. flavovirens* and *F. robustus* have retained several putatively plesiomorphic features, such as many-branched caespitose stems of small diameter with few ribs, few slender spines, and seed with a tabular testa. Likewise, *F. flavovirens* has been placed in a basal position within sect. *Bisnaga* due to the lack of specialized development of the glandular spines (Taylor 1987). Contrary to the hypothesis of an ancestral position for *F. flavovirens* and/or *F. robustus*, our study does not support either of these species as basal within *Ferocactus*. To evaluate the presumably basal position of these species following traditional taxonomic treatments (Taylor 1984), when *F. flavovirens* was forced in a basal position relative to the rest of *Ferocactus*, the tree length increased eight steps (L = 325); similarly, when *F. flavovirens* and *F. robustus* were placed as basal lineages in each of the clades in which they appeared in the maximum parsimony tree, the overall tree length increased 16 steps (L = 333). Thus, the lack of support for *F. flavovirens* and *F. robustus* as basal taxa suggest that the most likely basal species in *Ferocactus* are found within the lineage represented by *F. glaucescens-F. histrix-E. grusonii* (Fig. 1); this correlates with the basal placement of *Echinocactus* as a sister genus of *Ferocactus* in the phylogenetic scheme presented by Barthlott and Hunt (1993).

The taxonomic circumscription of *Ferocactus* into two sections represents the major divisions within the genus based on morphological characters (Taylor 1984). One lineage, (sect. *Bisnaga*), whose members are related to *F. flavovirens*, includes species distributed mainly in central Mexico and areas of the putative center of origin of the genus (Tehuacan Valley), while the other (sect. *Ferocactus*) includes species closely related to *F. robustus* and distributed in northern Mexico, Baja California, and southwestern U.S. Morphologically, the two sections are distinguished on the basis of fruit characters; members of sect. *Ferocactus* have dry fruits that dehisce by a basal pore, while the fruits of species in sect. *Bisnaga* are juicy and indehiscent, or occasionally splitting irregularly (Taylor 1984; Barthlott and Hunt 1993). Our phylogeny suggests that *F. flavovirens* and *F. robustus* appear to have evolved independently as suggested by Taylor and Clark (1983).

From our study, it is evident that at least three primary lineages have evolved within *Ferocactus* as currently circumscribed. Although sampling within *Ferocactus* was limited to selected representative taxa, the strict consensus tree (Fig. 1) shows areas of taxonomic disagreement with previous sectional delimitations in *Ferocactus*: one lineage includes taxa from sect. *Ferocactus* plus two species [*F. hamatacanthus* and *F. echidne* (DC.) Britton and Rose] placed in sect. *Bisnaga*. Therefore, monophyly in section *Ferocactus* is accepted with the inclusion of *F. hamatacanthus* and *F. echidne*. The second lineage corresponds to sect. *Bisnaga* which (as currently defined) is polyphyletic. Support for the monophyly of each of the *Ferocactus* and *Bisnaga* clades (including *E. grusonii*) is weak (decay value = 1), but when *E. grusonii*, *F. histrix* and *F. glaucescens* are excluded, sections *Ferocactus* and *Bisnaga* are more strongly supported by a decay value = 3 (Fig. 1). In addition to disagreement in the taxonomic position of *F. hamatacanthus* and *F. echidne*, *F. histrix* and *F. glaucescens* are grouped with *E. grusonii* in a position sister to the rest of *Ferocactus* (Fig. 1). Based on shared morphological and molecular synapomorphies, we are inclined to consider the complex *F. histrix-F. glaucescens-E. grusonii* as the third evolutionary lineage and sister to all ferocacti examined. Preliminary studies of DNA sequence data from the intron of the chloroplast gene *rpl*16 including two species of *Echinocactus* (*E. grusonii* and *E. platyacanthus*) indicate close relationship of *Ferocactus* and

Echinocactus, supporting in part a shared common *Echinocactus*-like ancestor early in the divergence of the Echinocactinae (Cota and Wallace, unpubl. data).

Relationships of* Ferocactus *with Columnar Cacti of Subtribe Stenocereinae. Cladistic analysis of our restriction site data (Fig. 1) provides no evidence to support a direct phylogenetic relationship between the columnar cacti *E. chiotilla* (or other Stenocereinae) and *Ferocactus* as hypothesized by Gibson (1992). As such, the presence of chartaceous scales and confluent areoles in both North American and South American columnar cacti and *F. flavovirens* appears to provide one more example of morphological parallelism in the Cactaceae. Gibson (1992) suggested that "if relatively short flowers were primitive for the *Escontria*-type columnar lineage, then the evolution of even shorter flowers in *Myrtillocactus* and *Ferocactus* of Mexico required only a short step." However, evolutionary changes in these lineages may not have been that simple and may have required multiple changes. Our results provide evidence that these sclerified scales have evolved in parallel in these lineages, and that their presence in distantly related taxa does not represent common ancestry.

