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A phylogenetic study of Ferocactus Britton and Rose (Cactaceae: Cactoideae)

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A phylogenetic study of *Ferocactus* Britton and Rose (Cactaceae: Cactoideae)

by

Jorge Hugo Cota-Sánchez

A dissertation submitted to the graduate faculty in
partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Botany (Systematics and Evolution)
Major Professors: Robert S. Wallace and Jonathan F. Wendel

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For the Graduate College

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CHAPTER ONE

GENERAL INTRODUCTION

Statement of Research

It is widely accepted that convergent evolution in both vegetative and reproductive structures obscure the assessment of phylogenetic relationships in various plant groups of angiosperms (Rensch, 1959; Gibson and Nobel, 1986). The Cactaceae, a New World plant family, is not an exception to the occurrence of convergent evolution of similar structures in distantly related taxa, e.g., floral attributes such as pollination syndromes, sclerified scales of the pericarpel, and growth habit, among others. In fact, much of the taxonomic confusion and lack of phylogenetic understanding that characterizes this family is due to parallelism, to the overwhelming number of "new" taxa which have been described over the years, and to the lack of fossil records for reconstructing phylogeny and the evolutionary history of the family.

The use of molecular techniques in the last decades has been transcendental in the phylogenetic studies of flowering plants. Among these techniques, restriction site analysis and DNA sequencing of coding and non-coding regions of the chloroplast genome (cpDNA) have been useful to infer phylogeny and elucidate evolutionary relationships at different taxonomic levels (Jansen and Palmer, 1987; Palmer et al., 1988; Palmer, 1991; Downie and Palmer, 1992; Chase et al., 1993; Olmstead and Palmer, 1994; Clark et al., 1995; Downie et al., 1996). These approaches have also been important in the phylogenetic studies of the Cactaceae. Two of the main reasons which justify the application of molecular tools to study cactus phylogeny are: 1) the number of characters generated with molecular methods is much higher than those obtained from morphology, and 2) because of the inherited nature of the molecules, the amount of homoplasy due to convergent evolution is minimal, and thus homology assessment and phylogenetic reconstruction is less problematic. Consequently, as in other major plant groups, molecular tools have provided new and significant insights into the origin and evolution of the major lineages of this important New World plant family. Previous work in molecular systematics of the Cactaceae (Wallace, 1995; Wallace and Forquer, 1995; Dickie, 1996; Cota and Wallace, 1996, in press; Wallace and Cota, 1996) and ongoing molecular phylogenetic studies at different taxonomic levels have proven their effectiveness providing evidence about the origin and evolution of the cactus family and new insight into the patterns of divergence in some lineages.

This dissertation describes a phylogenetic study of the genus *Ferocactus* Britton and Rose (Cactaceae), and includes species representing the major lineages within the genus. In addition, various taxa from within the subfamily Cactoideae (primarily from tribe Cacteae and tribe Pachycereeae) were included in the study to investigate the degree of relationship of *Ferocactus* with allied genera, particularly with *Echinocactus* Link and Otto and *Stenocactus* (K. Schum.) A. W. Hill.

Dissertation Organization

The main body of this dissertation is organized into four chapters, each consisting of a journal article manuscript. The first manuscript "A review of *Ferocactus* Britton and Rose" is a book chapter published in 1996 in *The Genus Ferocactus: Taxonomy and Ecology*, pp. 35-80. The second manuscript, " Chromosome numbers in *Ferocactus* (Cactaceae: Cactoideae)" was published in the journal *Cytologia* (1996. 61: 431-437) of the Japanese Mendelian Genetics Society. In this paper, Jon P. Rebman and Robert S. Wallace appeared as second and third authors, respectively. The third manuscript, "Chloroplast DNA evidence for divergence in *Ferocactus* and its relationships to North American columnar cacti (Cactaceae: Cactoideae)" is in press in the journal *Systematic Botany* and will appear late this year. The fourth manuscript entitled "Phylogenetic relationships of *Ferocactus* (Cactaceae) and allied taxa based on sequences of non-coding DNA" will be submitted to the journal *Molecular Biology and*

Evolution. Following the last manuscript there is a chapter integrating independent findings and presenting them as the general conclusions of this dissertation.

The first paper (book chapter) as the title suggests "A review of *Ferocactus* Britton and Rose") includes an historical perspective and general account the taxonomic and phylogenetic knowledge of this genus based on an extensive literature review of previous work. New cytological and preliminary molecular data are presented, and their repercussions in the taxonomy and evolution of *Ferocactus* are addressed. This chapter also includes an extensive discussion of the first phylogenetic hypotheses and putatively basal lineages in the genus, in addition to a description of the problems faced by taxonomists to infer phylogeny in the Cactaceae.

The second paper describes the cytology of *Ferocactus*, particularly the documentation of new chromosome numbers in the genus based on both mitotic and meiotic material. The occurrence of hybridization is addressed in those taxa for which hybridization has been hypothesized; an issue which has not been resolved in part because of the observation of normal pairing and meiotic configurations, and because of limited taxonomic and population sampling. Chromosome morphology based on mitotic chromosomes is described for the species investigated.

The third paper is based on a restriction site analysis of the chloroplast DNA conducted to infer major phylogenetic lineages within *Ferocactus* and to clarify its hypothesized phylogenetic relationships with North American columnar cacti of tribe Pachycereeae. This paper has partially solved several issues addressed as main objectives. It does provide preliminary evidence of paraphyly in *Ferocactus*, which most likely shared a common ancestor with *Echinocactus*. It also demonstrates that *Ferocactus* is not phylogenetically related to any of the North American columnar cacti examined, and that the presence of chartaceous scales in the flowers of *Escontria chiotilla* (F. A. C. Weber) Rose and *F. flavovirens* (Scheidw.) Britton and Rose represents another case of convergence in this plant family. Consequently, this study has a couple of taxonomic implications. First, the paraphyly of *Ferocactus*, and second, within the columnar cacti, our study suggests that *Stenocereus dumortieri* (Scheidw.) Buxb. is phylogenetically distinct from the rest of *Stenocereus* (A. Berger) Riccob. Thus, the early generic name *Isolatocereus* proposed by Backeberg should be maintained because this species diverged early in the evolution of the Stenocereinae.

The fourth paper is a continued effort to clarify the origin and evolution of *Ferocactus* and demonstrates the utility of DNA sequences from two non-coding regions of the chloroplast genome to addressing these issues. The rate of evolution of the non-coding regions studied (intergenic spacer region of the *trn*L-*trn*F genes and *rpl*16 intron) is appropriate to resolve phylogenetic problems at higher taxonomic levels. DNA sequence analyses confirm paraphyly in *Ferocactus* and its close relationship with *Echinocactus*. The repeated result of paraphyly in *Ferocactus* adds more taxonomic complexities to the study. According to the International Code of Botanical Nomenclature, priority is given to the name formally described first. In this case, *Echinocactus* has priority, and because *E. grusonii* Hildm. appears basal to *F. histrix* (DC.) G. E. Linds. and *F. glaucescens* (DC.) Britton and Rose, the generic name *Echinocactus* would apply at least those two *Ferocactus* species sister to *E. grusonii*, assuming they are segregated from *Ferocactus*. The taxonomic implications of their exclusion from *Ferocactus* would convey to a monophyletic *Ferocactus*, but at the same time, a paraphyletic *Echinocactus*. From a biogeographic standpoint, DNA sequences of this study groups taxa by geographic provinces, such as northern and central species, which are discussed according to the hypothetical scenario of patterns of radiation in the evolutionary history of *Ferocactus*.

The last section of my dissertation includes general conclusions of my research and aspects in which future work may be focused.

The second paper has multiple authorship with Dr. Jon P. Rebman and Dr. Robert S. Wallace. The third and fourth paper are co-authored with Dr. Robert S. Wallace who supervised the generation and analysis of the molecular data. Together the four papers form a comprehensive account of the results of my Ph.D. project, which encompassed both traditional taxonomy and molecular systematics to study phylogenetic and evolutionary aspects in the North American barrel cactus *Ferocactus*.

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CHAPTER TWO

A REVIEW OF *FEROCACTUS* BRITTON AND ROSE

A book chapter published in *The Genus* Ferocactus: *Taxonomy and Ecology* [1]

J. Hugo Cota

INTRODUCTION

More than four decades have elapsed since Lindsay (1955a) wrote his doctoral dissertation, a study of the taxonomy and ecology of *Ferocactus* Britton and Rose. Lindsay's studies represent a valuable source of field documented material in which he includes both morphological and ecological data, and his own concepts about the classification of *Ferocactus*. His extensive field work at that time was challenging especially in remote regions of the Baja California Peninsula and surrounding islands as well as in mainland Mexico in the 1950's. The extent of his field work proves that there was no obstacle which could stop George from accomplishing his goals and conducting his field studies. His passion for cactus taxonomy and adventurous spirit have inspired other people to follow his example. George has been one of my mentors and his support and advice during my graduate studies and career in cactus taxonomy have been meaningful. Many fruitful discussions with him have given me a broader perspective of the genus and encouragement to seeking the answers of the taxonomic relationships and evolutionary history of *Ferocactus*. I feel honored to contribute with this chapter of the book, in particular because of my personal interest in *Ferocactus* and because

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Lindsay's pioneer field studies of the genus represent an excellent example of his love an devotion for plants and his understanding of their taxonomic relationships.

Subsequent to the conclusion of Lindsay's dissertation, several authors have conducted separate studies dealing with diverse biological aspects of the genus. Such studies have considered a single species or major taxonomic groups within the genus and have referred to nomenclatural, biological, ecological, or physiological matters (e.g., Blom and Clark, 1980; Bravo-Hollis, 1966; Cota and Wallace, 1996; Nobel, 1977; Ruffner and Clark, 1986; Taylor, 1979a, 1979b, 1980, 1984, 1987; Taylor and Clark, 1983; Unger, 1992). Lindsay contributed descriptions of new taxa, nomenclatural changes, and ecology of *Ferocactus* (e.g., 1942, 1955b, 1955c, 1955d, 1964, 1965, 1968).

In the following sections, I briefly describe the main topics that have been investigated in *Ferocactus* from 1955 to the present. My objectives in this chapter are to summarize the knowledge of the genus in different areas of study and to discuss the importance of understanding its phylogenetic relationships at the interspecific, generic and tribal levels. It is my intent to review Lindsay's taxonomic treatment and associate it with the results from modern techniques (molecular studies) used in phylogenetic analyses of *Ferocactus*. I hope that the up-to-date view of *Ferocactus* presented here is useful to anyone interested in cacti.

Because of the diverse array of topics investigated since 1955, several studies have been omitted not because they are irrelevant, but because their goals are considered distant from the main issues addressed in this chapter. In addition, to mention every paper dealing with the genus would imply the discussion of several areas of research that are not my specialty. Therefore, and with my apologies to those authors who have contributed to the knowledge of the genus in the topics excluded herein, I restrict myself mainly to those studies that in one way or another are more closely related to the understanding of the biosystematics of *Ferocactus*. At the end of this chapter there is a list of additional bibliographic references dealing with various aspects of the genus.

Ferocactus is distributed in arid and semiarid regions of central and northern Mexico and southwestern United States (Fig. 1), and it has been hypothesized to occupy a critical phylogenetic position in the classifications of the Cacteae (Fig. 2) (Buxbaum, 1951, 1958; Barthlott, 1988; Barthlott and Hunt, 1993). Within the Cacteae, *Ferocactus* is the third largest genus in number of species after *Mammillaria* Haw. and *Coryphantha* (Engelm.) Lem. (Gibson and Nobel, 1986; Barthlott and Hunt, 1993).

The understanding of the phylogeny of *Ferocactus* and its phylogenetic position within the Cacteae (the largest tribe within subfamily Cactoideae in North America), along with its patterns of speciation, is significant for understanding the relationships at the interspecific and tribal level (in particular between the tribes Pachycereeae and Cacteae). The development of a robust phylogenetic hypothesis for the genus will provide a basis for the subsequent determination of its evolutionary patterns *vis a vis* other genera of the Cacteae as well as clarifying the relationships between the tribes Pachycereeae and Cacteae. Likewise, a methodical study of *Ferocactus* will help to explain the interspecific phylogenetic relationships and most likely provide a scenario of the evolutionary patterns of radiation.

TAXONOMIC BACKGROUND

The taxonomic history of *Ferocactus* is addressed by Lindsay earlier in this book, and no further discussion is needed. At present, the taxonomy of the genus remains in a somewhat confused state. Different taxonomic philosophies, disagreement as to species boundaries, and under-representation of some taxa in herbarium collections have contributed to the complexity of its taxonomic circumscriptions. Another factor involved is the relatively high level of morphological homoplasy, which obscures the degree of relatedness due to convergence. Additionally, cactus taxonomy in general is complex because morphological variability is associated with gradients in habitats and geographic ranges (Cody et al., 1983). In *Ferocactus*, the use of primarily vegetative morphological characters (which are likely to display different degrees of plasticity in response to local environmental conditions) has also contributed to its taxonomic difficulty. As a result, natural subpopulations subject to varied selective pressures may diverge both morphologically and genetically, occasionally providing discontinuities that are useful to distinguish taxonomic complexes in the process of speciation. As a consequence of natural variation and taxonomic delineations, the number of species circumscribed in the genus has been a matter of disagreement and is reflected in several taxonomic treatments (Table 1).

Prior to Lindsay, Britton and Rose (1922) proposed a taxonomy in which they included 30 species [Lindsay (1955a: 40) indicates 31]. Lindsay's classification (1955a) considered a total of 25 species and ten varieties. More recently, in a taxonomic treatment of *Ferocactus sensu stricto* Taylor and Clark (1983) included 29 species; Taylor (1984) recognized 23 species and 20 infraspecific taxa, while Bravo and Sánchez-Mejorada (1991) accepted 29 species and 25 varieties. Finally, Unger (1992) provided a review of the genus in which descriptions and distributional data are discussed. Although his taxonomic delimitations were based on previous treatments, he presented a classification scheme which included four sections (Table 1) and a list of naturally occurring hybrids. In his broad circumscription of the genus, Taylor (1980) transferred some species of *Stenocactus* (K. Schum.) A. W. Hill to *Ferocactus*, based on morphological affinities such as similarities in rib pattern and presence of glandular areoles, and created the subgenera *Ferocactus* (in which are placed all of Lindsay's species) and *Stenocactus*.

Despite the substantial amount of taxonomic work conducted during the past seven decades, the controversy in species boundaries within the genus is evident: no classifications are identical yet the number of species circumscribed are similar (e.g., 30, 29, 28, 25, 23). Neither Britton and Rose (1922) nor Lindsay (1955a) used infrageneric categories, whereas Bravo and Sánchez-Mejorada (1991), Taylor (1984), Taylor and Clark (1983), and Unger (1992) classified the genus using other taxonomic hierarchies, such as sections, groups and subgenera (Table 1). A common feature of these classifications is that the delimitation of species has been based primarily on gross morphological features of the stem, flower and fruit.

Of these taxonomic treatments, those by Taylor and Clark (1983) and Taylor (1984) are of special importance because they have associated patterns of gross morphology with micromorphological seed coat characters. These studies have led to the establishment of sectional boundaries and the consideration of new ideas about the evolution of *Ferocactus*. In spite of Taylor's (1984) comprehensive treatment combining all the data available at that time, a robust phylogeny of the genus has not been presented to date, and key issues regarding its origin and subsequent radiation remain unresolved.

The extensive use of varietal rank in the different classifications is further indication of the taxonomic confusion of this genus. To some extent, the use of varietal rank may be helpful in distinguishing infraspecific groups in *Ferocactus*, and might accurately reflect the relatively recent origin of some species groups, especially for some Baja Californian species. Indeed, Lindsay (1955a, 1965) and Taylor (1984) indicated that those species occurring in mainland Mexico are taxonomically well-defined, but several taxa from northwestern Mexico and the Baja California peninsula remain problematic, probably due to incipient processes of speciation.

In the following sections, I review new data from cytological and molecular sources that reveal preliminary information about the relationships at the interspecific level as well as the inferred relationships of *Ferocactus* with the columnar cacti of Tribe Pachycereeae. Both sources of information are used to discuss the issues of phylogeny and hybridization. To avoid confusion and to facilitate the taxonomic circumscriptions of *Ferocactus*, the specific epithets and authorities used in this chapter are those included in Lindsay's classification for two reasons. First, to honor him for contributing to the understanding of the infra- and interspecific categories of the genus and second, because the other taxonomies (Taylor and Clark, 1983; Taylor, 1984; Bravo and Sánchez-Mejorada, 1991; Unger, 1992), also recognize the majority of species proposed by Lindsay. This is not meant to discredit the valuable taxonomic contribution of these authors. Also, it is worth mentioning the outstanding contributions of Taylor (1979a, 1979b, 1984, 1987) in the nomenclature of the genus. He clarified the application of several specific epithets and authorities that should be discontinued. I must repeat, however, that although I am aware of these nomenclatural changes, here I follow those names used by Lindsay (1955a) for the reasons indicated above.

To date, the most modern taxonomic treatments of *Ferocactus* are those of Taylor (1984) and Taylor and Clark (1983), in which phylogenetic interpretations of the evolution and radiation of the genus based on vegetative and reproductive characters, geography, and macro- and micromorphology of the seed coat have been proposed. This information is essential to understanding the modern concept of classification of the genus, and these recent evolutionary hypotheses will be used to discuss phylogenetic aspects of *Ferocactus*, especially in the section which includes molecular studies.

REPRODUCTIVE BIOLOGY IN *FEROCACTUS*

Reproductive strategies in plants are numerous and they are reflected in an array of floral morphology and breeding systems, which have evolved in response to genetic, environmental, and selective forces. In sexually reproducing plants, as is the case in *Ferocactus*, breeding systems determine, in part, patterns and levels of genetic variation. Although little is known regarding the pollination biology and breeding systems of the genus, in the following paragraphs I summarize our current knowledge of the topic.

Floral Biology and Pollination

The flowers of *Ferocactus* are perfect and bowl-shaped (Fig. 3); they open during the day, and have a slight fragrance (Bravo and Sánchez-Mejorada, 1991). The color spectrum varies from yellow, orange, red, pink, lavender, to purple (Fig. 3); the stamens are numerous

(Fig. 3A), usually with yellow anthers that mature before the style becomes receptive, thus they are protandrous (Richards, 1986). The plants bloom from early spring to late summer, and the flowers remain open for several days (Bravo and Sánchez-Mejorada, 1991).

In *Ferocactus* there is an apparent coadaptation of pollinating agents and its flowers. The floral attributes such as the funnelform to bell-shaped, diurnal, bright yellow, medium-to large-sized flowers, with nectar and little or no fragrance at all, are characteristic of bee pollinated flowers (Rowley, 1980a). The flowers of *Ferocactus* are pollinated by several species of bees. Bee pollinated flowers have been documented in *F. wislizeni* (Engelm.) Britton and Rose (Grant and Grant, 1979a) which is usually pollinated by medium sized bees of the family Megachilidae (*Megachile sidalcea* Cockerell and *Lithurge echinocacti* Cockerell) and Apiidae (*Diadasia australis* Cresson) (Grant and Grant, 1979b). Cockerell (1900) also indicated that *Auglochlora neglectula* Cockerell visits the flowers of *F. wislizeni*. Unidentified bees have been observed crawling around the stamens and stigmas of the flowers of *F. histrix* (DC.) Lindsay and *F. robustus* (Otto) Britton and Rose in areas of central Mexico (pers. obs.). Although the flowers of *Ferocactus* are also visited by other species of insects, the pollination syndrome has evolved to promote bees as the principal vector. In addition to bees, beetles are common visitors of *Ferocactus* flowers and feed on pollen and may accidentally pollinate the flower (Grant and Grant, 1979b) acting as secondary pollinators.