Other authors (Buxbaum 1951; Gibson and Nobel 1986) have also suggested that homoplasious character transformations within *Ferocactus* may be common, as has been reported in other groups of the Cactaceae. Similarly, if there was any direct phylogenetic relationship between *Ferocactus* and pachycereoid columnar cacti, the taxa which may "link" these groups may have gone extinct or diverged significantly from their original form, making assessments of relationships difficult.

Phylogeny of Tribe Pachycereeae. Several studies involving North and South American columnar cacti of tribe Pachycereeae (Buxbaum 1958; Gibson 1982; 1988a; Gibson and Horak 1978; Gibson et al. 1986) have been conducted to elucidate its phylogeny. The wide geographic distribution, morphological variability and species diversity has made the classification of this tribe difficult, and the evolutionary history has yet to be resolved. In this regard, Gibson et al. (1986) indicated that tribe Pachycereeae can serve as a model to show the systematic complexities of the Cactaceae. The Pachycereeae may be characterized by predominately columnar species having silica bodies and pearl cells in the epidermal tissues (Gibson and Horak 1978; Gibson et al. 1986). Our molecular phylogeny provides 11 restriction site changes which support the monophyly of tribe Pachycereeae (Fig. 1). In addition, the data support the recognition of subtribes Pachycereinae and Stenocereinae, proposed by Gibson (1988a) and Gibson and Horak (1978). Some of the morphological features that have been used to define the subtribe Pachycereinae, such as the lack of funicular pigment cells, the absence of stem triterpenes, and seeds with smooth testa are interpreted as symplesiomorphies and cannot be used to define the subtribe phylogenetically (Gibson et al. 1986). Despite the absence of clearly identified synapomorphic morphological characters, our study provides strong support for the monophyly of this subtribe as indicated by relatively high bootstrap and decay values (Fig. 1).

Unique chemical and morphological characters have previously been used to support the monophyletic origin of the subtribe Stenocereinae. These include presence of specific stem triterpenes, epidermal silica bodies, special funicular pigment cells and areoles with red trichomes, all of which are not presumed to be under environmental influence (Gibson 1982; 1988a; Gibson and Horak 1978). Further division of the Stenocereinae has been based on the presence versus absence of epidermal silica bodies. The close relationship among *Escontria*, *Polaskia* and *Myrtillocactus* was first established by Gibson and Horak (1978) due to the lack of epidermal silica bodies in these taxa. The cpDNA phylogeny (Fig. 1) confirms those authors' phylogenetic concepts of the Stenocereinae with the presence of the subclade *Escontria*, *Polaskia*, and *Myrtillocactus* and supports the conclusions that *Stenocereus* should be considered as a separate monophyletic lineage. Finally, the cpDNA phylogeny supports the uniqueness of *S. dumortieri*, which had been also indicated by Gibson and Horak (1978) and Gibson (1991).

***Uniqueness of* Stenocereus dumortieri.** The genus *Isolatocereus* Backeb. was proposed by Backeberg (1942) and has been placed in synonymy under *Stenocereus* (Bravo 1978). Recently, Gibson (1991) presented the morphological similarities and differences between *Isolatocereus* and *Stenocereus* and concluded that although *S. dumortieri* exhibits three of the main characteristics of subtribe Stenocereinae (stem triterpenes, epidermis of the funiculus with idioblastic pigment cells, and *Stenocereus*-like seeds with verrucose testa), it should be recognized as a monotypic genus and separate from *Stenocereus*. The presence of a unique stem triterpene (oleanane triterpene dumortierigenin) placed it as a specialized and distinct taxon (Gibson

and Horak 1978). Our phylogeny agrees with Gibson's recognition of *Isolatocereus* as monotypic genus. In our phylogeny *S. dumortieri* appears in a basal position within subtribe Stenocereinae, which is well supported by decay analysis (Fig. 1). These results support the hypothesis of Gibson (1991), who stated "I must hypothesize that *Isolatocereus* diverged as an evolutionary branch before the origin of *Stenocereus*." We, therefore, favor the proposal of segregating *S. dumortieri* from *Stenocereus* and resurrecting *Isolatocereus*, in which its only species, *I. dumortieri*, diverged early in the evolution of the Stenocereinae. The morphological differences among this particular species and *Escontria chiotilla*, *Polaskia chende* (Gosselin) A. C. Gibson and E. Horak, and *Myrtillocactus schenckii* (Purpus) Britton and Rose are also reflected in our phylogeny based on cpDNA variation. The core of the Stenocereinae is composed of two major lineages; one containing the *Escontria-Polaskia-Myrtillocactus* clade and the other clade containing *Stenocereus* s. str.

In conclusion, our study provides insight into a possible paraphyletic or polyphyletic origin of *Ferocactus* from an *Echinocactus*-like ancestor, and the evolution of at least three major lineages within the genus. Although preliminary, these results lead to the possibility of new taxonomic circumscriptions if these results are confirmed in future studies including a larger number of terminal taxa in the *Ferocactus-Echinocactus-Stenocactus* complex. Finally, our results also provide evidence that the Pachycereoid columnar cacti, in particular *Escontria chiotilla*, are distantly related to *Ferocactus* and confirms the occurrence of homoplasious floral characters in these two phylogenetically distant lineages.

ACKNOWLEDGMENTS. We would like to thank the following persons and institutions for supplying plant material for this study. Charles Glass, Lee Hughes, Mario Mendez, Robert Ross, Liz Slauson, El Charco Botanic Garden (San Miguel de Allende, Guanajuato), The Desert Botanical Garden (Phoenix, AZ), and The Huntington Botanic Garden (San Marino, CA). The comments and suggestions made by Lynn G. Clark, Jonathan F. Wendel, Rich Cronn, and two anonymous reviewers in an early version of this manuscript resulted in a number of improvements for which we are thankful. This research was supported by grants from The Cactus and Succulent Society of America and The International Organization of Succulent Plant Study to JHC, and from The National Science Foundation, DBS/DEB-9207767 to RSW, which are gratefully acknowledged.

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APPENDIX 1. Restriction site changes in the cpDNA in taxa investigated. Region corresponds to tobacco cpDNA clone numbers. *See Table 1 for species names.

Enzyme	Region	Mut. no.	Site gain/loss	Observed fragments (kb)	Variant taxa*
*Ava*I	2/3	1	L	0.7 + 1.7 = 2.4	23, 25–32
*Ava*I	7	2	G	6.2 = 3.6 + 2.6	33, 34
*Ava*I	7	3	L	0.6 + 1.3 = 1.9	31
*Ava*I	7	4	G	5.7 = 1.5 + 4.2	29
*Ava*I	8	5	G	3.8 = 1.7 + 2.1	6
*Ava*I	9/10	6	G	5.4 = 1.4 + 4.0	27, 29
*Ava*I	12	7	G	8.6 = 3.3 + 5.3	6
*Ava*I	12	8	G	8.6 = 2.2 + 6.4	12
*Ava*I	14	9	G	1.7 = 1.2 + 0.5	33
*Ava*I	16	10	L	3.2 + 1.8 = 5.0	16, 17, 21, 26, 27, 29, 30, 32, 33
*Ava*I	20 A, B/21	11	G	13.2 = 6.0 + 7.2	11
*Ava*I	20 A, B/21	12	G	9.1 = 6.0 + 3.1	19, 22
*Ava*I	22	13	G	6.0 = 2.1 + 3.9	33
*Ava*I	24/25	14	G	8.5 = 5.2 + 3.3	8–15 (Stenocereinae) & 16–34 (Cacteae)
*Ava*I	27/28	15	G	2.9 = 1.4 + 1.5	8
*Ava*I	31/32	16	G	4.2 = 3.0 + 1.2	8–15 (Stenocereinae)
*Ava*I	31/32	17	L	1.1 + 1.3 = 2.4	16–34 (Cacteae)
*Ava*I	32/33	18	G	1.2 = 1.1 + [0.1]	2–15 (Pachycereeae)
*Ava*I	34	19	G	1.2 = 0.9 + [0.3]	12
*Ava*I	34	20	L	0.9 + 1.2 = 2.1	32
*Ava*I	34	21	G	1.2 = 0.7 + 0.5	33
*Ava*I	34	22	G	1.9 = 1.2 + 0.7	Cacteae (16–34) except 18 and 32
*Ava*I	34	23	L	1.2 + [0.2] = 1.4	29
*Ava*I	37	24	G	5.0 = 3.7 + 1.3	8
*Ava*I	37	25	G	5.0 = 2.7 + 2.3	32

APPENDIX 1. Continued.