Mechanisms such as herkogamy (the spatial isolation of stamens and stigma lobes) and protandry in the flowers of *Ferocactus*, coupled with the active behavior of bees, are important factors that discourage self-fertilization and promote gene exchange. In addition, there are several stigma lobes that expand when they are receptive and provide a landing platform for pollinators (Fig. 3), facilitating cross-pollination. Therefore, open floral morphology and full exposure of anthers and stigmatic surface probably allows pollination by more than one bee species. Similar floral attributes have been reported for *Echinocereus* Engelm. (Cota, 1993) and the cactus flora of the southwestern United States (Grant and Grant, 1979b).

Other floral characteristics in *Ferocactus* are nectar and pollen rewards. Nectar production seems to be relatively low, and despite the lack of experimental tests, measurable amounts of this fluid are difficult to detect (at least by the human eye!). Similarly, low nectar production has been reported in the flowers of *Echinocactus grusonii* Hildm. and other genera of the Cactoideae (Scogin, 1985). Low nectar amount has also been observed in *Echinocereus engelmannii* (Parry) Lem. (Cota, 1993), species which shares similar floral attributes to *Ferocactus*. It is likely that low nectar production in the flowers of *Ferocactus* is compensated for by the correspondingly abundant pollen (as evidenced by the numerous stamens) which probably is the major floral reward. The same pattern (i.e., little nectar and abundant pollen) has been reported in *Opuntia lindheimeri* Engelm. (Grant and Hurd, 1979). Moreover, pollen amount is also associated with pollinator efficiency: high amounts of pollen produced per flower correlate with higher pollinator efficiency (Richards, 1986). In the wild, the anthers of the numerous stamens in the flower of *Ferocactus* produce massive amounts of pollen which satisfy demands by pollinators and assure its transfer to another flower. Correspondingly, seed production usually occurs in large numbers, which seems to be sufficient for the perpetuation of the species in nature.

Ants and Extrafloral Nectaries

Extrafloral nectaries as the name implies, are glands that secrete nectar from different parts of the plant, i.e., other than the flower. These glands are characteristic in *Ferocactus* and they occur apically in the areole, in particular in actively growing areoles where new floral structures are in the process of development. The number of extrafloral nectaries per areole varies from one to five in *F. gracilis* Gates (Blom and Clark, 1980). These glandular structures produce an ant-attracting nectar of varied sugar composition (glucose, sucrose, and fructose) of which only glucose has been detected in higher concentrations early in fruit production in *F. acanthodes* (Lem.) Britton and Rose (Ruffner and Clark, 1986).

It is generally accepted that extrafloral nectaries play a role in a mutualistic relationship between plants and protective ants (Formicidae) (Bentley, 1977a, 1977b; Pickett and Clark, 1979). In this protectionist scenario, ants are attracted by nectar produced in these glands, and while foraging for nectar they protect the plants from potential herbivores. Ruffner and Clark (1986) indicated that ant visitation and activity is correlated with nectar production and phenology of the plant. For instance, in *F. acanthodes* var. *lecontei* (Engelm.) Lindsay, the composition and amount of extrafloral nectar changes according to plant phenology, and ant visitation is more common during the blooming and fruiting periods. Also, ants depending on liquid food in desert environments may respond to fluctuations in the water content of their food (Ruffner and Clark, 1986).

Though several species of ants visit the plant, some species are more frequent visitors than others. Among the species of ants visiting extrafloral nectaries in *Ferocactus*, the honey ants (*Crematogaster depilis* Wheeler) have been reported to be the most frequent in *F. gracilis* (Blom and Clark, 1980) and *F. acanthodes* var. *lecontei* (Ruffner and Clark, 1986). In addition, del Castillo (1982, 1988) documented the presence of *Irydomyrmex* and *Dorymyrmex* in *F. histrix* in mainland Mexico. Other species associated with the extrafloral nectaries of *F. gracilis* are *Iridomyrmex pruinosum*, *Camponotus ocreatus*, and *Pheidole grallipes* (Blom and Clark, 1980). Unidentified ants have been observed visiting flowers and extrafloral nectaries in wild plants of *F. histrix*, *F. latispinus* (Haw.) Britton and Rose, *F. recurvus* (Miller) Y. Ito, and *F. robustus* (pers. obs.).

SEED MORPHOLOGY

Both seeds and pollen grains are important sources of morphological characters useful for elucidating evolutionary trends and phylogenetic lineages. The importance of the architectural design of these structures is evident when they are applied to the classification of taxa in which characters based on gross morphology are not the best source of data to assess the degree of relationship.

In the Cactaceae, seed macro- and micromorphology have been successfully applied at different taxonomic levels (e.g., Barthlott and Voit, 1979; Friedrich and Glaetzle, 1983; and Glaetzle and Prestlé, 1986). The shape, variation in the testa cells and wall sculpture of seeds provide information of taxonomic importance. In *Ferocactus,* studies on seed coat attributes are limited; the most relevant is that of Taylor and Clark (1983), in which the importance of seed characters was demonstrated in the classification of the genus. In their study, the authors correlated characteristics of the fruit and the hilum-micropilar rim (HMR) which allowed them to define the two sections of *Ferocactus*, namely section *Ferocactus* (seeds with massive HMR, fruit dry, dehiscent by a basal pore, and glossy seed coat) and section *Bisnaga* (Orcutt) N. P. Taylor (seeds with narrow HMR, juicy fruit and shiny seed coat). To date, the most complete description of the distribution of seed coat attributes in 31 species of the genus is found in Taylor and Clark (1983), which also provide hypotheses about the evolutionary trends of *Ferocactus* based on micromorphology of seeds, plant habit, and stem morphology.

In terms of the current knowledge of macro- and micromorphological seed characters, the following are some of the defining seed attributes of *Ferocactus* (variations to this pattern should be expected due in part to convergent evolution, malformation, and genetic factors among others). According to Taylor and Clark (1983), the seeds of *Ferocactus* subgenus *Ferocactus* are in general mussel-shaped, and range in size from 1 to 3 mm; the seed coat may be smooth to rugose (with testa-cells tabular to tabular-concave). Some species (*F. robustus* and *F. wislizeni*) have a reticulate pattern with verrucose periclinal walls. In the case of *F. rectispinus* (Engelm.) Britton and Rose and *F. acanthodes* var. *lecontei* the seeds have tabular testa cells (Fig. 4). The seed coats of the species analyzed exhibited cracks (Fig. 4B) probably due to the exposure of seed to the beam of the scanning electron microscope; the same situation was reported by Taylor and Clark (1983).

POLLEN MORPHOLOGY

Palynology, the study of pollen grains and spores, is another source of comparative data for taxonomic purposes practically at all levels of hierarchy. Reliable palynological data are related to the pollen grain itself, e.g., aperture number, shape and position of apertures, and exine sculptural patterns. In the Cactaceae, it has been shown that the distribution of some morphological features of the pollen grain are useful in defining major lineages. For instance, the three subfamilies (Pereskioideae, Opuntioideae and Cactoideae) can be distinguished based on pollen types and exine structural design (Kurtz, 1948; Tsukada, 1964). Moreover, pollen grain morphology is relatively uniform at the tribal level (Leuenberger, 1976).

At the specific level, pollen analyses are limited in *Ferocactus*. According to Kurtz (1948) and Tsukada (1964) the pollen grains of *F. acanthodes*, *F. covillei*, and *F. wislizeni* are tricolpate with a punctibaculate ektexine (finely pitted exine), and equatorial diameter ranging from 48-68 (85) µm. Whether these morphological patterns in pollen are common throughout the genus will remain unclear until further studies are conducted in a broader range of species.

CYTOLOGICAL STUDIES

In general, the classification of the Cactaceae has used traditional methods and has been based on shared morphological characters. In the last 40 years, several tools for addressing questions in plant systematics and for reconstructing phylogeny at different taxonomic levels have blossomed. Cytotaxonomy and modern molecular techniques based on DNA analyses are clear examples of the revolution that plant systematics has experienced to produce genetic based phylogenies.

The study of chromosomes (structures that contain the genetic material) is a reliable source of comparative data in plant classification. Cytological data of various types (chromosome number, behavior at meiosis, size and shape of chromosomes, and DNA amount) are widely used in plant taxonomy. In the Cactaceae, cytological studies have provided insight into polyploid complexes, hybrid lines, chromosome morphology, and chromosomal rearrangements. Additionally, studies of mitotic and meiotic chromosomes have shown that species of *Ferocactus* have a base number (lowest haploid number) of $x = 11$ (Fig. 5) (which is also the base number for the family; Pinkava et al., 1977). Thus far, all chromosome counts for *Ferocactus* reported in the literature (17 species) are consistent with the base number for the family (Table 2). The number of taxa analyzed to date represent 68% of the total of species recognized in Lindsay's taxonomy.

Overall, the morphology of mitotic chromosomes among the species of *Ferocactus* is homogeneous: primarily metacentric, with a few pairs of submetacentric chromosomes, and size ranging from 3 to 7 µm (Figs. 5B-C). The chromosomes of *Ferocactus* happen to be the largest among those taxa surveyed in the Cactaceae (Cota et al., 1996), however, the significance of large chromosomes is unclear. Some taxa, *Ferocactus acanthodes* var. *lecontei, F. chrysacanthus* (Orcutt) Britton and Rose (Fig. 5B), and *F. wislizeni* exhibit satellites or secondary constrictions (small extensions at the end of the arms of the chromosomes) (Cota et al., 1996). Similar patterns in chromosome morphology have been observed in unrelated genera of the Cactaceae such as *Echinocereus* Engelm. (Cota, 1991; Cota and Wallace, 1995), *Mammillaria prolifera* (Miller) Britton and Rose (Johnson, 1980), and *Nyctocereus* (Berger) Britton and Rose (Palomino et al., 1988).

The uniformity in shape and number of chromosomes and lack of satellites does not necessarily reflect evidence of close phylogenetic relationship. Chromosomal rearrangements at the molecular level may account for the distinctiveness of some species. Alternatively, the presence of satellites in some northern species, such as *F. acanthodes* var. *lecontei* , *F. chrysacanthus*, and *F. wislizeni* indicates that satellites might be used as taxonomic markers (Cota et al., 1996), and that further analyses of chromosome morphology might be advantageous to understand interspecific relationships in *Ferocactus*.

As mentioned previously, some of the taxonomic confusion that characterizes *Ferocactus* has been attributed to hybridization and introgression events. Lindsay (1955a) and more recently Taylor (1984) have both indicated that Baja Californian species are taxonomically problematic due to probable introgressive processes, which brings new genetic attributes to actively evolving species. This hypothesis is based upon observations of individuals with intermediate morphologies in areas where two species are sympatric. Indeed, cases of intermediacy have been suggested in two related species groups: *F. gracilis* and the *F. peninsulae* (Engelm.) Britton and Rose group (Taylor, 1984). Polymorphism in spine shape and color in addition to intermediacy of stem and flower characters are common in individuals of the same population. At present, it is unclear to what degree this variation is genetically and/or environmentally induced. Analyses of meiotic configurations in different individuals of *F. gracilis* (Fig. 5A) and *F. peninsulae* failed to provide evidence for hybridity (Cota et al., 1996) at least for the populations examined. Contrary to those hypothesized introgressive events that have contributed to the taxonomic confusion in some Baja Californian species, chromosomal analyses of these taxa at different stages of meiosis show normal pairing and formation of bivalents at diakinesis, which may suggest the lack of hybridization in sympatric species (Cota et al., 1996) or simply a lack of genetic barriers. In addition, previous meiotic counts of some Baja Californian and Mexican mainland species (Beard, 1937; Pinkava et al., 1973, 1977, 1985) have not reported abnormalities during meiosis. To date, in spite of cases of morphological intermediacy, there is no cytological evidence such as univalents, trivalents, chromosome bridges, fragments, lagging chromosomes, or any other abnormalities during cell division to support the possibility of hybridization. Also, hybrids may not show meiotic abnormalities because there is little chromosomal differentiation between species. It should be mentioned however, that even though there is no evidence of hybridization based on chromosome behavior and morphology, this event can not be ruled out until further analyses are conducted on the remaining species, especially in those problematic populations indicated by Taylor (1984).

In addition to hybridization, polyploidy has been indicated as an important factor in the evolution of certain cacti, e.g., some species of *Opuntia* (Tournef.) Miller (Pinkava et al., 1985) and *Echinocereus* (Cota and Philbrick, 1994; Cota and Wallace, 1996). Both processes are yet to be documented in *Ferocactus* and is it likely that they have not contributed substantially to speciation events in this genus (Cota et al., 1996). The frequency of polyploidy in the subfamily Cactoideae in which *Ferocactus* is circumscribed is low: approximately 12.5% (Pinkava et al., 1985). Unlike some other genera of the subfamily in which polyploidy is relatively common, e.g., *Echinocereus* (Cota and Philbrick, 1994; Pinkava et al., 1992; Weedin and Powell, 1978), to date all the species of *Ferocactus* which have been cytologically investigated are diploid (Table 2). Moreover, *F. robustus*, one of the putatively least derived species (Taylor and Clark, 1983) has not experienced changes in chromosome number. The same apparently stable diploid number has been observed in putative sister genera of *Ferocactus* such as *Echinocactus grusonii* and *Stenocactus* (*Echinofossulocactus*) *crispatus* (DC.) Berger (Katagiri, 1952); *Echinocactus polycephalus* (Engelm.) Bigel. (Pinkava et al., 1977); *E. horizonthalonius* Lem. and *E. texensis* Hoppfer (Weedin and Powell, 1978); and *S. pentacanthus* (Lem.) Berger (Pinkava and Parfitt, 1982). The homogeneity of chromosome numbers in *Ferocactus* and allied taxa does not provide useful information for understanding interspecific and intergeneric relationships by correlating it with patterns of chromosomal evolution. Cota et al. (1996) suggest that the relatively recent origin of the genus may account for the lack of polyploid individuals in *Ferocactus* and its suspected sister taxa (*Echinocactus* Link. and Otto and *Stenocactus*), and that changes in chromosome number have not played a major role in the evolution of *Ferocactus*. They have hypothesized that genetic evolution in the genus is likely taking place at the molecular level, and consequently, chromosome rearrangements remain cryptic. Indeed, the non-coding region (intron) loss of the chloroplast gene *rpo*C1 has been reported in representative members of the Cactoideae, including *F. flavovirens* (Wallace and Cota, 1996). Further cytological investigations with a wider taxonomic sampling within *Ferocactus* may be useful in determining the extent of the diploid condition and the occurrence of chromosomal rearrangements. In addition, studies of crossability to examine the genetic basis of taxonomic characters and isozyme analyses to estimate the genetic distance among taxa might be effective detecting cases of natural hybridization and/or introgression in populations where individuals with intermediate morphology are common.

On the other hand, and in spite of the lack of evidence to document natural hybrids based on the study of meiotic figures, artificial hybridization is an approach from which cactophiles obtain bizarre specimens. Intergeneric hybrids involving *Ferocactus* are precious novelties, and documented cases are those crosses between *F. acanthodes* and *Leuchtenbergia principis* Hook. to produce the hybrid X *Ferobergia* (Glass, 1966), and the cross between *Stenocactus* (*Echinossofulocactus* sp.) X *Ferocactus* sp. that produced X *Ferossofulocactus* (Rowley, 1980b). Disregarding fertility issues in these intergeneric hybrids, the ability to recover viable offspring from intergeneric crosses probably indicates a relatively low degree of genetic divergence; therefore, the apparent lack of reproductive isolation allows chromosomes to pair at meiosis to create artificial hybrids. If artificial and even naturally occurring intergeneric hybrids are created within certain groups of cacti, then it is quite likely that interspecific hybrids of *Ferocactus* do occur in the wild as suggested by Lindsay (1955a) and Taylor (1984), nonetheless, sources of evidence (other than intermediacy) are still missing.

CURRENT MOLECULAR PHYLOGENETIC KNOWLEDGE

Several molecular biological techniques involving macromolecules (in particular chloroplast and nuclear DNA), are being applied to the study of phylogenetic relationships and evolution of certain plant groups. Because the advantages of molecular tools in plant systematics and evolution are discussed at length in several papers (e.g., Avise, 1994; Palmer, 1987; Palmer et al., 1988; Olmstead and Palmer, 1994; Whitkus et al., 1994) I will only mention two of their strengths: 1) they provide virtually unlimited number of characters to analyze and compare in relatively short periods of time, and 2) they are less subject to convergent evolution, so that homoplasy is minimized and phylogenetic reconstruction is less problematic. Previous papers (Wallace, 1986, 1995a, 1995b; Wallace and Cota, 1996; Cota and Wallace, 1996) have explained the importance and application of macromolecular techniques to the study of cactus evolution in more detail.

Pioneering studies using molecular techniques in the systematics of the Cactaceae at different taxonomic levels were initiated in the early 1990's at Iowa State University under the direction of Robert S. Wallace, and are providing new insights into the systematics, evolution, and phylogeny of the cactus family. In this facility, various molecular methods [in particular gene sequencing and restriction site variation studies of chloroplast DNA (cpDNA)] are being applied to both North and South American groups of cacti in order to generate a phylogeny of this family. Among the numerous promising projects in progress in this lab are:

1. Within the subfamily Cactoideae, a major study using different regions of the chloroplast genome to elucidate the interspecific relationships in *Ferocactus* and its relationships with North American columnar cacti of Tribe Pachycereeae. The preliminary results of this project are discussed in the following sections.
2. The assessment of the phylogenetic relationships and major lineages between North and South American columnar cacti.
3. In the subfamily Opuntioideae, a study of the interspecific phylogenetic relationships in *Opuntia* and related taxa (*Pereskiopsis* Britton and Rose, *Pterocactus* MacDouglas and Mir., *Quiabentia* Britton and Rose, and *Tacinga* Britton and Rose), using several regions of the chloroplast genome represents a

major effort which is providing new insight to clarify the taxonomy of this complex group.

4. The phylogenetic study of the Tribe Rhipsalidae (including *Hatiora* Britton and Rose, *Lepismium* Pfeiffer, *Rhipsalis* Gartn., *Rhipsalidopsis* Britton and Rose, and *Schlumbergera* Lem.) is attempting to determine the putative terrestrial lineage from which this epiphytic tribe evolved and the pattern of divergence within the tribe.
5. Analyses of the major lineages within the tribe Hylocereeae to clarify their phylogenetic relationships and intergeneric boundaries.
6. An intergeneric study in members of the South American tribes Trichocereeae, Cereeae, and Browningieae to investigate their phylogenetic relationships has been initiated recently. Additionally, several other projects that will complement those outlined here are being planned for the near future.

Relevant descriptions and preliminary results of the main projects in progress in Wallace's lab in diverse lineages of the Cactaceae, and a review of the major techniques in the molecular systematics of this family using cpDNA are provided in Wallace (1995a, 1995b), Wallace and Cota (1996), and Cota and Wallace (1996). The reader is advised to consult these references for a more comprehensive understanding of terminology and methods used in the following section.

Molecular Studies in *Ferocactus*

The putatively basal phylogenetic position in which *Ferocactus* has been placed in the Tribe Cacteae of the Subfamily Cactoideae (Fig. 2) (Buxbaum, 1958; Barthlott, 1988; Barthlott and Hunt, 1993), suggests the possibility that several lineages evolved from it. However, the pattern of intergeneric relationships within the members of the Cacteae and possibly taxa of other tribes remain unclear despite a few shared morphological characters that suggest some degree of relationship. The phylogenetic reconstruction of *Ferocactus* confronts a series of issues, some of which are related to the following questions: Is this genus monophyletic and a basal lineage within the Cacteae? Did other genera arise from *Ferocactus* or evolve in parallel? If so, What are those? and finally, Does the presence of chartaceous scales in the flowers of *F. flavovirens* (Scheidw.) Britton and Rose suggest any phylogenetic relationship with North American columnar cacti of the Pachycereeae which also display this character? The answers to these enigmas are certainly significant in elucidating the origin, evolution and phylogeny of this controversial genus. In this section, it is my aim to address some of these issues, namely the monophyly of *Ferocactus* and its relationships with columnar cacti of North America, based on preliminary molecular data obtained from cpDNA restriction site analysis and DNA sequences of coding (exon) and non-coding (intron) regions of the chloroplast genome. Because these studies are still in progress, it is necessary to note that the relationships presented in the following discussions are subject to future changes as new data becomes available from within the genus and within the tribe. Also, the arguments approached are only briefly discussed because they are at present based on preliminary data; detailed discussions are in preparation for publication elsewhere.