Enzyme	Region	Mut. no.	Site gain/loss	Observed fragments (kb)	Variant taxa*
*Bam*HI	1	26	L	2.2 + 1.1 = 3.3	16–34 (Cacteaae)
*Bam*HI	1	27	L	1.4 + 8.6 = 10.0	24
*Bam*HI	1	28	G	1.4 = 1.0 + [0.4]	25
*Bam*HI	2	29	G	9.5 = 1.2 + 8.3	19, 22
*Bam*HI	9 A/B	30	G	5.2 = 3.0 + 3.2	16–34 (Cacteae)
*Bam*HI	9 A/B	31	G	2.5 = 1.4 + 1.1	2–15 (Pachycereeae)
*Bam*HI	10	32	G	2.2 = 1.7 + 0.5	18, 22
*Bam*HI	12/13	33	L	2.7 + 2.9 = 5.6	34
*Bam*HI	18/19	34	L	2.2 + 1.8 = 4.0	2–7 (Pachycereinae)
*Bam*HI	18/19	35	G	7.0 = 3.5 + 3.5	19, 22, 31
*Bam*HI	18/19	36	G	11.0 = 7.0 + 4.0	3, 7–10, 12–15, 16–34
*Bam*HI	21	37	G	0.8 = 0.6 + [0.2]	16–34 (Cacteae)
*Bam*HI	22/23	38	L	2.9 + 4.5 = 7.4	16–18, 20, 21, 23–30
*Bam*HI	24/25	39	G	3.5 = 2.7 + 0.8	2–7 (Pachycereinae)
*Bam*HI	24/25	40	G	4.4 = 2.1 + 2.3	16, 30
*Bam*HI	27/28	41	L	1.5 + 1.2 = 2.7	Stenocereinae (8–15), 21, 25
*Bam*HI	31	42	G	1.9 = 1.6 + [0.3]	3, 6
*Bam*HI	31	43	L	0.9 + [0.3] = 1.2	14, 15
*Bam*HI	31	44	G	2.9 = 2.3 + 0.6	23, 28, 32
*Bam*HI	32/33	45	L	1.2 + 3.5 = 4.7	5, 7, 11
*Bam*HI	34	46	G	1.7 = 1.2 + 0.5	2–15 (Pachycereeae) & 16–34 (Cacteae)
*Bam*HI	34	47	G	1.2 = 1.0 + [0.2]	Stenocereinae (8–15)
*Bam*HI	34	48	G	9.0 = 8.3 + 0.7	34
*Bam*HI	34	49	L	1.2 + [0.2] = 1.4	32
*Bam*HI	34	50	G	9.0 = 3.5 + 5.5	11, 13
*Bam*HI	37/38	51	G	11.8 = 2.8 + 9.0	8, 10
*Bam*HI	39	52	L	9.0 + 2.0 = 11.0	16–31
*Bam*HI	39	53	G	2.1 = 1.3 + 0.8	2–15 (Pachycereeae)
*Ban*II	3	54	L	0.5 + 1.0 = 1.5	16–34 (Cacteae)
*Ban*II	4/5	55	G	5.9 = 1.9 + 4.0	9
*Ban*II	7	56	G	3.0 = 2.0 + 1.0	16–34 (Cacteae)
*Ban*II	7	57	G	1.0 = 0.7 + [0.3]	31
*Ban*II	8	58	L	1.6 + [0.4] = 2.0	8, 9, 10, 11, 13, 14, 15
*Ban*II	9	59	G	1.8 = 1.0 + 0.8	31
*Ban*II	13	60	L	2.2 + [0.2] = 2.4	22
*Ban*II	16	61	L	3.4 + 2.0 = 5.4	8–15 (Stenocereinae)
*Ban*II	16	62	G	3.0 = 1.8 + 1.2	24
*Ban*II	22	63	L	2.7 + 0.9 = 3.6	11, 13
*Ban*II	22	64	G	2.5 = 1.9 + 0.6	32
*Ban*II	25/26	65	G	4.0 = 3.2 + 0.8	2–15 (Pachycereeae)
*Ban*II	25/26	66	G	2.7 = 2.0 + 0.7	2–7 (Pachycereinae)
*Ban*II	25/26	67	G	2.7 = 2.3 + [0.4]	11, 13, 14, 15
*Ban*II	25/26	68	L	4.1 + 2.9 = 7.0	32
*Ban*II	25/26	69	G	4.0 = 2.7 + 1.3	23, 28
*Ban*II	27/28	70	L	1.2 + 2.4 = 3.6	23, 28
*Ban*II	30/31	71	L	5.0 + 1.4 = 6.4	18, 23, 24, 25, 28, 34
*Ban*II	30/31	72	L	0.9 + [0.3] = 1.2	3
*Ban*II	30/31	73	L	1.4 + [0.5] = 1.9	16, 17, 20, 21, 22, 26, 27, 29, 30, 31, 32
*Ban*II	34	74	L	1.7 + 3.6 = 5.3	16–34 (Cacteae)
*Ban*II	34	75	L	1.0 + [0.7] = 1.7	2–15 (Pachycereeae) except 12
*Ban*II	34	76	L	1.7 + [0.2] = 1.9	2–7 (Pachycereinae)
*Ban*II	34	77	L	5.3 + 1.8 = 6.1	23, 28
*Ban*II	37/38	78	L	4.9 + 4.5 = 9.4	16, 17, 20, 21, 30
*Bgl*II	1	79	L	3.4 + 9.0 = 12.4	2–7 (Pachycereinae)
*Bgl*II	1	80	G	9.0 = 4.6 + 4.4	23, 24, 25, 28, 31

APPENDIX 1. Continued.

Enzyme	Region	Mut. no.	Site gain/loss	Observed fragments (kb)	Variant taxa*
*Bgl*II	1	81	G	8.0 = 5.3 + 2.7	16, 17, 20, 30
*Bgl*II	3	82	G	3.4 = 2.2 + 1.2	23, 28
*Bgl*II	4/5	83	G	3.4 + 9.0 = 12.4	8, 9, 10, 11, 13, 14, 15
*Bgl*II	9/10	84	G	9.1 = 5.2 + 3.9	3, 5, 7
*Bgl*II	9/10	85	G	9.1 = 6.0 + 3.1	11
*Bgl*II	12	86	G	8.7 = 6.7 + 2.0	16–34 (Cacteae)
*Bgl*II	13/14	87	G	4.4 = 3.4 + 1.0	29
*Bgl*II	14/14	88	L	2.0 + 2.4 = 4.4	33
*Bgl*II	16	89	G	1.1 = 0.9 + [0.2]	2–7 (Pachycereinae)
*Bgl*II	16	90	G	1.1 = 0.8 + [0.3]	16, 17, 18, 20, 21, 23–30
*Bgl*II	16	91	G	3.0 = 2.7 + [0.3]	17, 26, 27, 29
*Bgl*II	16	92	G	3.0 = 2.9 + [0.1]	16, 30
*Bgl*II	18/19	93	G	1.8 = 1.1 + 0.7	3
*Bgl*II	18/19	94	G	1.8 = 1.0 + 0.8	16–34 (Cacteae)
*Bgl*II	18/19	95	G	2.3 = 1.3 + 1.0	29
*Bgl*II	23	96	L	2.1 + 3.6 = 5.7	8–15 (Stenocereinae)
*Bgl*II	25	97	L	3.4 + 8.6 = 12.0	2–7 (Pachycereinae)
*Bgl*II	25	98	G	3.4 = 2.2 + 1.2	8, 9, 10, 11, 13, 14, 15
*Bgl*II	29/30	99	G	3.4 = 2.8 + 0.6	27, 29
*Bgl*II	29/30	100	G	3.4 = 2.4 + 1.0	3
*Bgl*II	29/30	101	G	3.0 = 0.9 + 2.1	16, 17, 20, 21, 30, 33
*Bgl*II	34	102	G	2.7 = 1.8 + 0.9	16–30 (Ferocactus), 31
*Bst*NI	2/3	103	G	6.1 = 3.3 + 2.8	24
*Bst*NI	5	104	G	3.6 = 1.2 + 2.4	11, 13, 14, 15
*Bst*NI	5	105	G	3.6 = 1.2 + 2.4	24
*Bst*NI	8	106	G	0.9 = 0.6 + [0.3]	2, 4, 5, 7, 8, 9, 10
*Bst*NI	9	107	G	2.5 = 1.5 + 1.0	2