Molecular Phylogenetics of *Ferocactus* and its Relationships with North American Columnar Cacti of North America (Tribe Pachycereeae) Based on Restriction Site Variation of cpDNA and Gene Sequence Analyses of the Chloroplast Gene *ndh*F

The enormous diversity encompassed by *Ferocactus* and the presence of similar morphological structures in other genera (*Echinocactus* and *Stenocactus*) raises the possibility that this diverse assemblage may not be monophyletic. Initially, no specific hypotheses were suggested about the origin of *Ferocactus*. Lindsay (1955a) considered the genus to be a "non-natural group," interpreted today to mean perhaps polyphyletic; later, Taylor (1980), based on morphological similarities in rib pattern and presence of areolar glands in *Ferocactus* and

Stenocactus coptonogonus (Lem.) Berger, considered that these two lineages should be united because the affinities suggested a common origin and close evolutionary relationship. Consequently, four species previously placed in *Stenocactus* were transferred to his newly established subgenus *Stenocactus* of *Ferocactus* (Taylor, 1980) in the broad sense of the genus. It is remarkable that these authors' ideas converged in the same assumption of a paraphyletic, possibly polyphyletic *Ferocactus*. Moreover, the presence of morphologically similar flower scales have suggested some degree of relationship between *Ferocactus* and columnar cacti of North America (Gibson, 1992). Sclerification in the bract tips or scales of the pericarpel and floral tube occur in both Mexican columnar cacti of the Tribe Pachycereeae, subtribe Pachycereinae [*Pachycereus* (Berger) Britton and Rose], and subtribe Stenocereinae [*Escontria chiotilla* (F. A. C. Weber) Rose, *Myrtillocactus cochal* (Orcutt) Britton and Rose, *Stenocereus stellatus* (Pfeiff.) Riccob., and Tribe Cacteae [*F. flavovirens* (Scheidw.) Britton and Rose]. Some authors (Buxbaum, 1951; Gibson and Nobel, 1986) have suggested that homoplasious character transformation in the Cactaceae may be common. Molecular data in the next section demonstrate that the presence of sclerified scales in distantly related taxa is due to parallelism.

The molecular part of a biosystematic study of *Ferocactus* based on cpDNA restriction site variation and sequence of the gene *ndh*F, to investigate the monophyly of the genus, its interspecific relationships, and major lineages (as proposed by Taylor, 1984) has provided new insight into its taxonomy and phylogeny. The gene *ndh*F is located at one end of the small single copy region of the chloroplast (Fig. 6), and encodes a subunit of the NADH dehydrogenase (Scotland et al., 1995)]. In the next paragraphs I combine the preliminary results obtained from these projects because both studies focus on the same objectives. In both the restriction site variation and gene sequence studies, selected taxa from throughout the genus have been examined along with representative columnar cacti of North America (Tribe

Pachycereeae) and other cylindric cacti of Tribe Cacteae to clarify the relationships within the genus and the allied taxa of these tribes.

The 250 mutations (representing changes in restriction sites) detected in the restriction site study and the sequences obtained from the *ndh*F gene were analyzed independently using the principle of maximum parsimony with the program PAUP [Phylogenetic Analysis Using Parsimony (Swofford, 1993)] to construct trees. Because taxonomic sampling in the sequencing study is still limited, the sequencing results must be considered preliminary.

Because the restriction site study includes a larger number of taxa from the different lineages, the relationships determined from this study will be used to discuss aspects related to the phylogeny of *Ferocactus*. Although different in topology and species investigated, both phylogenies will be used in concert to discuss the relationships of *Ferocactus* with North American columnar cacti. For the restriction site study, a modified molecular phylogeny [*F. hamatacanthus* (Muehlenpf.) Britton and Rose was excluded as in Lindsay's treatment)] of that published by Cota and Wallace (1996; in press) obtained from this analysis is shown in Figure 7. In turn, Figure 8 shows the most parsimonious tree with the preliminary phylogeny obtained from sequences of the *ndh*F gene. Both phylogenies suggest the following:

1. *Ferocactus* is a paraphyletic assemblage as presently circumscribed in the different classifications, i.e., *Echinocactus grusonii* is included within the *Ferocactus* clade and is basal to *F. glaucescens* (DC.) Britton and Rose and *F. histrix*, making *Ferocactus* paraphyletic (this result is not evident in the *ndh*F phylogeny, and *E. grusonii* has not yet been sequenced for *ndh*F phylogeny).
2. There is no direct phylogenetic relationship of *Ferocactus*, in particular *F. flavovirens*, with columnar cacti of the Pachycereeae. This evidence eliminates the possibility of relationships between *Ferocactus* and North American columnar cacti, particularly with *Escontria chiotilla* based on similarities of chartaceous flowers scales as indicated by Gibson (1992).

3. This evidence confirms the monophyletic origin of Subfamily Cactoideae as proposed by Wallace and Cota (1996).
4. The North American columnar cacti of Tribe Pachycereeae and its two subtribes (Pachycereinae and Stenocereinae) are all monophyletic lineages.

The most remarkable result in this study is the possibility of paraphyly of *Ferocactus* as presently circumscribed, which confirms in part Lindsay's and Taylor's earlier suspicions on the origin of the genus. If *Ferocactus* were a monophyletic group it should include all of the descendants (which is not the case as *E. grusonii* has traditionally been treated as a separate taxonomic unit). It is evident that this preliminary phylogeny will likely raise more controversy. If we favor monophyletic groups, making *Ferocactus* monophyletic (including *E. grusonii*) requires a new taxonomic treatment and consequently, nomenclatural changes would be necessary. That is, the International Code of Botanical Nomenclature (Greuter et al., 1994) calls for the principle of priority of the earliest name formally published. In fact, the oldest valid generic name is *Echinocactus* because Engelmann in 1884 originally described the type species for the genus as *Echinocactus wislizeni*, and it was later proposed as *Ferocactus wislizeni* by Britton and Rose in 1922, when they segregated it from *Echinocactus*. Since this phylogeny is preliminary, and the remaining species need to be investigated, no further discussion is provided here to avoid more taxonomic confusion and misinterpretation. Some species of *Ferocactus* are still missing from this study as well as of species from *Echinocactus* and allied taxa from within the Cacteae, therefore, it is premature to ascertain the paraphyly of *Ferocactus*, until the study is completed.

The lack of molecular evidence to support the association between *Ferocactus* and the columnar cacti based on the presence of floriferous chartaceous scales led to a new hypothesis about the origin of *Ferocactus*. As suggested by Cota and Wallace (in press), it is feasible that *Ferocactus* probably evolved early in the divergence of the present Cacteae, and most likely shared a common ancestor with *Echinocactus* and *Stenocactus* as evidenced by some shared morphological characters (e.g., *Ferocactus* and *Stenocactus* have flattened central spines and naked scales of the pericarpel), features that might also indicate their close phylogenetic relationship. In addition, the similarity in rib pattern and glandular spines in *S. coptonogonus* and *Ferocactus* reflect close relationship (Taylor, 1980).

Based on the molecular data, to date, the phylogenetic relationships of *Ferocactus*, *Stenocactus* and *Echinocactus* appear to be clarified. The molecular evidence based on restriction site analysis of the cpDNA genome indicates that within the Subtribe Echinocactinae, *Stenocactus* is in a basal position relative to *Ferocactus* and *Echinocactus* (Fig. 7) (Cota and Wallace, in press). Conversely, on morphological grounds it is difficult to demonstrate the basal position of any one of these three genera. Nonetheless, in early classifications of the Cacteae, *Echinocactus* has been placed in a putatively basal position (Buxbaum, 1958; Barthlott and Hunt, 1993).

Although gene sequencing is one of the most effective methods to estimate the amount of DNA variation by comparing the order of nucleotides in different taxa, analysis of preliminary data failed to confirm the phylogenetic relationships at the interspecific level in *Ferocactus*. Two factors may account for this. First, a limited number of taxa were sampled, and second, the *ndh*F gene is evolving rapidly so that the phylogenetic signal might not be the most appropriate for estimating the degree of relationship. It has been reported that this gene is evolving 2-3 times faster that the *rbc*L (large subunit of ribulose biphosphate carboxylase/oxygenase) gene (Olmstead and Sweere, 1994). Similar results were obtained in the comparative analysis based on sequences of the non-coding region of the *trn*L-*trn*F (intergenic region of the transfer RNA genes) with 17 species of *Ferocactus* (Cota and Wallace, unpub. data): the degree of relatedness was unresolved (cladogram not included). However, the phylogenetic relationships of *Ferocactus* with columnar cacti corroborated those obtained from the restriction site and *ndh*F phylogenies, confirming that the presence of sclerified scales in distantly related lineages represents one more example of convergence in the Cactaceae.

THE BIOGEOGRAPHY OF *FEROCACTUS*

The geological history of Baja California explains in part the origin as well as the composition and affinities of the flora of the Sonoran Desert. Axelrod (1979) provides an excellent interpretation of the geological scenario that was involved in the origin and evolution of the flora of this area. Because the geographic distribution of *Ferocactus* includes northwestern mainland Mexico, the Baja California Peninsula, and southwestern U.S., it also possible to explain, in part, its geographic range based on the series of geological events that took place during the Pliocene and Pleistocene as described below. For a complete review of the geological history of this area, the reader should consult Axelrod (1979), Gastil et al. (1983), and Wiggins (1960) plus references included therein.

Before peninsular California was separated by the Gulf of California (Sea of Cortéz), it was connected to mainland Mexico in western Sonora, Sinaloa, and Nayarit. During the middle Miocene the peninsula first separated from continental Mexico (Axelrod, 1979) beginning the formation of the Gulf of California. The tip of the Peninsula (La Paz and Los Cabos regions) were presumably attached to Jalisco during the Miocene and joined the peninsula in the Pleistocene (Gastil et al., 1983). As the incipient gulf expanded, the islands formed through several natural processes such as submergence, uplift, erosion, and volcanism. Throughout this time, the islands probably acted as land bridges contributing to the expansion and colonization of new areas for plants. In others cases, they may act as centers in which divergence and thus speciation events were favored due to geographic isolation. It is quite likely that the islands in the Sea of Cortéz played a major role in the diversification of *Ferocactus* as they provided new ecological conditions and suitable habitats for establishment of immigrant individuals. In addition, time may have been a major ally in the divergence and differentiation of these populations that became isolated from their mainland relatives. Examples of these are those endemic species [e.g., *F. chrysacanthus*, *F. diguetii* (Weber) Britton and Rose, *F. gatesii* Lindsay, and *F. johnstonianus* (Britton and Rose) Fosb.] that probably arose from a common stock but diverged as they became geographically isolated. It is remarkable that these taxa appear to have mainland relatives and that morphological differences are subtle or incipient. Indeed, some of these island endemics have been described as varieties by some authors, which may reflect the actively evolving status of the genus.

The occurrence of 11 of the 25 species (44%) recognized by Lindsay (1955a) in the Baja California peninsula and surrounding islands is evidence that this area has been important in the diversification of the genus. To date, it is unknown whether these species originated after the separation of the peninsula from the Mexican coast. As a manner of speculation, a possible scenario of the distribution of the species of *Ferocactus* in Baja California can be explained by a bi-directional, possibly tridirectional pattern (Figs. 9 and 10). That is, if the species originated after the formation of the Sea of Cortéz, it is possible that some taxa (e.g., *F. acanthodes*) entered Baja California from the north, whereas the Baja California relatives of *F. covillei* and *F. wislizeni* reached the peninsula via a migratory route through the islands in the young Gulf of California. These islands probably acted as land bridges promoting the migrations of species into new habitats, which through time, most likely they diverged and evolved into new entities. Conversely, if some species evolved in the peninsula itself, then the ancestors of the extant floristic elements drifted along with the peninsula and then became isolated by subsequent geological events. Figures 9 and 10 show the hypothetical routes of radiation that might have taken place early in the evolutionary history of *Ferocactus* from the putatively center of origin in central Mexico.

Although the ideas I present here lack evidence such as fossil records to document migratory pathways, they are supported in part by plate tectonic events related to the origin of the Baja California peninsula itself, and to the affinities of its flora to mainland Mexico [e.g., *F. robustus* (from central Mexico) has similar architectural design of the seed coat with that of *F. wislizeni* (from northwestern Mexico) and the Baja Californian species *F. townsendianus* (Taylor and Clark, 1983), and other genera of the Cactaceae (*Cochemiea* (Brandegee) Walton and *Stenocereus* Riccob.) are allied with mainland genera of cacti (Wiggins, 1960) among other flowering plants]. Also, some floristic elements in southern Baja California (La Paz and Cabo San Lucas) are similar to plant species distributed in mainland Mexico in the areas of the Pacific Coast (Axelrod, 1979; Rzedowski, 1973). These affinities imply that some elements of the flora probably originated in mainland Mexico and migrated west towards the Baja California peninsula and southwestern U.S. (Axelrod, 1979). Moreover, the similarity in ecological conditions between the desert areas of Baja California, Sonora and Sinaloa, with those of Central Mexico, in particular the Tehuacán Valley suggested the same predominant conditions for the semideserts of the Tertiary (Axelrod, 1979).

It is interesting to note the parallelism in ecological conditions in these two desert areas because this information adds more insight into the patterns of radiation of *Ferocactus*. Taylor and Clark (1983) hypothesized that two major evolutionary lines (section *Ferocactus* and section *Bisnaga*) arose independently. The former originated from *F. robustus* and the latter from *F. flavovirens*, two species that have retained plesiomorphic characters in gross morphology of stem and growth habit. Interestingly, the authors suggested the Tehuacán Valley as the center from which *Ferocactus* evolved. In section *Bisnaga*, most of the species are restricted to east-central Mexico and appear to be well defined. However, according to Taylor and Clark (1983) and Taylor (1984) those taxa in section *Ferocactus* radiated and evolved into northwestern Mexico and Baja California, and have little morphological resemblance with their putative ancestor (*F. robustus*) from which they became isolated. It is likely that the "linking species" is missing, as indicated by the question mark in Fig. 9. Could it then been possible that the connection between the taxa of this phylad was lost during evolution? Likewise, it could have became extinct during the geological events that took place during the origin of the peninsula of Baja California, enhancing the morphological divergence between the descendants and the putative ancestor. It is clear that the evolution of the genus was in the direction of similar desert environments from those predominant in the Valley of

Tehuacán. Although no additional evidence is available, this explanation seems reasonable and based on the geological history, provides a stronger basis for the interpretation of the origin and radiation of the genus as suggested by Taylor and Clark (1983). Clearly, more data are needed to fully understand the patterns of radiation of *Ferocactus*.

CONCLUSIONS

Ferocactus is indeed a complex taxonomic unit. In spite of the effectiveness of molecular tools in systematic studies of the Cactaceae at different hierarchical levels, the interspecific relationships in the genus will remain unclear until taxonomic sampling is completed. However, the application of these techniques in the systematics of *Ferocactus* has proven to be of importance in understanding the intergeneric relationships and the evolution of certain morphological traits, such as the presence of chartaceous scales in the flowers. It is possible that some change in taxonomic circumscription will be made as future studies are conducted. Those molecular systematic studies in progress may clarify the relationships at the infra- and interspecific levels and may lead to a phylogeny which provides more insight into character evolution. Also, it is important to keep in mind that after more than 70 years of taxonomic work, some of the taxonomic and evolutionary parts of this *Ferocactus* story are gradually coming together. Consequently, the evolutionary history and phylogenetic reconstruction of this genus in particular, and of the Cactaceae in general, is being possible in part, thanks to these revolutionary studies in molecular systematics. Although additional taxonomic sampling is needed, it is satisfying to see that the modern phylogeny based on cpDNA restriction site data correlates well with Lindsay's classification. Overall, most of the species considered in his taxonomy and other authors' taxonomies (with the exception of *F. histrix* and *F. glaucescens*) are included in the cpDNA phylogeny as a monophyletic group. In addition, the inclusion of these two species within the *Ferocactus* clade confirms previous ideas about the paraphyletic origin of the genus. The next step for clarifying the patterns of evolution should allow determination of the major lineages within *Ferocactus* and how they have diverged in time into such a morphologically plastic taxonomic unit.

Finally, I would like to mention that molecular studies for phylogenetic reconstruction are not being done to prove whether a certain taxonomic treatment is correct; they are done to test proposed taxonomies in terms of phylogeny, and more importantly, to understand the patterns of radiation in the different lineages, as well as the evolution of floral and other morphological characters. It is noteworthy that the existence of previous treatments based on classical taxonomy allow us to apply molecular techniques in concert with morphological traits to generate what we think will be a more sound phylogenetic foundation for the Cactaceae. Molecular characters in combination with morphology are rich sources of data which can explain evolutionary patterns and phylogenetic relationships in the cactus family.

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Table 1. Comparison of the taxonomic categories used in the different classifications of the genus *Ferocactus*. Bold numbers indicate number of species in each treatment. * Includes five natural hybrids.

Authority	**Subgenus**	**Section**	**Group**	**Species**
Britton and Rose, 1922				**30**
Lindsay, 1955a				**25**
Taylor, 1980	*Stenocactus*			**4**
Taylor and Clark, 1983	*Ferocactus*	*Ferocactus*	*F. robustus*	13
			F. pottsii	4
		Bisnaga (Orcutt) Taylor and Clark	*F. glaucescens*	5
			F. recurvus	7
				29
Taylor, 1984	*Ferocactus*	*Ferocactus*	*F. robustus*	9
			F. pottsii	3
		Bisnaga	*F. glaucescens*	5
			F. latispinus	6
				23
Bravo and Sánchez-M., 1991	*Ferocactus* Britton and Rose			27
	Hamatacanthus Bravo			1
	Pennisquama Buxb.			1
				29
Unger, 1992	*Ferocactus*	*Ferocactus*		13*
		Pottsia Unger		5
	Bisnaga (Orcutt) Unger	*Bisnaga* (Orcutt) Unger		5
		Glaucescenti Unger		5
				28

Table 2. Chromosome counts reported for *Ferocactus*. Taxonomy and authority names in the taxon column follow that of Lindsay (1955a). Asterisk indicates species excluded from Lindsay's classification.