*Bst*NI	9	108	G	2.5 = 2.3 + [0.2]	16–34 (Cacteae)
*Bst*NI	13	109	G	2.1 = 1.6 + 0.5	16–34 (Cacteae)
*Bst*NI	16	110	G	2.3 = 1.9 + 0.4	16, 17, 26, 27, 29, 30
*Bst*NI	16	111	G	2.3 = 1.8 + 0.5	33
*Bst*NI	18/19	112	G	1.2 = 1.1 + [0.1]	5, 7
*Bst*NI	20B	113	G	0.9 = 0.6 + 0.3	24
*Bst*NI	22	114	G	0.7 = 0.6 + [0.1]	8–15 (Stenocereinae)
*Bst*NI	24	115	G	5.9 = 3.5 + 2.4	3
*Bst*NI	27/28	116	L	1.2 + 2.2 = 3.4	3, 5, 7
*Bst*NI	27/28	117	G	1.2 = 0.9 + [0.3]	16–34 (Cacteae)
*Bst*NI	27/28	118	L	1.2 + 2.2 = 3.4	3, 5, 6, 7, 9, 11, 12, 13, 14, 15
*Bst*NI	27/28	119	L	3.4 + 1.0 = 4.4	3, 5
*Bst*NI	29A/B	120	L	1.1 + 0.2 = 1.3	8, 9, 10, 11, 13, 14, 15
*Cla*I	2/3	121	G	6.1 = 4.4 + 1.7	16–34 (Cacteae)
*Cla*I	2/3	122	L	1.7 + 3.9 = 5.6	24
*Cla*I	5	123	L	4.4 + 2.2 = 6.6	2–34
*Cla*I	7	124	L	1.1 + 2.2 = 3.3	25
*Cla*I	7	125	G	2.4 = 2.2 + [0.2]	4–7, 16–34
*Cla*I	8	126	G	1.8 = 1.0 + 0.8	2
*Cla*I	8	127	L	1.1 + 1.8 = 2.9	19, 27, 33
*Cla*I	10	128	L	1.4 + 1.0 = 2.4	32
*Cla*I	12/13	129	G	12.9 = 8.0 + 4.9	16–34 (Cacteae)
*Cla*I	16	130	L	0.7 + [0.5] = 1.2	4
*Cla*I	16	131	G	0.9 = 0.7 + [0.2]	2–7 (Pachycereinae)
*Cla*I	16	132	L	0.7 + 1.0 = 1.7	16, 17, 18, 20, 21, 23–30
*Cla*I	16	133	L	1.0 + 1.9 = 2.9	16, 17, 21, 26, 27, 29, 30
*Cla*I	16	134	L	2.9 + [0.2] = 3.1	16, 21, 30
*Cla*I	16	135	L	2.9 + 1.1 = 4.0	32
*Cla*I	18/19	136	L	2.8 + 1.6 = 4.4	16, 30