Taxon	**Reported as**	**Chromosome number**	**Reference(s)**
F. chrysacanthus (Orcutt) Britton and Rose	*F. chrysacanthus*	$2n = 22$	Cota et al. (1996).
F. acanthodes (Lem.) Britton and Rose	*F. acanthodes*		
var. *acanthodes*	var. *acanthodes*	$n = 11$	Pinkava et al. (1977).
	F. rostii	$n = 11$	Stockwell (1935).
var. *lecontei* (Engelm.) Linds.	var. *lecontei*	$n = 11$	Pinkava and McLeod (1971); Pinkava et al. (1977, 1992).
		$2n = 22$	Cota el al. (1996).
F. echidne (DC.) Britton and Rose	*F. echidne*	$2n = 22$	Katagiri (1952).
F. covillei Britton and Rose	*F. covillei*	$2n = 22$	Katagiri (1952).
F. fordii (Orcutt) Britton and Rose	*F. fordii*		
var. *fordii*	var. *fordii*	$n = 11$	Pinkava et al. (1973); Cota et al. (1996).
F. gracilis H. E. Gates	*F. gracilis*		
var. *gracilis*	var. *gracilis*	$n = 11$	Pinkava et al. (1973, 1977, 1985); Cota et al. (1996).
*F. hamatacanthus** (Muehlpf.) Britton and Rose	*F. hamatacanthus*	$n = 11$	Beard (1937).
var. *hamatacanthus**	var. *hamatacanthus*	$n = 11$	Weedin and Powell (1978).
F. histrix (D.C.) G. E. Linds.	*F. histrix*	$n = 11$	Cota et al. (1996).
F. latispinus (Haw.) Britton and Rose	*F. latispinus*	$2n = 22$	Katagiri (1952); Cota et al. (1996).
F. macrodiscus (Mart.) Britton and Rose	*F. macrodiscus*	$n = 11$	Cota et al. (1996).
F. peninsulae (Engelm. ex Weber) Britton and Rose			
var. *peninsulae*	var. *peninsulae*	$n = 11$	Cota et al. (1996).
F. pilosus (Galeotti) Werderm.	*F. pringlei*	$n = 11$	Pinkava et al. (1977).
	F. pilosus	$n = 11$	Pinkava and Parffit (1982).
	F. stainesii	$n = 11$	Katagiri (1952).

Table 2. Continued.

Taxon	Reported as	Chromosome number	Reference(s)
F rectispinus (Engelm.) Britton and Rose	*F. rectispinus*	2*n* = 22	Cota et al. (1996).
F. recurvus (Mill.) Y. Ito	*F. recurvus*	2*n* = 22	Cota et al. (1996).
F. robustus (Otto) Britton and Rose	*F. robustus*	2*n* = 22	Cota et al. (1996).
F. townsendianus Britton and Rose	*F. peninsulae* var. *townsendianus*	2*n* = 22	Cota et al. (1996).
F. viridescens (Nuttal) Britton and Rose	*F. viridescens*	*n* = 11	Pinkava et al. (1973; 1977).
	var. *viridescens*	*n* = 11	Cota et al. (1996).
F. wislizeni (Engelm.) Britton and Rose	*F. wislizeni*	*n* = 11	Pinkava et al. (1973).
		2*n* = 22	Katagiri (1952); Cota et al. (1996).

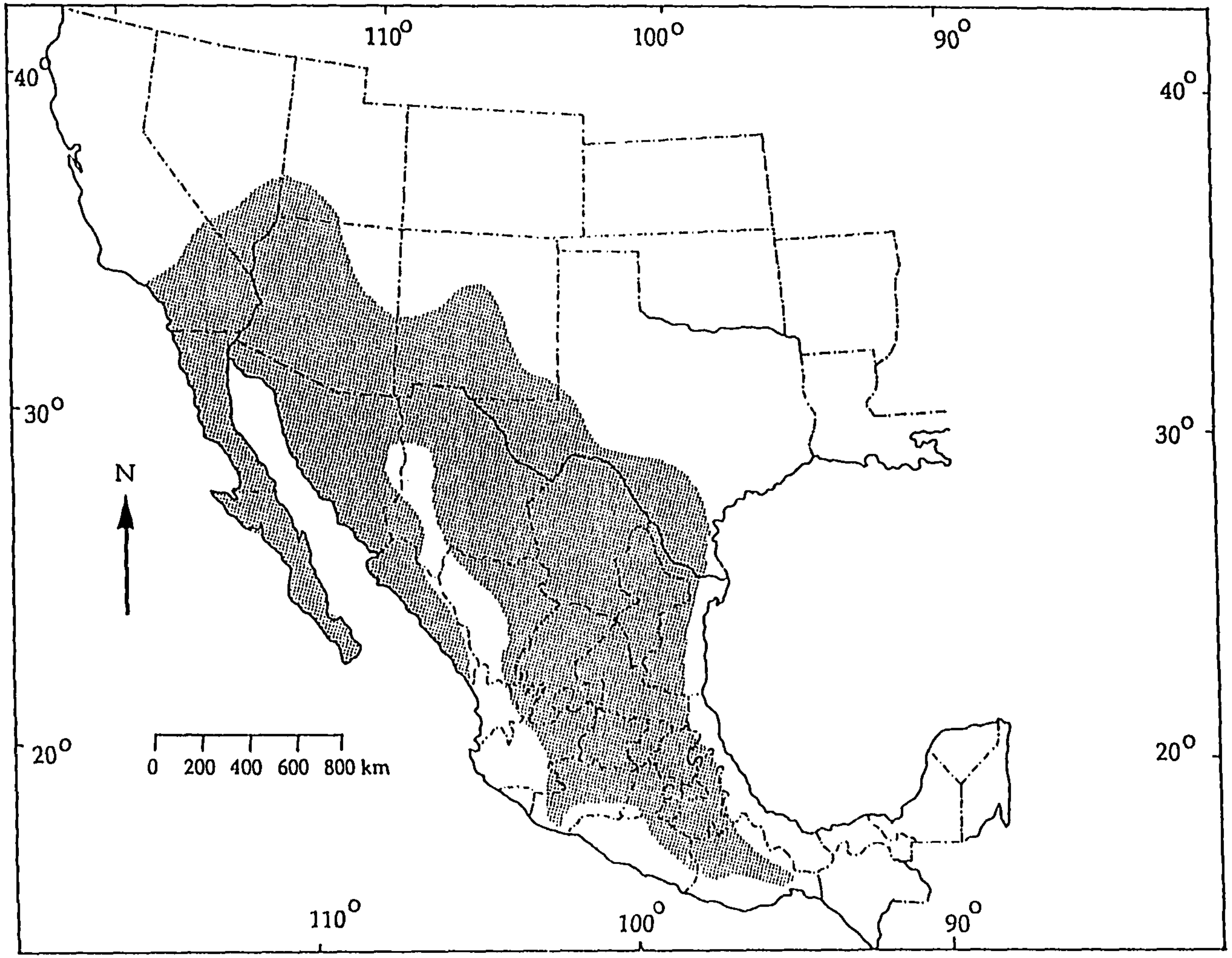

Figure 1. Overall geographic distribution of *Ferocactus* based on data from Lindsay (1955a) and Taylor (1984). The Baja California Islands have been omitted from the map.

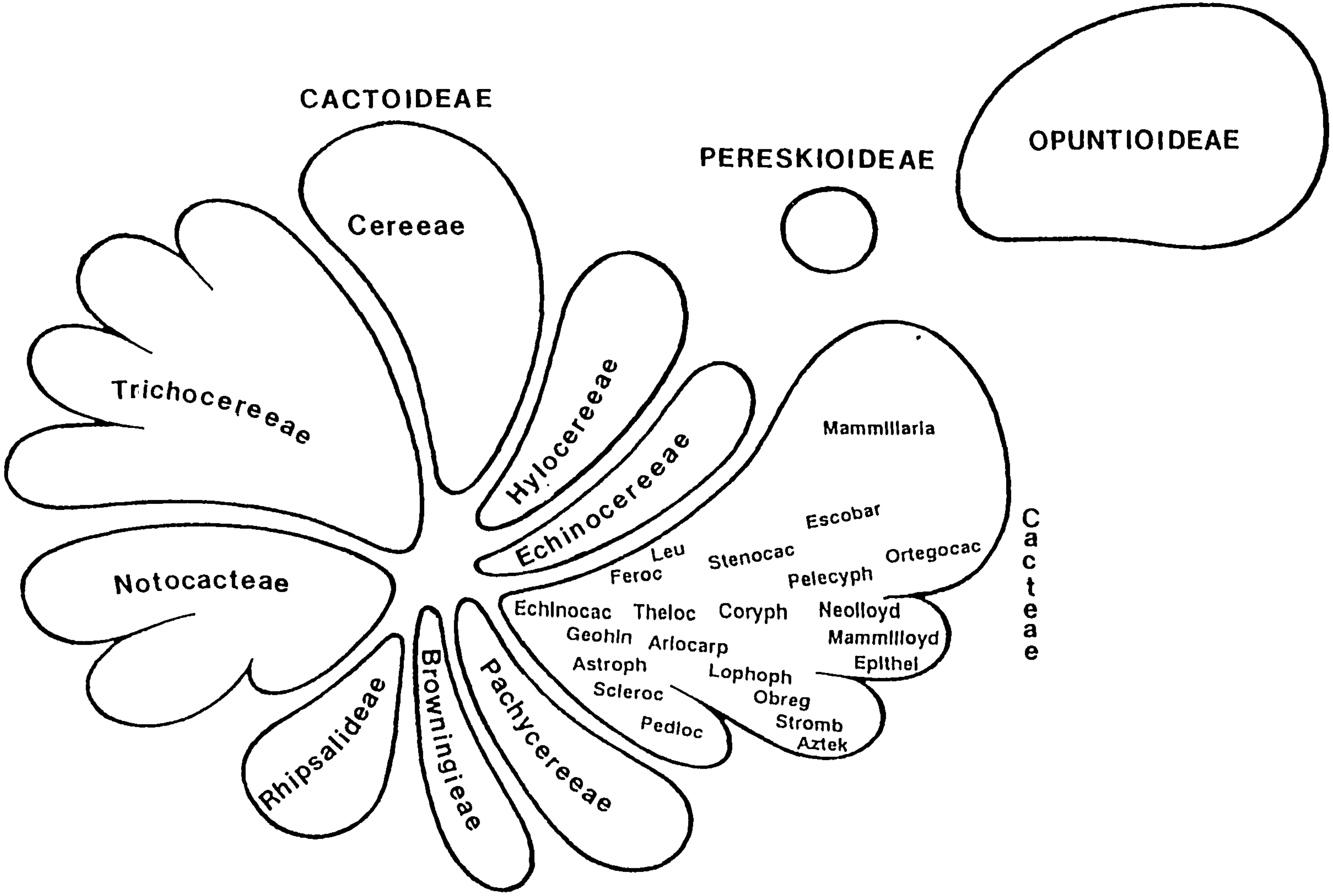

Figure 2. Diagram showing the presumably evolutionary position of *Ferocactus* within the Tribe Cacteae of the Subfamily Cactoideae. Note the position of *Ferocactus* relative to *Echinocactus* and *Stenocactus*. Figure modified from Barthlott and Hunt (1993).

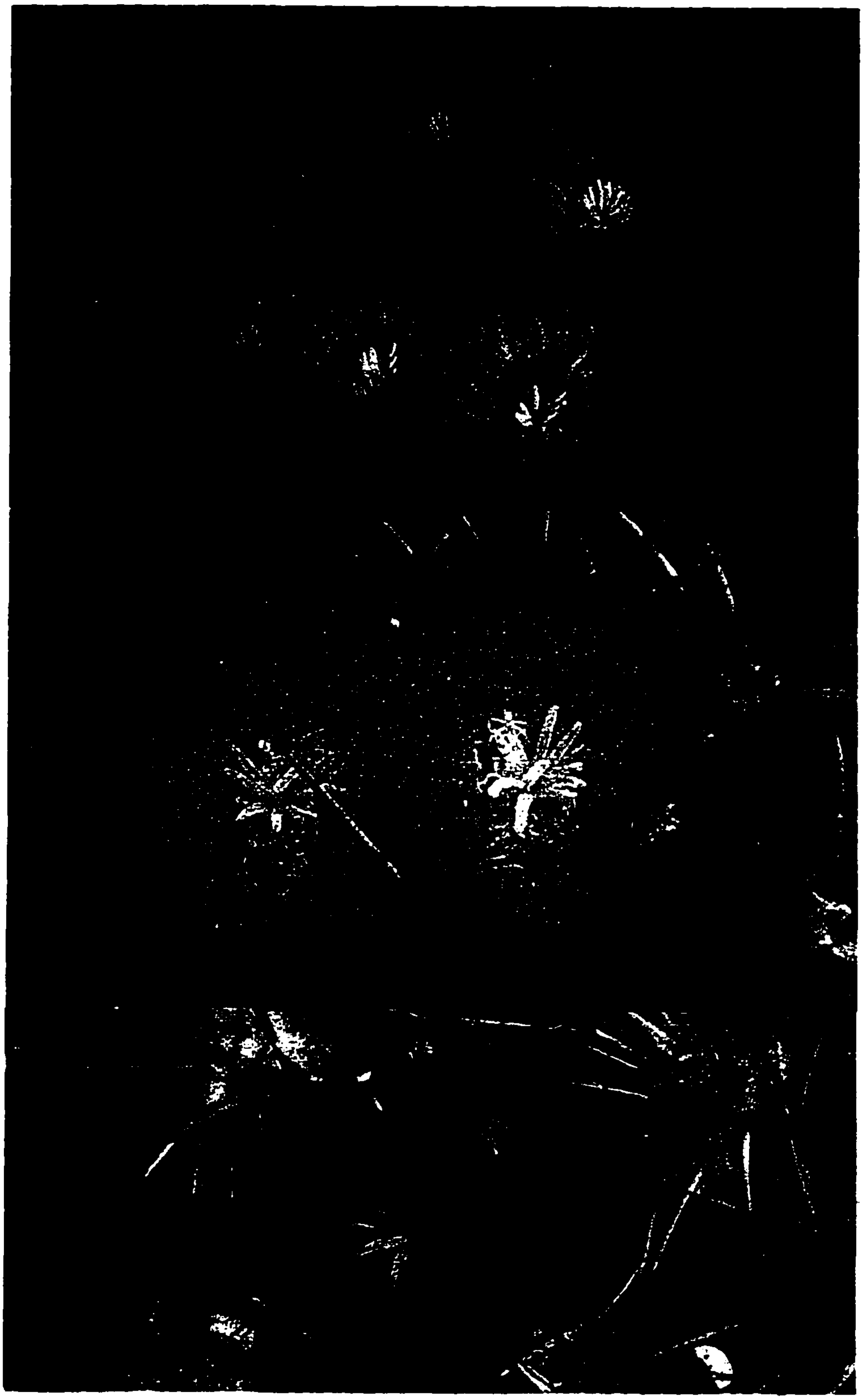

Figure 3. Floral morphology in *Ferocactus*. A. *F. macrodiscus*, G. Lindsay 2607. B. *F. echidne* var. *echidne* from the Barranca of Meztitlán, G. Lindsay, 2069. C. *F. flavovirens* from Zapotitlán Salinas, G. Lindsay, 2058.

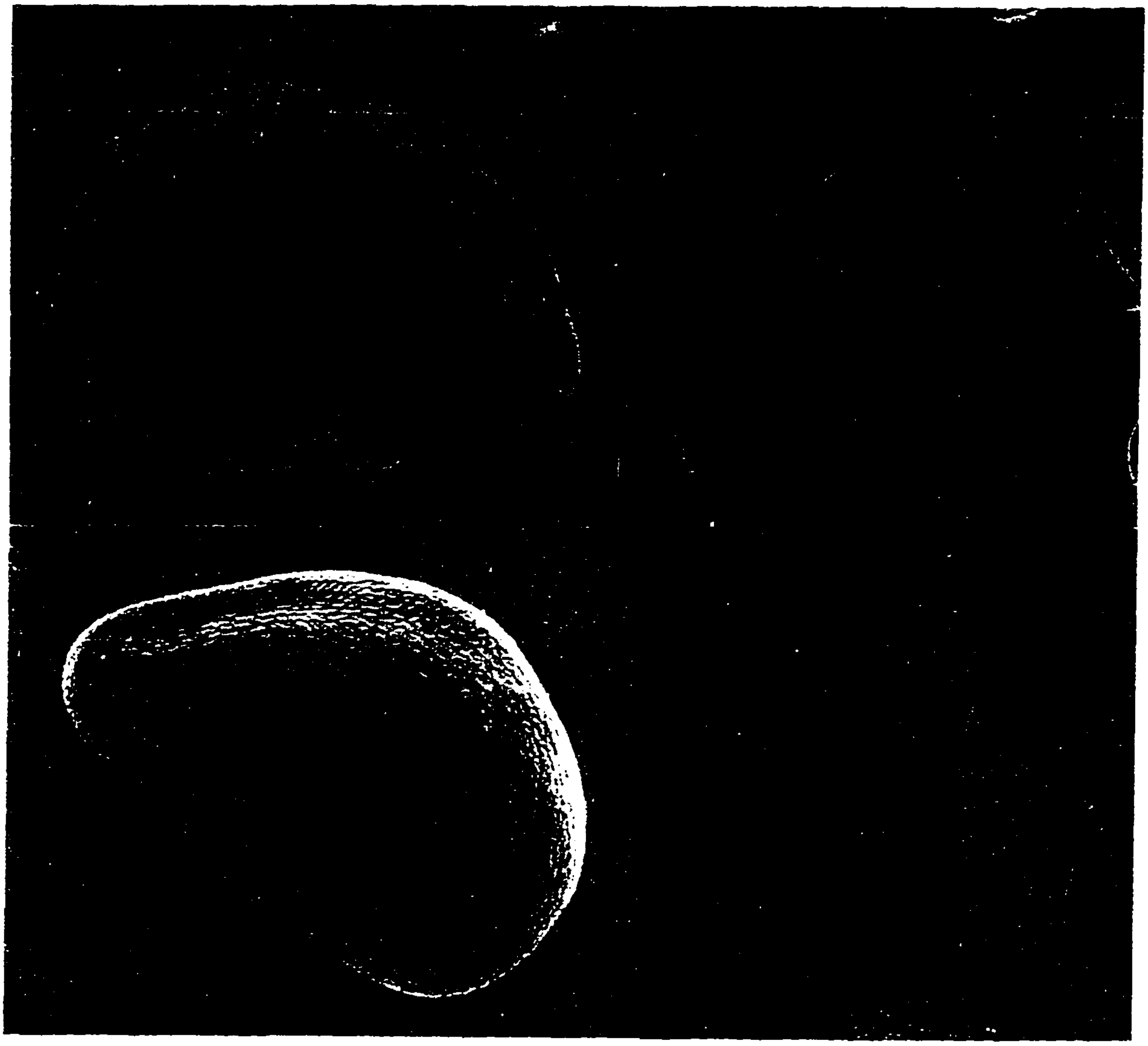

Figure 4. Seeds of *Ferocactus*. A. Seed of *F. rectispinus* (H. Cota, 8027) with tabular concave testa-cells. B. Detail of seed-coat of *F. rectispinus* showing portions of the testa-cells with cracks of the seed-coat. C. Seed of *F. acanthodes* var. *lecontei* (W. Wisura, s.n., RSA). D. Cross section of seed of *F. acanthodes* var. *lecontei*.

Figure 5. Representative species of *Ferocactus* showing meiotic and mitotic chromosomes.
A. *F. gracilis* var. *gracilis*, J. Rebman 2728, Metaphase II. Scale bar = 20 µm.
B. *F chrysacanthus*, H. Cota 7441. Arrows indicate satellites. Scale bar = 10 µm.
C. *F. latispinus*, H. Cota, 8039. Scale bar = 10µm.

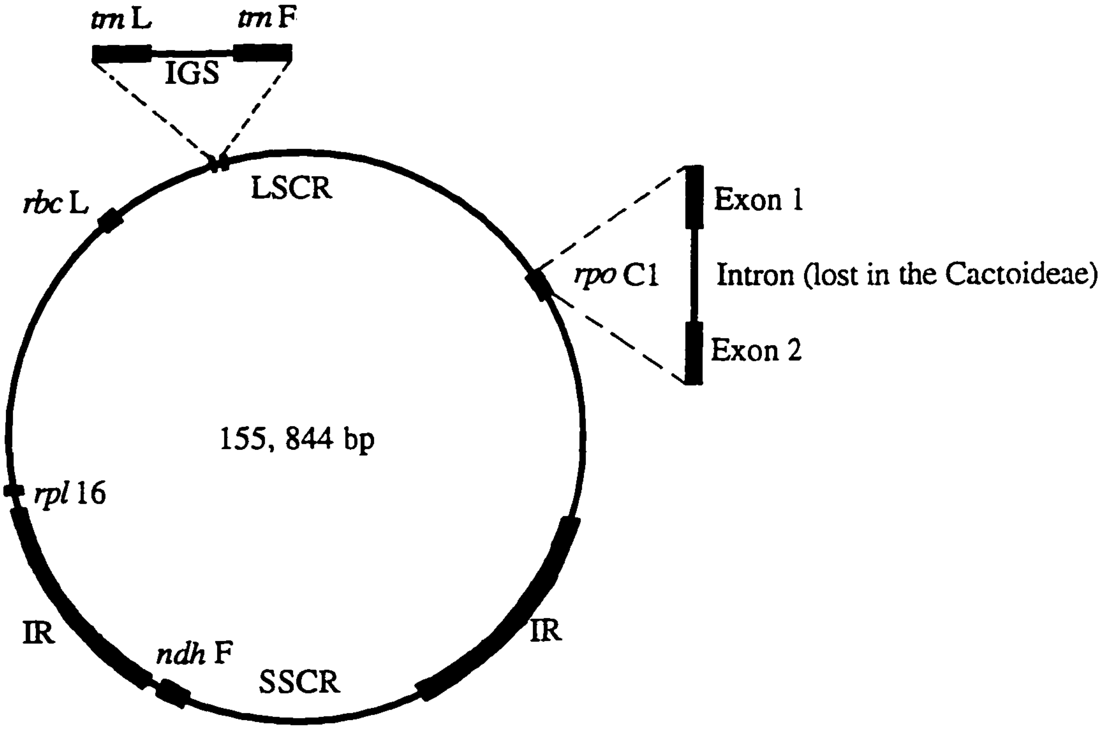

Figure 6. Diagram showing the circular molecule of the cpDNA in *Nicotiana tabacum* (modified from Shinozaki et al., 1986) and the approximate positions of the genes and regions used in the phylogenetic studies of the Cactaceae, including *Ferocactus*. LSCR = Large Single Copy Region, SSCR = Small Single Copy Region, IR = Inverted Repeat, IGS = Intergenic Spacer between the *trn*L (UAA) 3' exon and the *trn*F (GAA) genes. The abbreviations for the genes are: *ndh*F = NADH dehydrogenase, *rbc*L = Large subunit of rubisco, *rpl*16 = ribosomal protein, *rpo*C = RNA polymerase, *trn*L and *trn*F = transfer RNA genes. For details about the structure and gene order of the cpDNA molecule the reader is referred to Shinozaki et al. (1986).

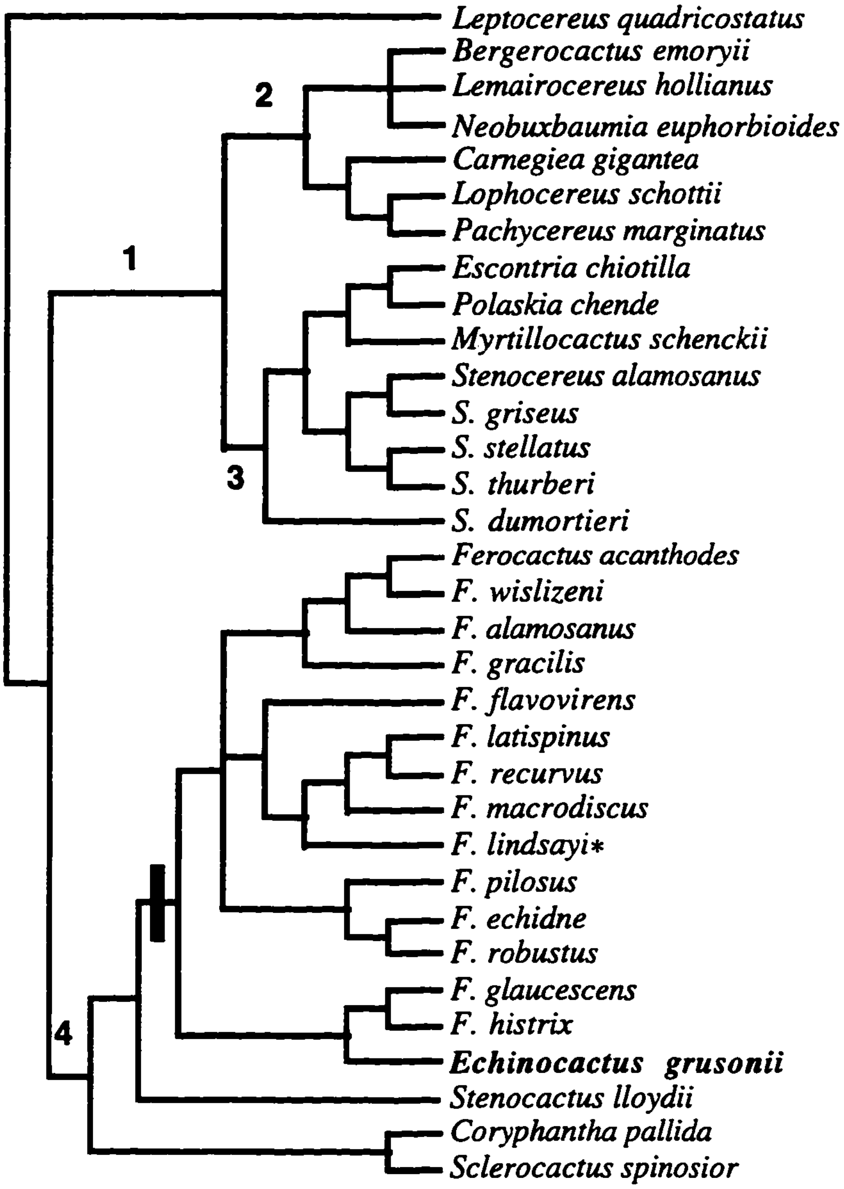

Figure 7. 50% Majority Rule consensus tree of 42 equally parsimonious trees (length = 320) and Consistency Index = 0.772) depicting the preliminary phylogeny for the genus *Ferocactus* obtained from cladistic analysis of cpDNA restriction site data (data from Cota and Wallace, submitted). The thick bar indicates the inclusion of *E. grusonii* within *Ferocactus* making it paraphyletic. Also, there is no relationship of any of the columnar cacti with *Ferocactus*. **1** = represents evidence of monophyly in the Pachycereeae; **2** = monophyly for the Pachycereinae; **3** = monophyly for the Stenocereinae; **4** = monophyly for the Cacteae. Asterisk represents species described by Bravo-Hollis (1966) after Lindsay's taxonomic treatment of the genus was proposed.

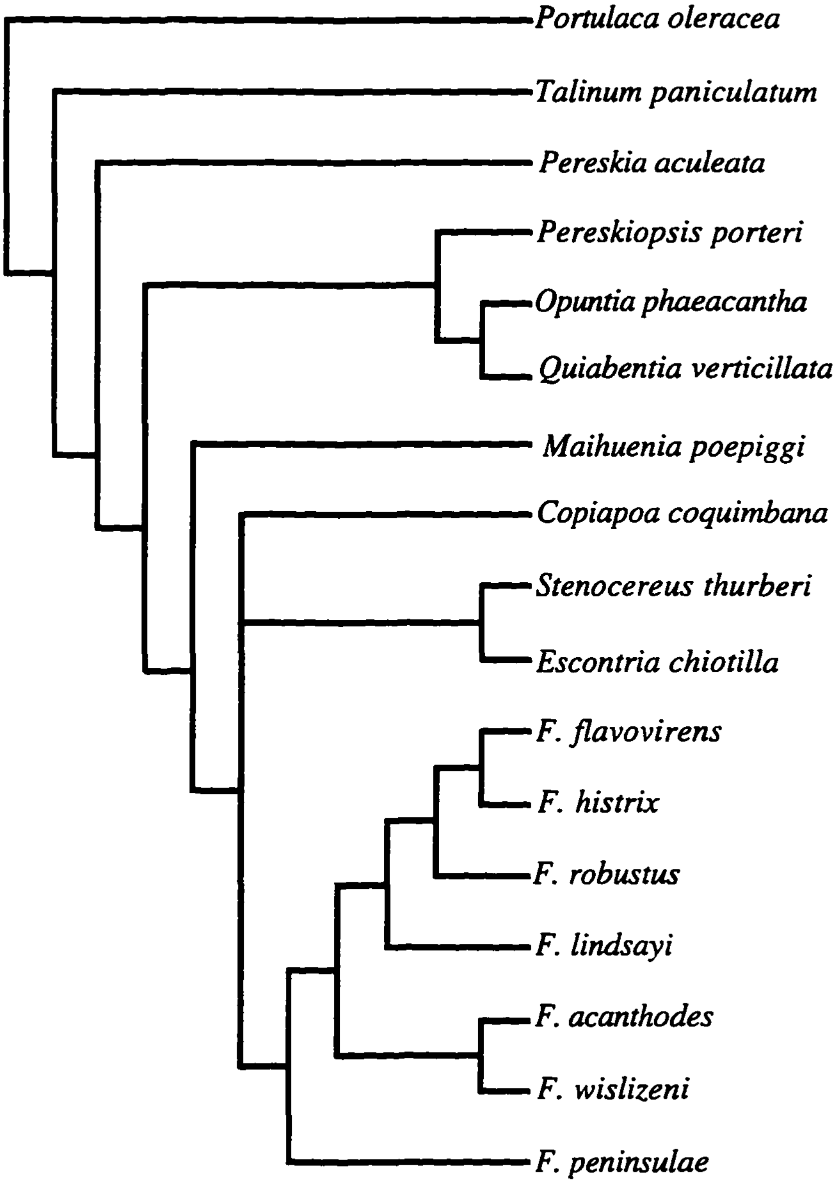

Figure 8. Strict consensus tree of three equally parsimonious trees (length = 1434 and Consistency Index = 0.79) showing preliminary phylogenetic relationships of *Ferocactus* based on sequences of the chloroplast gene *ndh*F (Cota and Wallace, unpub. data). See text for detailed explanation about the phylogenetic relationships of the cladogram.

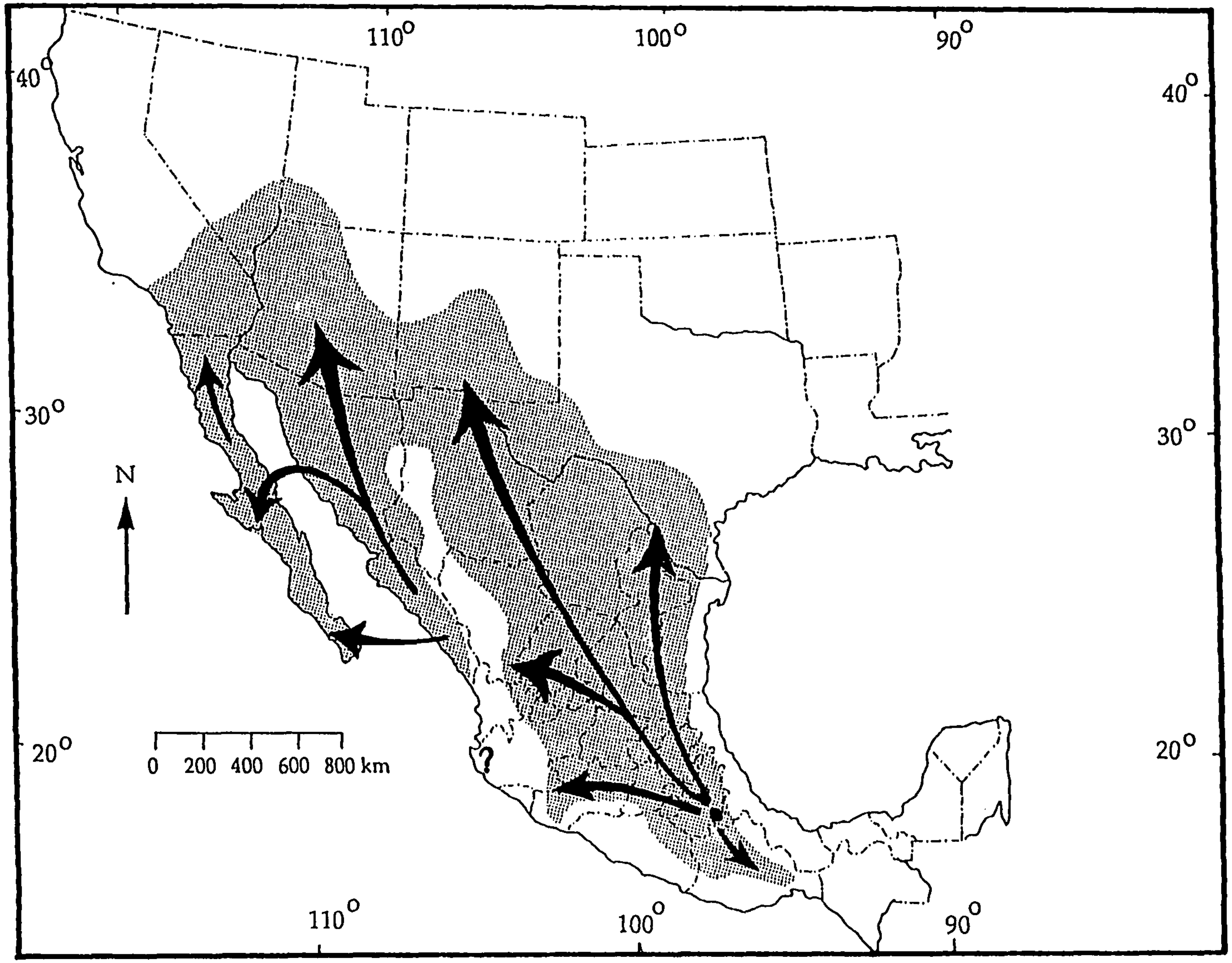

Figure 9. Hypothetical patterns of radiation in *Ferocactus*. The center of origin in this model is based on that proposed by Taylor and Clark (1983): the Tehuacan Valley in central Mexico.

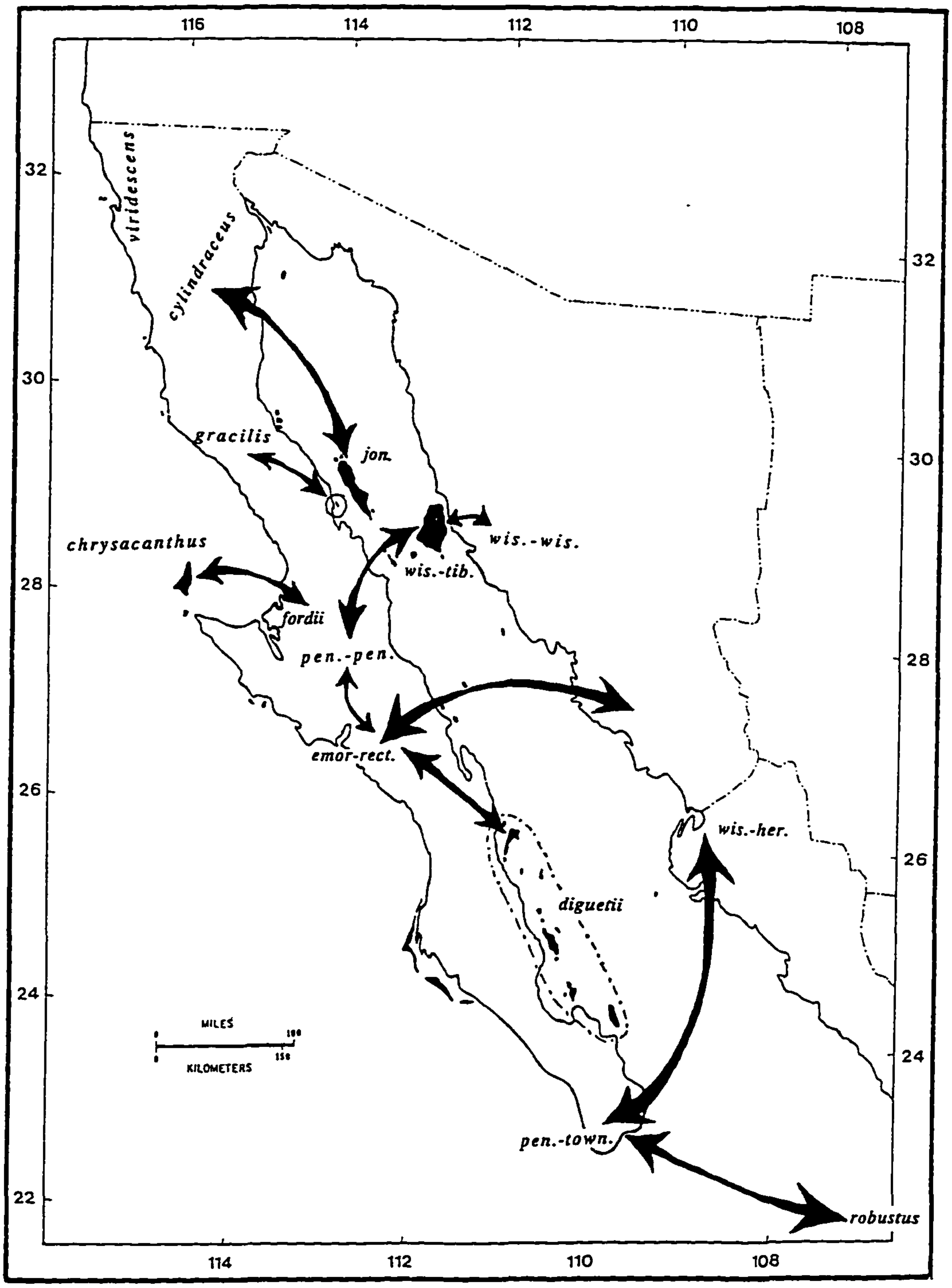

Figure 10. Hypothetical routes of migration of *Ferocactus* into the Baja California Peninsula and morphological affinities of Baja Californian species with island and mainland relatives (indicated by arrows).

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CHAPTER THREE

CHROMOSOME NUMBERS IN *FEROCACTUS* (CACTACEAE: CACTOIDEAE)[1]

A paper published in the Journal Cytologia[1]

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ABSTRACT

Meiotic and mitotic chromosome numbers were determined for 14 taxa of *Ferocactus*. Chromosome numbers are reported for the first time for seven species, including two varieties of *F. peninsulae*, and chromosome counts were confirmed for an additional six species. All taxa investigated were diploid and have a base chromosome number of $x = 11$. Within the Cactaceae, *Ferocactus* appear to have the largest chromosomes. Meiotic figures in Baja Californian and Mexican mainland species failed to document hybridization at least for those populations investigated. The morphological homogeneity of chromosomes and the apparently consistent diploid condition throughout the genus suggest that chromosome evolution in *Ferocactus* is taking place at the molecular level.

Key words: Cactaceae, Cactoideae, *Ferocactus*, diploid.

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INTRODUCTION

The Cactaceae is subdivided into three subfamilies (Pereskioideae, Opuntioideae, and Cactoideae) which are traditionally interpreted as monophyletic lineages (Barthlott and Hunt, 1993). The Cactoideae include 85% of the species diversity in the family and exhibit the greatest morphological extremes in habit and stem structure. This subfamily includes the tribe Cacteae, which is found mainly in arid or semiarid habitats in North America and contains the genus *Ferocactus* Britton and Rose.