APPENDIX 1. Continued.

Enzyme	Region	Mut. no.	Site gain/loss	Observed fragments (kb)	Variant taxa*
*Cla*I	22	137	L	3.5 + 2.2 = 5.7	4
*Cla*I	22	138	G	5.7 = 3.5 + 2.2	2–34
*Cla*I	22	139	L	2.9 + 2.5 = 5.4	19, 20
*Cla*I	22	140	G	2.9 = 0.6 + 2.3	18, 21, 23, 24
*Cla*I	22/23	141	L	2.2 + [0.3] = 2.5	16–34 (Cacteae)
*Cla*I	22/23	142	G	3.5 = 2.9 + 0.6	16–34 (Cacteae)
*Cla*I	26/27	143	L	7.9 + 7.1 = 15.0	29
*Cla*I	27/28	144	G	7.9 = 5.0 + 2.9	24
*Cla*I	29/30	145	L	1.2 + [0.2] = 1.4	3, 5, 7
*Cla*I	30	146	L	1.2 + 0.7 = 1.9	18, 23, 24, 25, 28
*Cla*I	30	147	G	1.3 = 0.9 + [0.4]	23, 25, 28
*Cla*I	32/33	148	G	1.2 = 1.1 + [0.1]	26, 27, 29
*Cla*I	33/34	149	G	8.4 = 3.2 + 5.2	2
*Cla*I	35	150	L	0.5 + 0.1 = 0.6	16–34 (Cacteae)
*Dra*I	2/3	151	L	2.4 + 3.0 = 5.4	16–34 (Cacteae)
*Dra*I	5/6	152	L	5.0 + 2.0 = 7.0	7, 9, 10, 11, 14, 15, 16–34
*Dra*I	8/9	153	G	4.5 = 2.0 + 2.5	8, 10
*Dra*I	16	154	L	2.5 + 1.5 = 4.0	23, 28
*Dra*I	21/22	155	L	4.0 + 6.8 = 10.8	2–7 (Pachycereinae)
*Dra*I	21/22	156	G	4.0 = 3.0 + 1.0	31, 34
*Dra*I	21/22	157	L	1.7 + 2.3 = 4.0	16–34 (Cacteae)
*Dra*I	35	158	L	5.0 + 1.5 = 6.5	16–34 (Cacteae)
*Dra*I	35	159	G	5.0 = 4.4 + 0.6	14, 15
*Dra*I	35	160	L	2.4 + 5.0 = 7.4	4, 6
*Dra*I	37	161	G	2.3 = 1.4 + 0.9	2–15 (Pachycereeae)
*Eco*O109	5/6	162	L	7.5 + 2.3 = 9.8	16–34 (Cacteae)
*Eco*O109	9	163	L	1.5 + [0.3] = 1.8	11, 13, 14, 15
*Eco*O109	13	164	L	3.5 + 0.8 = 4.3	8, 9, 10, 11, 13, 14, 15
*Eco*O109	22	165	G	1.2 = 0.9 + 0.3	16–34 (Cacteae)
*Eco*O109	25/26	166	L	1.0 + 0.3 = 1.3	2–15
*Eco*O109	29/30	167	G	5.1 = 4.0 + 1.1	5, 7
*Eco*O109	31	168	G	1.2 = 0.6 + 0.6	11
*Eco*O109	34	169	L	1.0 + 1.2 = 2.2	8–15 (Stenocereinae)
*Eco*O109	34	170	L	2.0 + 1.2 = 3.2	29
*Eco*O109	37	171	L	4.0 + 2.2 = 6.2	8
*Eco*RI	1	172	G	4.2 = 2.6 + 1.6	16–34 (Cacteae)
*Eco*RI	1	173	L	1.6 + 1.9 = 3.5	26, 27, 29
*Eco*RI	3	174	L	1.0 + 0.9 = 1.9	33, 34
*Eco*RI	8	175	G	6.7 = 5.2 + 1.5	33
*Eco*RI	12	176	L	2.2 + 3.3 = 5.5	19, 24
*Eco*RI	12/13	177	L	2.2 + 0.7 = 2.9	23, 25, 28
*Eco*RI	13	178	L	4.3 + 6.2 = 10.5	8, 16, 17, 18, 20, 21, 22, 23, 27, 30
*Eco*RI	16	179	G	4.8 = 1.3 + 3.5	3
*Eco*RI	20 A/B	180	G	3.4 = 2.3 + 1.1	19, 22
*Eco*RI	20 A/B	181	G	4.0 = 2.3 + 1.7	31, 34
*Eco*RI	22	182	G	2.1 = 1.2 + 0.9	16–34 (Cacteae)
*Eco*RI	22	183	G	1.3 = 1.2 + [0.1]	16, 17, 18, 20, 21, 23–30
*Eco*RI	22	184	L	1.3 + [0.4] = 1.7	19, 22
*Eco*RI	22	185	G	1.3 = 1.0 + [0.3]	32
*Eco*RI	22	186	G	0.9 = 0.6 + [0.3]	33
*Eco*RI	27/28	187	L	1.4 + 4.3 = 5.7	4, 5, 7
*Eco*RI	27/28	188	L	1.4 + 2.9 = 4.3	23, 24, 28
*Eco*RI	29 A/B	189	G	4.7 = 2.5 + 2.2	23, 28
*Eco*RI	29 A/B	190	G	4.7 = 3.5 + 1.2	19, 22
*Eco*RI	29 A/B	191	L	1.9 + [0.4] = 2.3	8–15 (Stenocereinae)
*Eco*RI	34	192	G	4.0 = 2.5 + 1.5	12

APPENDIX 1. Continued.