The taxonomy of the genus remains in a somewhat confused state due to several factors. As for most groups within the Cactaceae, the lack of extensive field research to document variability, the relatively high levels of morphological homoplasy, and the use of primarily vegetative morphological characters, make the establishment of species boundaries difficult. The number of species recognized in the genus has been a matter of disagreement and is reflected in several taxonomic treatments that have been produced over the last seven decades. Britton and Rose (1922) proposed a classification in which they included 30 species; subsequently, Lindsay (1955) proposed that the genus was comprised of 25 species. More recently, Taylor (1984) recognized 23 species, while Bravo and Sánchez-Mejorada (1991) accepted 29. Finally, Unger (1992) presented a classification scheme for 20 species based on the previous treatments. Of these classifications, that of Taylor (1984) represents a systematic treatment of the genus *sensu stricto*, and is the most widely accepted by cactus taxonomists because it includes detailed descriptions of floral, fruit, and seed coat characters.

The phylogeny of *Ferocactus* is uncertain; however, two major evolutionary lineages [section *Bisnaga* (Orcutt) N. P. Taylor and J. Y. Clark and section *Ferocactus*] have been proposed for the genus. The former probably derived from *F. flavovirens* (Scheidw.) Britton and Rose and the latter from *F. robustus* (Link and Otto) Britton and Rose (Taylor and Clark, 1983). These two species are distributed in south-central Mexico, a region that is thought to be the center of origin of the genus (Taylor, 1984), and have apparently retained plesiomorphic features that place them as putatively basal species within the genus. On morphological grounds, the two sections are distinguished on the basis of a fruit character: in section *Ferocactus* the fruits dehisce by a basal pore, and in section *Bisnaga* the fruits are juicy and indehiscent, occasionally splitting irregularly (Barthlott and Hunt, 1993; Taylor, 1984).

This study was undertaken to document chromosome numbers in *Ferocactus* and to discuss the causes of its apparently stable diploid condition.

MATERIALS AND METHODS

Fourteen taxa of *Ferocactus*, representing sections *Bisnaga* and *Ferocactus* were examined in this study. For these species, flower buds and seeds were field collected and fixed from natural populations. When possible the selection of the taxa investigated was based on geographic distribution and their taxonomic position in the two sections as proposed by Taylor (1984).

The preservation of flower buds for meiotic chromosome counts and analyses follows that of Pinkava *et al.* (1977). Buds were fixed in Farmer's solution (3 ethanol: 1 glacial acetic acid, v/v) for at least 24-48 hrs, then washed and stored in 70% ethanol under refrigeration. The anthers were dissected from floral buds, squashed in iron-aceto-carmine, and mounted in Hoyer's medium following Beeks (1955). For mitotic counts, seedling radicles and chromosome squashes were obtained as described by Cota and Philbrick (1994). Chromosome observations and analyses were conducted with a Leitz phase-contrast microscope and a 100X oil objective. Voucher specimens are deposited in ASU, BCMEX, CANTE, DES, ENCB, HUMO, and ISC herbaria as indicated in Table 1. Nomenclature follows that of Taylor (1984) with the exception of *F. rectispinus* and *F. recurvus*, specific epithets that are maintained according to Bravo and Sánchez-Mejorada (1991) and Lindsay (1955).

RESULTS

In this study chromosome numbers are reported for 14 taxa (Table 1). Of these, eight represent first-time counts: *Ferocactus chrysacanthus*, *F. histrix*, *F. macrodiscus*, *F. peninsulae* var. *peninsulae* and var. *townsendianus*, *F. rectispinus*, *F. recurvus*, and *F. robustus*. Chromosome counts for *F. cylindraceus* var. *lecontei*, *F. fordii* var. *fordii*, *F. gracilis* var. *gracilis*, *F. latispinus*, *F. viridescens* var. *viridescens*, and *F. wislizeni* are confirmed. All chromosome counts reported here are consistent with the base chromosome number for the family ($x = 11$). Our analyses of meoitic figures show no disruption of cell divisions (Figs. 1-6). Mitotic chromosomes arrested in metaphase are homogeneous in overall morphology, from 3-7 µm in length, and mostly metacentric and submetacentric (Figs. 7-14); some species have chromosomes bearing one pair of satellites (Figs. 10, 11, 14).

DISCUSSION

At present, most of the cytological data for *Ferocactus* has been presented as chromosome counts and all previously investigated species are diploid and have the base number $x = 11$ (Beard, 1937; Katagiri, 1952; Pinkava and McLeod, 1971; Pinkava and Parfitt, 1982; Pinkava *et al.*, 1973; 1977, 1985; Stockwell, 1935; Weedin and Powell, 1978). No cases of aneuploidy or polyploidy have been documented.

Some of the taxonomic confusion that characterizes *Ferocactus* has been attributed to hybridization and introgression events. Lindsay (1955) and more recently Taylor (1984) have both indicated that Baja Californian species are taxonomically problematic due to probable introgressive processes. This hypothesis is based upon observations of individuals with intermediate morphologies in sympatric areas. Indeed, the possibility of gene exchange between *F. gracilis* and *F. peninsulae* has been suggested (Taylor, 1984). Polymorphism in spine shape and color in addition to intermediacy of stem and flower characters are common in individuals of the same population. At present, it is unclear to what degree this variation is genetically and/or environmentally induced. Our analyses of meiotic configurations in different individuals of *F. gracilis* and *F. peninsulae* failed to provide evidence for hybridity at least for the populations investigated. In addition, previous meiotic counts of some Baja Californian and Mexican mainland species (Beard, 1937; Pinkava *et al.*, 1973, 1977, 1985) exhibited normal pairing. Nonetheless, hybridization should not be ruled out until further cytological analyses are conducted in those central Baja Californian populations of *F. gracilis* and *F. peninsulae* for which gene exchange has been suggested by Taylor (1984).

In terms of chromosome size, chromosomes in *Ferocactus* are relatively small when compared with other plants, e.g., *Allium* L. however, they appear to be the largest in the Cactaceae. Their relatively uniform morphology and size seem not to be correlated with plant size. Similar patterns in chromosome morphology have been observed previously in unrelated genera of the Cactaceae such as *Echinocereus* Engelm. (Cota and Wallace, 1995), *Mammillaria prolifera* (Miller) Britton and Rose (Johnson, 1980) and *Nyctocereus* (Berger) Britton and Rose (Palomino *et al.*, 1988). The cytological uniformity in shape and number does not necessarily reflect evidence of close phylogenetic relationship, but the presence of satellites in some northern species, such as *F. chrysacanthus* (Fig. 10), *F. cylindraceus* var. *lecontei* (Fig. 11), and *F. wislizeni* (Fig. 14) indicates that satellites can be used as taxonomic markers, and that further analyses of chromosome morphology might be advantageous to understand the interspecific relationships in the genus.

The frequency of polyploidy in the Cactoideae (12.5%) is considered low (Pinkava et al., 1985), and unlike other genera of the subfamily in which polyploidy is relatively common, such as *Echinocereus* (Cota and Philbrick, 1994; Pinkava *et al.*, 1992; Weedin and Powell, 1978), all the species of *Ferocactus* which have been cytologically investigated are diploid. Our findings indicate that *F. robustus*, one of the putatively ancestral species (Taylor, 1984) has not experienced changes in chromosome number. The same apparently stable diploid number has been observed in *Ferocactus*' putative sister genera such as *Echinocactus grusonii*

Hildm. and *Stenocactus* (*Echinofossulocactus*) *crispatus* (DC.) Berger (Katagiri, 1952); *Echinocactus polycephalus* (Engelm.) Bigel. (Pinkava *et al.*, 1977); *E. horizonthalonius* Lem., and *E. texensis* Hoppfer (Weedin and Powell, 1978); and *S. pentacanthus* (Lem.) Berger (Pinkava and Parfitt, 1982).

Based on our findings, it appears that changes in chromosome number do not play a major role in the evolution of *Ferocactus*; it is conceivable that chromosome evolution in the genus may be taking place at the molecular level and, consequently, chromosome rearrangements remain cryptic. Preliminary data on DNA sequences of the chloroplast gene *ndh*F indicate that the rate of nucleotide substitution in the Cactoideae, including *Ferocactus*, is higher than in the Pereskioideae and Opuntioideae (Cota and Wallace, unpublished data). Also, the intron loss within the chloroplast gene *rpo*C1 has been found in the Cactoideae, including *F. flavovirens* (Wallace and Cota, 1996). In addition, DNA sequences of non-coding regions of the *rpl*16 and *trn*L-*trn*F genes of the chloroplast genome show sequence divergence (insertions and deletions events) in several species of *Ferocactus* (Cota and Wallace, unpublished data), supporting chromosomal evolution at the molecular level. Further cytological and molecular investigations with a wider taxonomic sampling within *Ferocactus* may be useful to determine the extent of the diploid condition and to detect cases of hybridization in populations where individuals with intermediate morphology are common.

ACKNOWLEDGMENTS

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Table 1. Chromosome counts for *Ferocactus* and voucher information. Symbols: * first report for the species, n = meiotic counts, $2n$ = mitotic counts, ASU = Arizona State University, BCMEX = Universidad Autónoma de Baja California, CANTE = Jardín Botánico CANTE, DES = Desert Botanical Garden, ENCB = Escuela Nacional de Ciencias Biológicas, HUMO = Universidad Autónoma del Estado de Morelos, ISC = Ada Hayden Herbarium of Iowa State University, RSA = Rancho Santa Ana Botanic Garden.

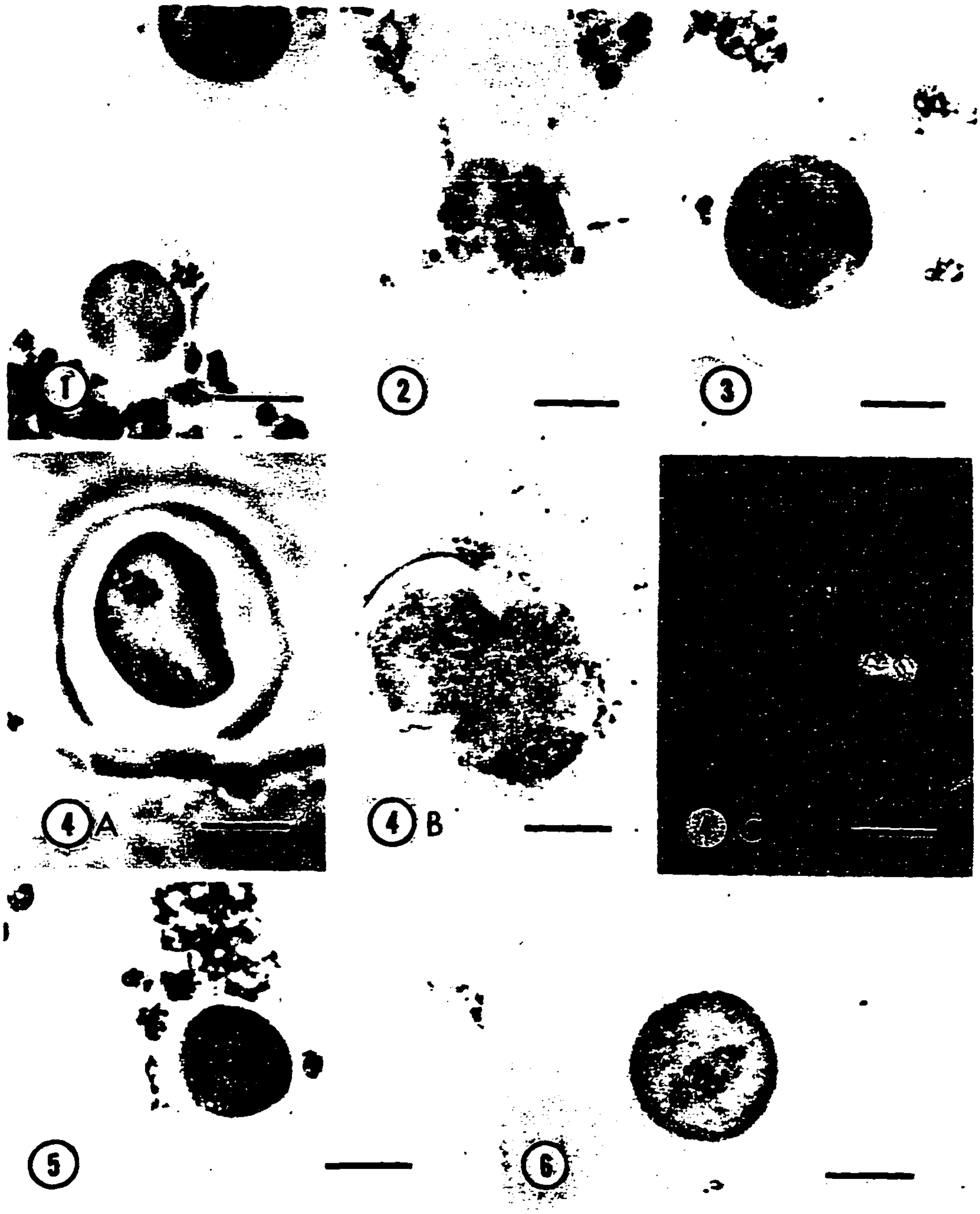
Taxon	Chromosome count	Source, Herbaria
Section *Bisnaga* (Orcutt) N. P. Taylor and J. Y. Clark		
* *F. histrix* (DC.) G. E. Lindsay	$n = 11$	MEXICO. Guanajuato: Rancho Alcocer, approx. 5 km SE San Miguel de Allende. 12 Jul. 1993, H. Cota 8037 (CANTE).
F. latispinus (Haw.) Britton and Rose	$2n = 22$	MEXICO. Guanajuato: Rancho Alcocer, approx. 5 km SE San Miguel de Allende. 12 Jul. 1993, H. Cota 8039 (CANTE).
* *F. macrodiscus* (Mart.) Britton and Rose	$n = 11$	MEXICO. Guanajuato: Rancho Alcocer, approx. 5 km SE San Miguel de Allende. 12 Jul. 1993, H. Cota 8038 (CANTE).
* *F. recurvus* (Miller) G. E. Lindsay	$2n = 22$	MEXICO. Puebla: Tehuacán Valley, between Tehuacán and Zapotitlán Salinas. 17 Jul. 1993, H. Cota 8049 (HUMO).
* *F. robustus* (Link and Otto) Britton and Rose	$2n = 22$	MEXICO. Puebla: Tehuacán Valley, between Tehuacán and Zapotitlán Salinas. 17 Jul. 1993, H. Cota 8045 (HUMO).
Section *Ferocactus*		
* *F. chrysacanthus* (Orcutt) Britton and Rose	$2n = 22$	MEXICO. Baja California: Cedros Island, north end of Island. 28 Dec. 1985, H. Cota 7441 (ENCB).
F. cylindraceus (Engelm.) Orcutt var. *lecontei* (Orcutt) Britton and Rose	$2n = 22$	U.S.A. California: San Bernardino Co., Clark Mts. May 1992, W. Wisura s.n. (RSA).

Table 1. Continued.

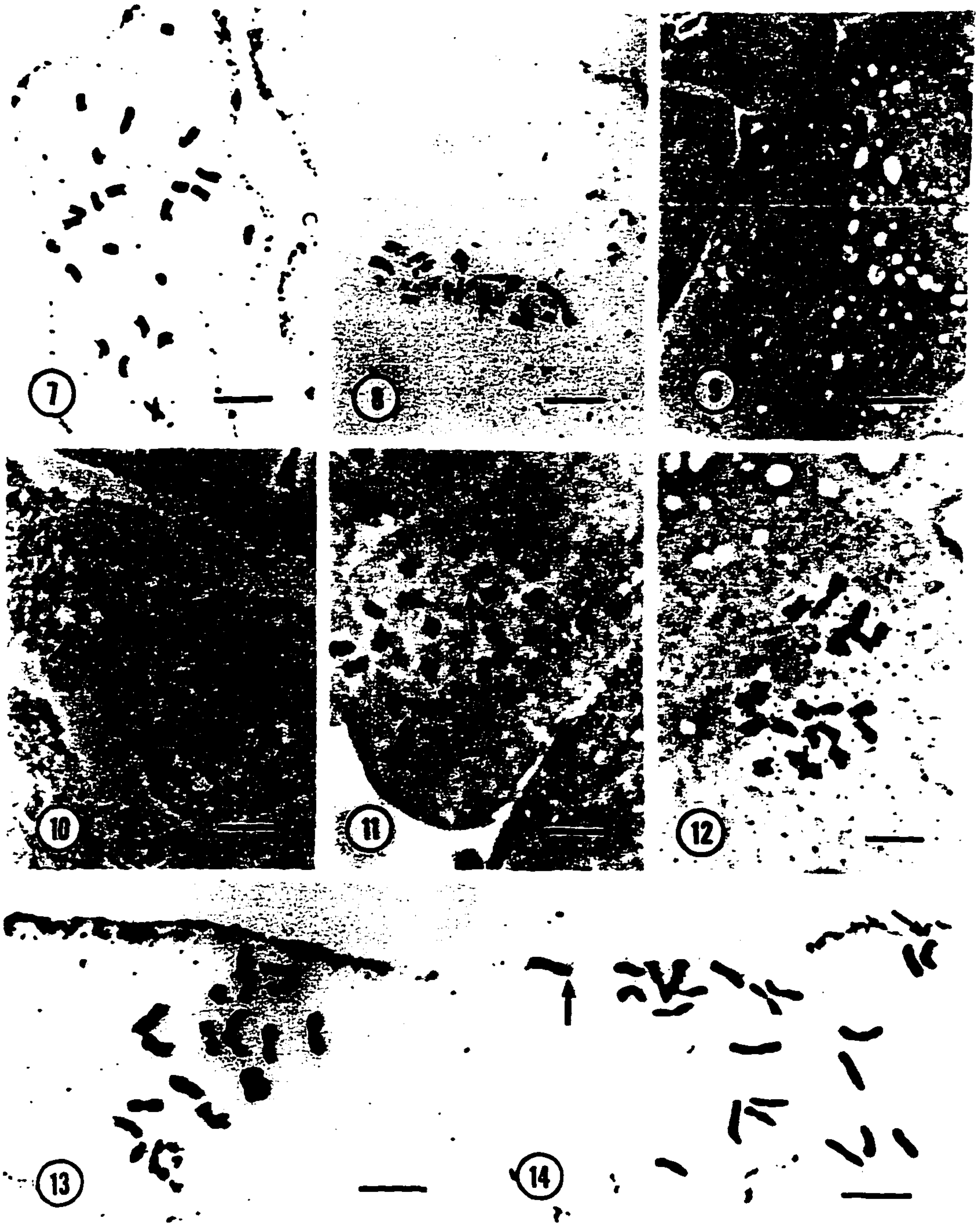
Taxon	Chromosome count	Source, Herbaria
F. fordii (Orcutt) Britton and Rose		
var. *fordii*	$n = 11$	MEXICO. Baja California: Between El Rosario and San Quintín, approx. 10 mi N of El Rosario. 17 May 1994, J. Rebman 2707 (ASU, BCMEX, ISC).
F. gracilis H. E. Gates		
var. *gracilis*	$n = 11$	MEXICO. Baja California: Rte 1, Km marker 105, between Cataviña-El Rosario. 29 May 1992, H. Cota 8034 (ISC); 1.2 mi NE Rte 1, S of km marker 166. 19 May 1994, J. Rebman 2728 (ASU, BCMEX, ISC); E of El Rosario, along road to Sierra San Borja. 25 Apr. 1994, J. Rebman 2635 (ASU, BCMEX, ISC).
* *F. peninsulae* (F. A. C. Weber) Britton and Rose		
var. *peninsulae*	$n = 11$	MEXICO. Baja California: Sierra San Francisco, S of Rancho Los Crestones, E of San Ignacio. 25 Apr. 1994, J. Rebman 2629 (ASU, BCMEX, ISC).
* var. *townsendianus* (Britton and Rose) N. P. Taylor	$2n = 22$	MEXICO. Baja California: 0.5 mi E and just S of km marker 89, on road to Todos Los Santos, along Hwy 19, N of Cabo San Lucas. 17 May 1992, H. Cota 8014 (ISC).
* *F. rectispinus* (Engelm.) Britton and Rose	$2n = 22$	MEXICO. Baja California: 10 mi from Hwy 1, along road to San Isidro. 25 May 1992, H. Cota 8027 (ISC).
F. viridescens (Torrey & A. Gray) Britton and Rose		
var. *viridescens*	$n = 11$	MEXICO. Baja California: Arroyo San Carlos, SE of Ensenada. 3 May 1994, J. Rebman, 2643 (ASU, BCMEX, ISC); Punta Morro, S of Ensenada. 26 May, 1994, J. Rebman 2733 (ASU, BCMEX, ISC).

Table 1. Continued.

Taxon	**Chromosome count**	**Source, Herbaria**
F. wislizeni (Engelm.) Britton and Rose	$2n = 22$	MEXICO. Sonora: 25 km S of the city of Obregón. 12 May 1986, H. Cota 2687 (ENCB). U.S.A. Arizona: Pima Co. 11.1 mi S of junction Interstate 10 and Hwy 83. 19 Aug. 1992, L. Slauson 112 (DES).



Figures 1-6. Photomicrographs of meiotic chromosomes of selected taxa of *Ferocactus*. 1. *F. histrix*, Cota 8037. Metaphase I. 2. *F. macrodiscus*, Cota 8038. Metaphase I. 3. *F. fordii*, Rebman 2707. Metaphase II. 4A-C. F. gracilis var. *gracilis*. A. Cota 8034. Metaphase II. B. Rebman 2635. Metaphase II. C. Rebman 2728. Metaphase II. 5. *F. peninsulae* var. *peninsulae*, Rebman 2629. Metaphase I. 6. *F. viridescens* var. *viridescens*, Rebman 2643. Metaphase I. Scale bar = 20 µm.



Figures 7-14. Photomicrographs of mitotic chromosomes of selected taxa of *Ferocactus*. 7. *F. latispinus*, Cota 8039. 8. *F. recurvus*, Cota 8049. 9. *F. robustus*, Cota 8045. 10. *F. chrysacanthus*, Cota 7441. 11. *F. cylindraceus* var. *lecontei*, Wisura s.n. 12. *F. peninsulae* var. *townsendianus*, Cota 8014. 13. *F. rectispinus*, Cota 8027. 14. *F. wislizeni*, Slauson 112. Scale bar = 10 µm. Arrows indicate satellites.

CHAPTER FOUR

CHLOROPLAST DNA EVIDENCE FOR DIVERGENCE IN *FEROCACTUS* AND ITS RELATIONSHIPS TO NORTH AMERICAN COLUMNAR CACTI (CACTACEAE: CACTOIDEAE)

A paper in press in the Journal Systematic Botany

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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:

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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.

INTRODUCTION

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 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* [specific epithet under *F. latispinus* 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 EDTA, 1% bovine serum albumin, and 5 mM 2-mercaptoethanol. The homogenate was filtered through a fine cloth (Miracloth, Calbiochem) and pelleted at 2000 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 affects 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 indicate 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. flavovirens*), 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 subgenus *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* (Orcutt) N. P. Taylor and J. Y. Clark] 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* 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.

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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.) 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

TABLE 1. Continued.

TRIBE	SUBTRIBE	TAXON	SAMPLE NUMBER	SOURCE/VOUCHER
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

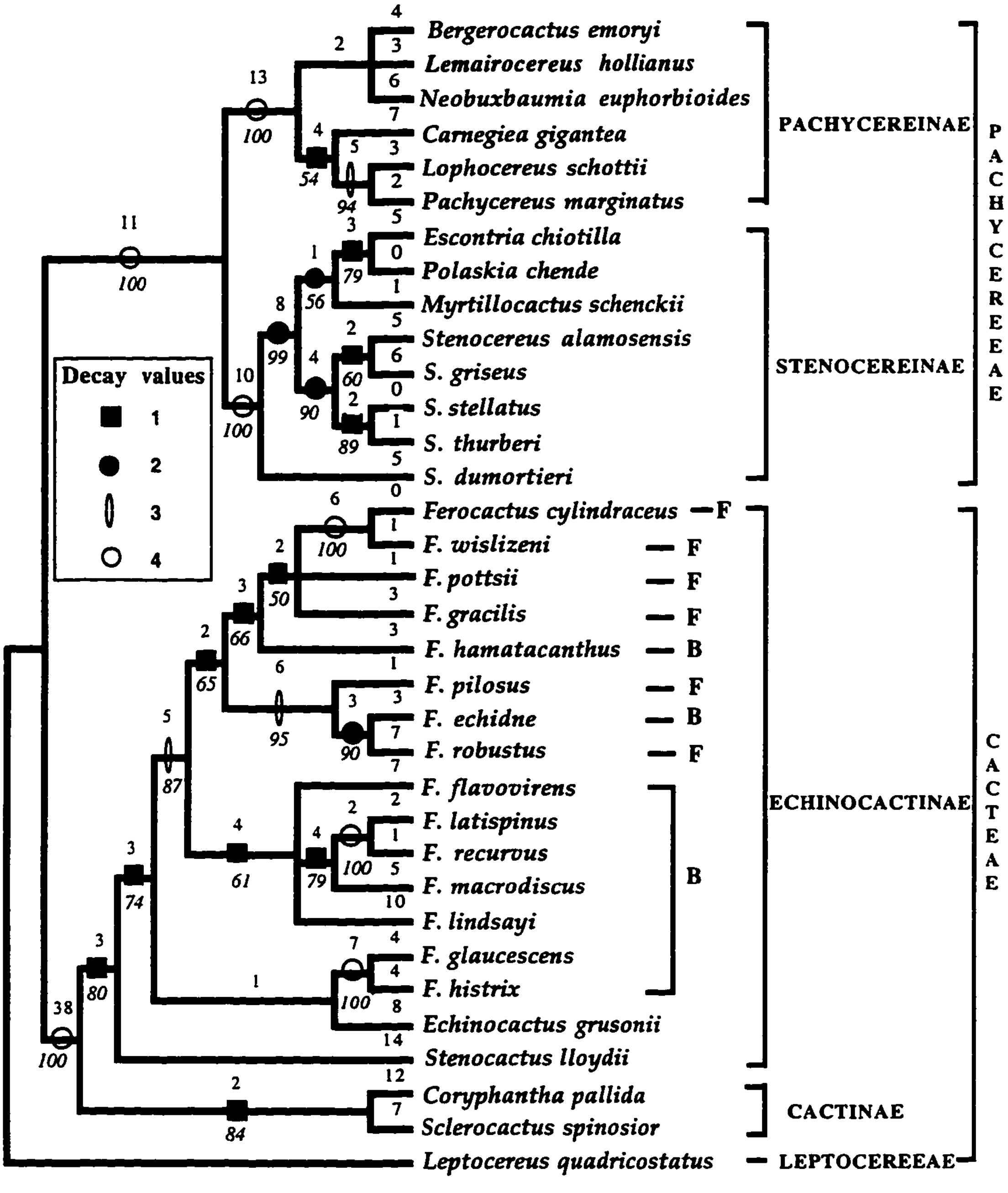

Figure 1. Strict consensus tree of four most parsimonious trees length 317, CI = 0.779 and RI = 0.939 including all characters. Numbers above branches indicate synapomorphic restriction site variants observed; italic numbers below the branches represent bootstrap percentages >50% obtained from 200 replicates; 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.

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

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*Ava*I	37	24	G	5.0 = 3.7 + 1.3	8
*Ava*I	37	25	G	5.0 = 2.7 + 2.3	32
*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	9A/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)

APPENDIX I. Continued

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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	Cacteae (16-34)
*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
*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

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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
*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
*Cla*I	26/27	143	L	7.9 + 7.1 = 15.0	29

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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
*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
*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

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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	20A/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	Cacteae (16-34)
*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	29A/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)

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*Eco*RI	34	192	G	4.0 = 2.5 + 1.5	12
*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	Cacteae (16-34)
*Hinc*II	12	215	L	1.9 + 2.4 = 4.3	16-32

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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

APPENDIX I. Continued.

Enzyme	Region	Mut. No.	Site Gain/loss	Observed fragments (kb)	Variant Taxa*
*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

APPENDIX II. DATA MATRIX FOR RESTRICTION SITE CHANGES OF THE cpDNA ANALYSIS

DIMENSIONS NTAX=34 NCHAR=247;
FORMAT MISSING=? GAP=-

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[                                      1         2         3         4     ]
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APPENDIX II. Continued.

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APPENDIX II. Continued.

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F. wislizeni	010100000011000001110000001000101010001001110
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Sclerocactus spinosior	000100000000000001100000001000101010001000000

APPENDIX II. Continued.

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APPENDIX II. Continued.

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Polaskia chende	0100101000100000100100
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Stenocactus lloydii	1001001000010000000010
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Sclerocactus spinosior	1001001000010000000010

CHAPTER FIVE

PHYLOGENETIC RELATIONSHIPS OF *FEROCACTUS* (CACTACEAE) AND ALLIED TAXA BASED ON SEQUENCES OF NON-CODING DNA

A paper to be submitted to the Journal of Molecular Biology and Evolution

J. Hugo Cota and Robert S. Wallace

ABSTRACT

Phylogenetic relationships among *Ferocactus* species were inferred using sequence data from two non-coding regions of the chloroplast DNA (cpDNA): the *rpl*16 intron and the intergenic spacer between the *trn*L-*trn*F genes. The monophyly of *Ferocactus* remains unresolved; few synapormophies support a monophyletic origin and the branching and assessment of phylogenetic relationships in some lineages is unclear. This suggests a rapid radiation of the genus, perhaps from central Mexico, to its extant geographic range of distribution in Mexico and southwestern U. S. The same situation was observed for the investigated taxa from the tribe Cacteae, thus, the phylogenetic position of *Ferocactus* within this tribe remains unclear. The topologies of the two phylogenies are somewhat congruent with the geographic distribution of taxa, distinguishing between the northern and southern species. The close phylogenetic relationships of *Ferocactus* with its sister taxon *Echinocactus grusonii* is supported by the two data sets, perhaps indicating paraphyly in *Ferocactus*. The taxonomic implications of this relationship are discussed. The two non-coding regions used in this study are AT-rich and appear to be evolving at the same evolutionary rate.

Key words: Non-coding DNA, *rpl*16, *trn*L-*trn*F, *Ferocactus*, *Echinocactus*

INTRODUCTION

Ferocactus Britton and Rose, perhaps the most attractive and best studied genera within the subfamily Cactoideae of the Cactaceae, has a complicated evolutionary history. Its taxonomy, systematics, and diverse aspects of its evolutionary history have long been addressed (e.g., Lindsay, 1955, 1965; Taylor, and Clark, 1983; Taylor, 1984; Bravo and Sánchez-Mejorada, 1991; Unger, 1992; Cota, 1996; Cota et al., 1996), yet there is no clear understanding of its phylogeny. Historically, *Ferocactus* has been assigned a basal phylogenetic position and closely allied to *Echinocactus* Link and Otto and *Stenocactus* (K. Schum.) A. W. Hill, in early and recent classifications of the tribe Cacteae (Buxbaum, 1951, 1958; Barthlott and Hunt, 1993).

The considerable taxonomic effort focused on this genus is an indirect reflection of the inability of morphological characters to clearly define species boundaries or to assess phylogenetic relationships. Several cases in which evolutionary relationships are obscured due to morphological similarities and parallelisms have been documented, e.g., Mexican columnar cacti of tribe Pachycereeae (Gibson and Horak, 1978), *Chusquea* (Kelchner, 1996). In the last decade, analysis of diverse regions of the chloroplast genome have increased the estimation and assessment of homology and elucidated phylogenetic issues in many plant groups. Methods in plant molecular systematics have been employed by several authors, e.g., Palmer, 1986; Olmstead and Palmer, 1994). The same principles and techniques have been successfully applied in the Cactaceae, e.g., Wallace, 1995, Wallace and Forquer, 1995; Cota and Wallace, 1996; Dickie, 1996. Recently, molecular studies of the chloroplast genome have provided more insight into the phylogeny of *Ferocactus*. Wallace and Cota (1996) in a survey for the Cactaceae have demonstrated monophyly for the tribe Cacteae (in which *Ferocactus* is circumscribed) based on the intron loss of the chloroplast gene *rpo*C1. In addition, phylogenetic inferences at lower taxonomic levels based on a study of restriction site analysis of the chloroplast DNA (cpDNA) provided preliminary evidence of paraphyly or possibly polyphyly in *Ferocactus* (Cota and Wallace, in press).

Two different types of non-coding DNA, introns and intergenic spacers, are currently being analyzed phylogenetically in plant systematics due to their valuable rapid evolutionary rate. Both types of sequences are nontranslated; introns are transcribed sequences that are excised during the processing of the primary transcript and the intergenic spacers are flanking sequences of coding genes or exons. Because of the different functional constraint in non-coding DNA versus coding DNA, the number or polymorphic sites is higher in the former type of DNA (Böhle et al., 1994). Non-coding sequences of the plastid genome such as intergenic spacers and introns are also characterized by their higher rate of evolution in terms of nucleotide substitution; also insertions and deletions are more often accumulated (Zurawski and Clegg, 1987; Gielly and Taberlet, 1994a), providing a good number of characters useful to reconstruct phylogeny. Recently, non-coding regions have been effective to address phylogenetic questions at different taxonomic levels. Diverse studies have demonstrated that non-coding sequences from the chloroplast gene encoding the ribosomal protein 16 (*rpl*16) (Kelchner and Wendel, 1996; Dickie, 1996) and the intergenic spacer between the *trn*L-*trn*F (Taberlet et al., 1991; Gielly and Taberlet, 1994b, 1996; Mes and t'Hart, 1994; van Ham et al., 1994) exhibit high rate of nucleotide substitution, and in some cases these regions evolve faster than coding genes, such as the *rbc*L (reviewed in Downie et al., 1996b).

This study represents a continued effort to evaluate the phylogenetic relationships of *Ferocactus*, and its affinities with its presumably sister genera such as *Echinocactus* and *Stenocactus*, but most important, our primarily objective is to verify whether *Ferocactus* is a monophyletic assemblage based on analyses of chloroplast DNA sequence data from the *rpl*16 intron and the intergenic spacer region between the *trn*L-*trn*F genes. These non-coding regions of the plastid genome were selected due to it easy of amplification via PCR, the size difference, which for the intergenic spacer, although relatively short (about 450 bp), is long enough to provide phylogenetic signal, conversely the *rpl16* intron is large enough and of a manageable size of about 1.1 kb (Kelchner, 1996) to estimate phylogeny. In addition, both regions are present in a single copy in most of the chloroplasts of higher plants (Doyle et al., 1995). In this paper we present the results of comparative sequence analyses from these two non-coding regions of the chloroplast genome to infer phylogenetic relationships in the genus *Ferocactus*. We have chosen these non-coding regions of the cpDNA because of their utility in providing information regarding genetic divergence useful for inferring phylogenetic relationships.

MATERIALS AND METHODS

Plant Material

Plant material for this study was obtained from different sources; some specimens were field collected, and other plants were obtained from diverse botanical institutions. Information relative to source and voucher specimens analyzed in this study is given in Tables 1 and 2. The taxonomic circumscriptions and nomenclatural authorities for *Ferocactus* follows that of Taylor (1984), and that of Barthlott and Hunt (1993) for the remaining taxa used in this study.

Ingroup Taxa

The accessions of *Ferocactus*, *Echinocactus*, and *Stenocactus* included in this study (Tables 1 and 2) were chosen because: 1) the *Ferocactus* species represent the major lineages for the genus recognized by Taylor (1984), and *Echinocactus* and *Stenocactus* are sister taxa of *Ferocactus* for which we expect to determine levels of genetic divergence, and 2) many of these species have been previously investigated systematically via restriction site analysis of the cpDNA (Cota and Wallace, in press), with which the results of the present study will be compared in the last section of this paper. Other taxa from within the tribe Cacteae, for which plant tissue became available after the data analysis of the intergenic spacer had been completed, were added for study in our generic survey in the analysis of the *rpl*16 intron to evaluate the phylogenetic position of *Ferocactus* within this tribe, and to elucidate the intergeneric phylogenetic relationships.

Outgroup Taxa

Representative taxa from the subfamilies Pereskioideae (*Pereskia*) and Opuntioideae (*Opuntia*) were chosen as outgroups because of their distant phylogenetic relationship with *Ferocactus* and allied taxa. In addition, representative members of the tribe Pachycereeae (sensu Gibson and Nobel, 1986; Barthlott and Hunt, 1993) were chosen as outgroups (Tables 1 and 2) because previous studies (Cota and Wallace, in press) have indicated that these columnar cacti are distantly related from the *Ferocactus*. Because of this, they are well suited as part of the outgroup.

DNA Extraction

Total genomic DNA was isolated from fresh plant material using a modified organelle-pellet method appropriate for mucilaginous plant tissues (Wallace, 1995) and further purified by ultracentrifugation with the cesium chloride-ethidium bromide gradients as described by Palmer (1986). The bands obtained from ultracentrifugation were dialyzed against TE, and stored at -20 ^{0}C.

DNA Amplification and Sequencing Strategy

The complete intergenic spacer region of the *trn*L-*trn*F genes and intron of the *rpl*16 were separately amplified in each genomic DNA via the polymerase chain reaction (CPR) using *Taq*I polymerase (Perkin-Elmer; Sequenase as part of the Amplitaq PCR kit). The PCR reaction mixture consisted of 10 μl of 10X reaction buffer, 10 μl of 25mM magnesium chloride solution, 8 μl of a 2.5 mM dNTP solution in equimolar ratio containing 1 μl (20 ng) of each primer, 5 µl of DNA template, and 0.5 units of *Taq* polymerase all in a total volume of 100 µl. Each set of reactions was monitored by the inclusion of a negative control. The double-stranded PCR products were produced via 24 cycles of denaturation (95° C for 5 min), primer annealing (50° C for 1 min), and extension (65° C for 4 min). A 10 min final extension cycle at 65° C followed the 24th cycle. The ensuing PCR fragments were analyzed by electrophoresis in 1.0 % agarose gels using 1X TAE buffer, stained with ethidium bromide and sized against digested lambda DNA standards. Successful PCR amplifications showed a single DNA band of 450 bp for the intergenic spacer region of the *trn*L-*trn*F genes and of 1.3 kb for the *rpl*16 intron. Double-stranded DNA was purified in Microcon 100 ultrafilters (Amicon, Beverly, Massachusetts) using 75 µl of PCR product plus 200 µl of water and spun at 3000 rpm for 20 min. Samples were then recovered by inverting microcons into a fresh Eppendorf tube and spinning at 16 000 rpm for 30 sec. Optical density (OD) using A260 reading was determined for each purified PCR product with a Beckman ultraviolet spectrophotometer and was diluted to a final concentration of 50 mg/µl for subsequent sequencing.

The complete *rpl*16 intron region and portion of the flanking exon regions in each genomic DNA was PCR-amplified using forward primer “F71” and reverse primer “637” following Posno et al. (1986). For sequencing of the intergenic spacer region the same set of universal primers (Taberlet et al., 1991) were used. No internal sequencing primers were necessary due to the relative short length of this region of the cpDNA. In both cases, 5 pmol of sequencing primers were used. The complete sequences of both amplification and sequencing primers used for the *rpl*16 intron and the intergenic spacer region between the *trn*L (UAA) 3’ exon and *trn*F (GAA) gene are given in Table 3 and Table 4 respectively.

DNA sequences were directly obtained from double-stranded DNA and produced by automated sequencing methods at the Iowa State University Nucleic Acids Facility using Perkin-Elmer/Applied Biosystems sequencing units.