Enzyme	Region	Mut. no.	Site gain/loss	Observed fragments (kb)	Variant taxa*
*Eco*RI	35	193	G	1.6 = 0.9 + 0.7	16–31
*Eco*RV	1	194	G	8.3 = 4.3 + 4.0	16–34 (Cacteae)
*Eco*RV	2	195	G	4.8 = 3.8 + 1.0	32
*Eco*RV	3	196	L	4.0 + 1.9 = 5.9	18, 26, 27, 29
*Eco*RV	4/5	197	G	4.6 = 1.3 + 3.3	13
*Eco*RV	4/5	198	G	4.6 = 4.2 + [0.4]	16–34 (Cacteae)
*Eco*RV	18/19	199	L	1.6 + 2.9 = 4.5	33
*Eco*RV	20	200	L	0.8 + 0.6 = 1.4	19, 22, 34
*Eco*RV	21/22	201	L	3.4 + 6.8 = 10.2	16–34 (Cacteae)
*Eco*RV	25/26	202	G	6.8 = 3.5 + 3.3	18
*Eco*RV	25/26	203	G	12.8 = 7.0 + 5.8	13
*Eco*RV	27/28	204	G	8.0 = 3.6 + 4.4	16–34 (Cacteae)
*Eco*RV	30/31	205	L	3.3 + 0.7 = 4.0	2–34
*Eco*RV	30/31	206	G	4.0 = 2.5 + 1.5	2–7 (Pachycereinae)
*Eco*RV	30/31	207	G	4.0 = 3.6 + [0.4]	8–15 (Stenocereinae)
*Eco*RV	30/31	208	G	4.0 = 3.3 + 0.7	31, 32
*Eco*RV	30/31	209	G	4.0 = 3.1 + 0.9	22
*Eco*RV	30/31	210	G	3.0 = 2.8 + [0.2]	26, 27, 29
*Eco*RV	35	211	G	5.8 = 3.3 + 2.5	2–7 (Pachycereinae)
*Hinc*II	6	212	G	5.0 = 2.2 + 2.8	34
*Hinc*II	7	213	G	2.9 = 1.4 + 1.5	16, 17, 19–34
*Hinc*II	8/10	214	G	15.4 = 9.4 + 6.0	16–34 (Cacteae)
*Hinc*II	12	215	L	1.9 + 2.4 = 4.3	16–32
*Hinc*II	13	216	L	2.2 + 0.6 = 2.8	2–7, 19, 22–26, 28, 31, 32, 33, 34
*Hinc*II	13	217	G	1.7 = 1.5 + [0.2]	12
*Hinc*II	14/15	218	G	2.3 = 1.6 + 0.7	2
*Hinc*II	21	219	G	3.0 = 2.5 + [0.5]	16–32
*Hinc*II	21	220	L	1.9 + [0.6] = 2.5	25, 27
*Hinc*II	23	221	G	8.4 = 5.7 + 2.7	13, 15
*Hinc*II	27/28	222	L	4.4 + 0.8 = 5.2	16–34 (Cacteae)
*Hinc*II	27/28	223	G	5.2 = 3.5 + 1.7	24
*Hinc*II	27/28	224	G	4.4 = 2.7 + 1.7	6
*Hinc*II	29	225	L	0.8 + 2.2 = 3.0	19, 22
*Hinc*II	30	226	G	3.4 = 1:2 + 2.2	16–34 (Cacteae)
*Hinc*II	30	227	L	3.4 + 0.5 = 3.9	8, 9, 10, 11, 13, 14, 15
*Hinc*II	30	228	L	1.2 + 0.5 = 1.7	16, 30
*Hinc*II	32/33	229	L	3.7 + 0.8 = 4.5	16–34 (Cacteae)
*Hinc*II	35	230	L	1.0 + 0.6 = 1.6	2–15 (Pachycereeae)
*Hinc*II	37	231	L	1.8 + 0.9 = 2.7	11, 14, 15
*Hinc*II	37	232	G	2.1 = 1.2 + 0.9	2–15 (Pachycereeae)
*Hinc*II	37	233	L	2.1 + 1.8 = 3.9	13
*Hinc*II	37	234	G	1.8 = 1.1 + 0.7	5
*Hinc*II	38	235	G	1.3 = 0.7 + 0.6	16, 17, 20, 30
*Hinc*II	38	236	L	2.4 + 6.6 = 9.0	2–15
*Hind*III	4	237	G	1.4 = 1.2 + [0.2]	16–34 (Cacteae)
*Hind*III	4	238	L	1.2 + 2.0 = 3.2	18
*Hind*III	6	239	G	2.0 = 1.8 + [0.2]	28
*Hind*III	8	240	L	5.0 + 10.2 = 15.2	16, 30
*Hind*III	12	241	G	10.3 = 6.8 + 3.5	33
*Hind*III	20/21	242	L	2.3 + 0.8 = 3.1	2–15 (Pachycereeae)
*Hind*III	29/30	243	G	10.3 = 5.7 + 4.6	5
*Hind*III	30	244	G	3.6 = 2.0 + 1.6	24, 27, 29
*Hind*III	32/33/34	245	G	1.7 = 1.4 + 0.3	8, 9, 10, 11, 13, 14, 15
*Hind*III	32/33/34	246	L	6.0 + 2.6 = 8.6	16–34 (Cacteae)
*Hind*III	32/33/34	247	G	10.7 = 5.5 + 5.2	29