Sequence Alignment and Phylogenetic Analysis

Multiple alignments of the non-coding regions (IGS of the *trn*L-*trn*F genes and *rpl*16 intron) were performed independently using the CLUSTAL program (Higgins et al., 1992) with subsequent manual alignment or corrections to minimize the number of gaps. When gaps were added to the sequences during the alignment process, the following criteria were considered: to minimize the number of nucleotide mismatches as a result of an insertion or deletion of a repeat unit, and gaps of equal length shared by two or more taxa were considered to have originated from a single mutation event. Insertions and deletions considered potentially phylogenetically informative were binary-coded as presence-absence characters and included in the sequence data matrix. As indicated by Dickie (1996), with this method the insertion-deletion (indel) events and substitutions are equally weighted.

In order to estimate the relative evolutionary rate of mutation given by the proportion of mutational (nucleotide substitutions or indels) events per site, the two data sets of these non-coding regions were analyzed independently using the program Molecular Evolutionary Genetics Analysis 1.0 (MEGA) (Kumar et al., 1993). The number of phylogenetically informative sites and the nucleotide composition of these sequences were also estimated with this program. The statistics of this comparative sequence analyses are shown in Table 5.

Maximum parsimony analysis for phylogenetic reconstruction was performed using PAUP 3.1.1. (Swofford, 1993). The phylogeny was assessed using the heuristic search algorithm with closest addition sequence, MULPARS on, and tree-bisection-reconstruction (TBR) branch swapping options. To search for islands of most parsimonious trees the search was repeated five times with RANDOM addition, saving 10 trees at each replication (Madison, 1991). To evaluate the robustness and stability of clades, bootstrap sampling with replacement method (Felsenstein, 1985) 200 replications, and decay analysis (Bremer, 1988) up to four steps longer than the most parsimonious tree were conducted.

RESULTS

Sequence Analysis

The length of the two non-coding regions used in the study is 3:1, as the *rpl*16 intron is three times longer than the IGS between the *trn*L-*trn*F genes. The full length including gaps is 1240 bp for the*rpl*16 intron and 461 bp for the IGS (Table 5). When gaps are excluded, the length of these regions is between 1151-1196 bp for the *rpl*16 and from 369-426 bp for the intergenic spacer (Table 5). The length for the *rpl*16 intron is similar to that reported in tobacco (Shinozaki et al., 1986), 1.1 kb in *Chusquea* (Kelchner, 1996), and 1.0 kb in the Opuntioideae (Dickie, 1996). The complete sequences for the examined taxa are included in Appendix I (*rpl*16 intron) and Appendix II (intergenic spacer).

The distribution of phylogenetically informative sites is somewhat uniform throughout these non-coding regions, with the exception of ambiguous regions at the 3' end of both regions (Appendix I and Appendix II). The *rpl*16 intron exhibits a relatively higher number of variable sites (527) towards the 3' end (Table 5; Appendix I) relative to the number of variable sites (187) in the IGS between the *trn*L-*trn*F genes (Table 5; Appendix II). Although the number of phylogenetically informative sites in both non-coding DNA regions seems to be different (*rpl*16 = 270 and IGS = 79; Table 5), statistically the evolutionary rate in these sequences is the same (1:1), considering that the length difference is 3:1 (Table 5).

Unlike coding sequences, the non-coding regions used in this study are AT-rich. The percentage of these nucleotides is A = 29.4 and T = 42.4 (A+T = 71.8) for the *rpl*16, and A = 31.8 and T = 31.0 (A+T = 62.8) for the IGS (Table 5). Similar percentage of A+T content (69.2-70.5%) was found in *Chusquea* (Kelchner, 1996), and in the *rpo*C1 intron (62.4-65.2%) of the subfamily Apioideae (Downie et al., 1996a) and tobacco (Shimada and Sugiura, 1991).

Parsimony Analysis

Parsimony analysis of equally weighted character states using the heuristic search of PAUP produced 696 most parsimonious trees with length = 1023 steps, Consistency Index (CI) = 0.599, and Retention Index (RI) = 0.588 for the *rpl*16. The strict consensus tree of this analysis was computed and it is shown in Figure 1.

For the *trn*L-*trn*F, the same type of analysis produced 32,700 most parsimonious trees with length of = 157 steps, Consistency Index (CI) = 0.803, and Retention Index (RI) = 0.767. For this data set, the 32,700 trees represent the functional limit for PAUP 3.1.1. A strict consensus tree was then computed from these trees and is shown in Figure 2.

DISCUSSION

The results obtained from the two analyses clearly show the close phylogenetic relationship of *Ferocactus* and *Echinocactus grusonii*, suggesting paraphyly in *Ferocactus* and a likely origin from an *Echinocactus*-like stock. Similar results based on restriction site analysis of the cpDNA were found by Cota and Wallace (in press), in which *E. grusonii* is basal to *F. histrix* and *F. glaucescens*, providing preliminary evidence of paraphyly in *Ferocactus*. Unlike the preceding results, in the phylogenies presented in this study *Ferocactus* remains unresolved as a natural monophyletic group. Instead, a polychotomy emerges, which makes *Ferocactus* polyphyletic with the rest of the genera of the tribe Cacteae.

The two non-coding regions analyzed in this study seem to be evolving sufficiently rapidly to provide phylogenetic resolution at this taxonomic level, nonetheless, it is possible that the lack of resolution at the base of the nodes is associated with a rapid and extensive radiation in *Ferocactus*, which encompassed morphological differentiation accompanied by genetic divergence characterizing single species. The accumulation of molecular autoapomorphies is reflected in long branches in the phylogenetic tree, which in turn have obscured the phylogenetic relationships at the interspecific level. Consequently, the lack of molecular synapomorphies contributed to the poor resolution of and support for terminal clades. A similar situation was reported in the Opuntioideae in a phylogenetic analysis using sequences from the *rpl*16 intron (Dickie, 1996).

The two phylogenies are congruent in terms of the geographic distribution of the species of *Ferocactus*; northern and southern species appear in different subclades, indicating perhaps several independent lines of evolution. The phylogenetic relationships hypothesized for these subclades are weakly supported by these data. The presumably northern radiation of *Ferocactus* is most evident in the Baja California peninsula, where 11 of the 23 species recognized by Taylor (1984) are distributed and appear to be actively evolving.

Non-coding DNA like introns (Palmer, 1991, Downie et al., 1996a) and intergenic spacer are AT-rich in base composition. This relative high proportion of A's and T's is correlated with the non-functional constraint and untranslated characteristic during the processing of a primary transcript. Conversely, coding regions are in general GC-rich, which provide the specific codons for the processing of amino acids during protein synthesis.

It appears that although non-coding regions evolve faster that coding genes, their application to answer phylogenetic questions at lower taxonomic levels does not always provide sufficient phylogenetic resolution, even when it has been demonstrated that they exhibit heterogeneous evolutionary rates. Factors other than functional constraints may be playing a major role in sequence evolution. Within *Ferocactus* and among the members of the tribe Cacteae the phylogenetic resolution provided from the *rpl*16 intron and intergenic spacer between the *trn*L and *trn*F genes was limited, thus, the intrageneric and intraspecific relationships in this tribe must be investigated. This study provided more insight into the phylogenetic scope and relationships between *Ferocactus* and *Echinocactus*. Further analyses with other molecular markers are needed before phylogenetic conclusions can be drawn for this controversial genus.

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Table 1. List of taxa examined in the study of sequence variation of the *rpl*16 intron. Specific epithets for *Ferocactus* follows Taylor (1984) taxonomic treatment and Gibson and Nobel (1986) and Barthlott and Hunt (1993) for the remaining taxa.

Taxon	Source and Voucher
Subfamily Pereskioideae	
Pereskia aculeata	BERLIN 249-02-85-30 - BERLIN
Subfamily Opuntioideae	
Opuntia phaeacantha	C. Christy 516 - DES
Subfamily Cactoideae	
Tribe Pachycereeae	
Pachycereus marginatus (DC.) Berger and Buxb.	HNT Bed 2N - HNT
Stenocereus dumortieri (Scheidw.) Buxb.	HNT 9550 - HNT
S. griseus (Haw.) Buxb.	DES 1953-4041-101- DES
Tribe Cacteae	
Ancistrocactus uncinatus	C. Glass 4866 - CANTE
Aztekium ritterii (Boedeker) Boedeker ex Berger	C. Staples s.n. - ISC
Coryphantha pallida Britton and Rose	H. Cota 8050 - HUMO
Echinocactus grusonii Hildm.	R. Wallace, s.n. - CONN
E. horizonthalonius Lem.	M. Mendez, 186 - CANTE
Ferocactus cylindraceus (Engelm.) Orcutt	L. Slauson 110 - DES
F. echidne (DC.) Britton and Rose	C. glass 7569 - CANTE
F. emoryi (Engelm.) Orcutt var. *rectispinus* (Engelm.) N. P. Taylor	DES 1984-0063-2136 -DES
F. flavovirens (Scheidw.) Britton and Rose	H. Cota 8051 - HUMO
F. fordii (Orcutt) Britton and Rose var. *fordii*	JIPE 050 CANTE
F. glaucescens (DC.) Britton and Rose	HNT 10339 - HNT
F. gracilis H. Gates	H. Cota 8034 - ISC
F. hamatacanthus (Muehlenpf.) Britton and Rose	C. Glass 6879 - CANTE
F. histrix (DC.) G. E. Linds.	H. Cota 8037 - CANTE
F. latispinus (Haw.) Britton and Rose	H. Cota 8039 - CANTE
F. lindsayi H. Bravo	M. Mendez 222 - CANTE
F. macrodiscus (C. Martius) Britton and Roe	C. Glass 6234 - CANTE
F. peninsulae (F. A. C. Weber) Britton and Rose	H. Cota 8014- ISC
F. pilosus (Galeotti ex Salm-Dyck) Werderm.	HNT 28036 - HNT
F. pottsii (Salm-Dyck) Backeb. var. *alamosanus* (Britton and Rose) G. Unger	HNT 39309 - HNT
F. recurvus (Miller) G. E. Linds.	H. Cota 8047 - HUMO
F. reppenhagenii G. Unger	HNT 7552-ISI 95-3 - HNT
F. robustus (Pfeif.) Britton and Rose	H. Cota 8045 - HUMO
F. viridescens (Torrey and A. Gray) Britton and Rose	HNT 49295 - HNT
F. wislizeni (Engelm.) Britton and Rose	L. Slauson 112 - DES
Geohintonia mexicana	C. Glass 6648 - CANTE
Glandulicactus crassihamatus	C. Glass 5201 - CANTE
Lophophora williamsii (Lem.) Coulter	D. Martinez, s.n. - HUMO
Mammillaria haggeana	H. Cota 8053 - HUMO
Obregonia denegrii Fric.	R. Wallace, s.n. ISC
Stenocactus lloydii (Britton and Rose) Berger	R. S. Wallace s.n. - CONN
Sclerocactus spinosior (Engelm.) Woodruf & L. D. Benson	Hughes 2 - ISC
Strombocactus disciformis (DC.) Britton and Rose	

Table 2. List of taxa examined in the study of sequence variation of the intergenic spacer region of the *trn*L-*trn*F genes. Specific epithets for *Ferocactus* follows Taylor (1984) taxonomic treatment and Gibson and Nobel (1986) and Barthlott and Hunt (1993) for the remaining taxa.

Taxon	Source and Voucher
Subfamily Pereskioideae	
Pereskia grandifolia	BERLIN 166-62-83-10 - BERLIN
Subfamily Opuntioideae	
Opuntia phaeacantha	C. Christy 516 - DES
Subfamily Cactoideae	
Tribe Leptocereeae	
Leptocereus quadricostatus (Bello) Britton and Rose	R. Ross s.n. - ISC
Tribe Pachycereeae	
Escontria chiotilla (Weber) Rose	H. Cota 8041 - HUMO
Pachycereus marginatus (DC.) Berger and Buxb.	HNT Bed 2N - HNT
Stenocereus griseus (Haw.) Buxb.	DES 1953-4041-101 - DES
Tribe Cacteae	
Echinocactus grusonii Hildm.	R. Wallace s.n. - CONN
Ferocactus cylindraceus (Engelm.) Orcutt	L. Slauson 110 - DES
F. echidne (DC.) Britton and Rose	HNT 6291 - HNT
F. emoryi (Engelm.) Orcutt var. *rectispinus* (Engelm.) N. P. Taylor	DES 1984-0063-2136 -DES
F. flavovirens (Scheidw.) Britton and Rose	H. Cota 8051 - HUMO
F. fordii (Orcutt) Britton and Rose var. *fordii*	JIPE 050 - CANTE
F. glaucescens (DC.) Britton and Rose	HNT 10339 - HNT
F. gracilis H. Gates	H. Cota 8034 - ISC
F. hamatacanthus (Muehlenpf.) Britton and Rose	C. Glass 6879 - CANTE
F. histrix (DC.) G. E. Linds.	H. Cota 8037 - CANTE
F. latispinus (Haw.) Britton and Rose	H. Cota 8039 - CANTE
F. lindsayi H. Bravo	M. Mendez 222 - CANTE
F. macrodiscus (C. Martius) Britton and Roe	C. Glass 6234 - CANTE
F. peninsulae (F. A. C. Weber) Britton and Rose	HNT 79316 - HNT
F. pilosus (Galeotti ex Salm-Dyck) Werderm.	HNT 28036 - HNT
F. pottsii (Salm-Dyck) Backeb. var. *alamosanus* (Britton and Rose) G. Unger	HNT 39309 - HNT
F. recurvus (Miller) G. E. Linds.	H. Cota 8047 - HUMO
F. robustus (Pfeif.) Britton and Rose	H. Cota 8045 - HUMO
F. wislizeni (Engelm.) Britton and Rose v. *herrerae*	C. Glass, s.n. - CANTE
F. wislizeni (Engelm.) Britton and Rose v. *wislizeni*	L. Slauson 112 - DES
Mammillaria voburnensis Scheer	Lippold, s.n. - CONN
Stenocactus albatus Dietrich) Knuth	HNT 5031 - HNT
Sclerocactus spinosior (Engelm.) Woodruf & L. D. Benson	Huges 2 - ISC

Table 3. Sequences of the primers used for the amplification and sequencing of the *rpl*16 intron. Numbers correspond to position in the sequence of *Spirodela oligorhiza* (Posno et al., 1986).

Primer	**Sequence 5' to 3'**
F71	GCT-ATG-CTT-AGT-GTG-TGA-CTC-GTT-G
R1661	CGT-ACC-CAT-ATT-TTT-CCA-CCA-CGA-C
R1516	CCC-TTC-ATT-CTT-CCT-CTA-TGT-TG
R637	GGT-TCG-TCG-TCC-GCC-ATC-C

Table 4. Sequences of the universal primers used for the amplification and sequencing of the intergenic spacer region between the *trn*L (UAA) 3' exon and *trn*F (GAA) gene. Primer names and code denotes the 3'-most base pair in the published tobacco cpDNA sequence (Taberlet, et al., 1991). The A and B in each code corresponds to each strand of DNA.

Primer	**Code**	**Sequence 5' to 3'**
e	B49873	GGT-TCA-AGT-CCC-TCT-ATC-CC
f	A50272	ATT-TGA-ACT-GGT-GAC-ACG-AG

Table 5. Sequence comparison between the *rpl*16 intron and the intergenic spacer region between the *trn*L (UAA) 3' exon and the *trn*F gene.

	Full length	**Full length**	**No. Var.**	**No. Inf.**	**Nucl. Composition (%)**			
	incl. gaps	**excl. gaps**	**Sites**	**Sites**	**A**	**T**	**C**	**G**
*rpl*16 intron	1240	1151-1196	527	270	29.4	42.4	15.4	12.8
IGS *trn*L-*trn*F	461	369-426	187	79	31.8	31.0	17.8	19.6

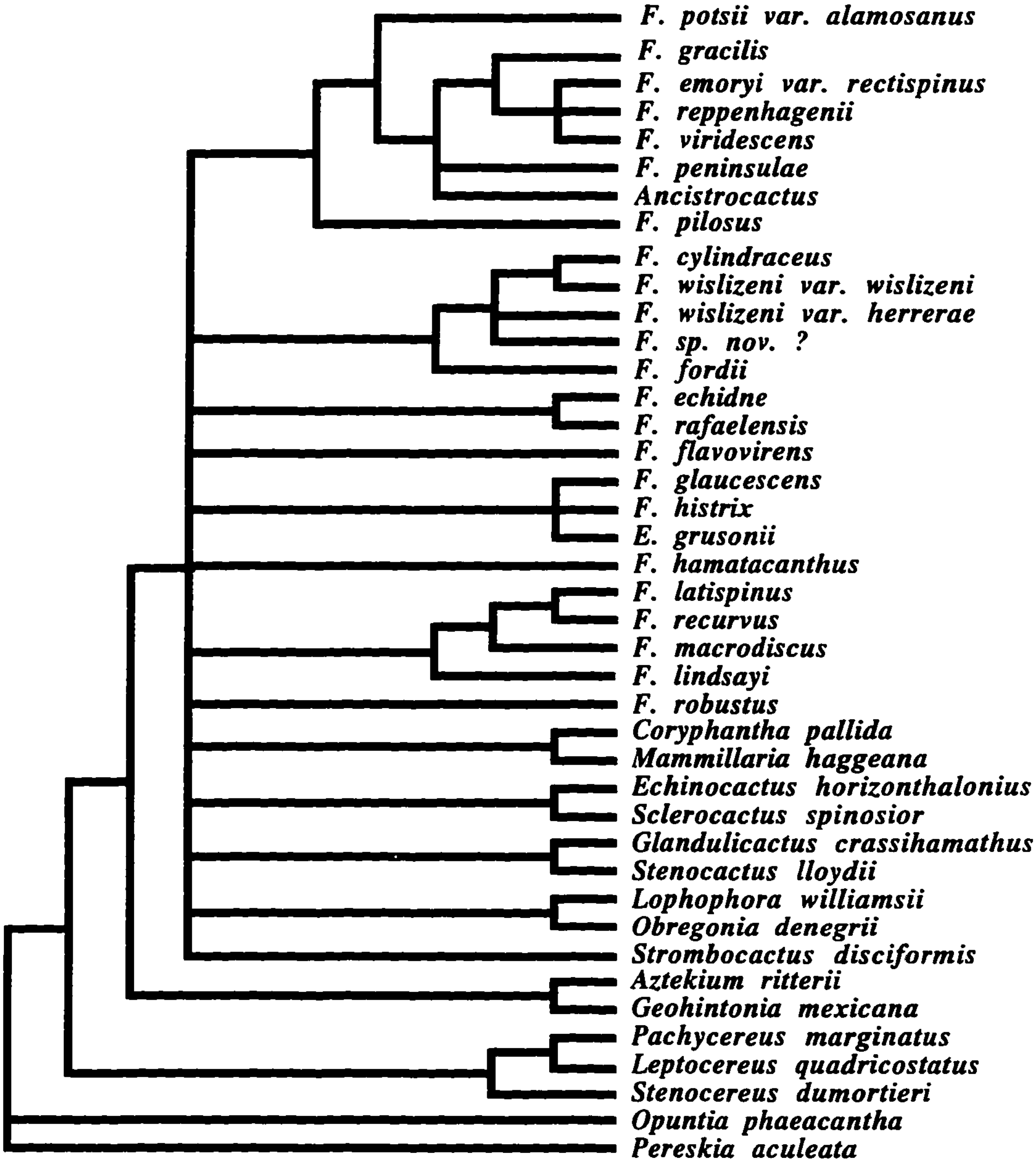

Figure 1. Strict consensus tree of 696 most parsimoniuos trees representing the phylogenetic relationships in *Ferocactus* based on DNA sequences analysis of the *rpl*16 intron. Tree length = 1023 steps, Consistency Index (CI) = 0.599; Retention Index (RI) = 0.588.

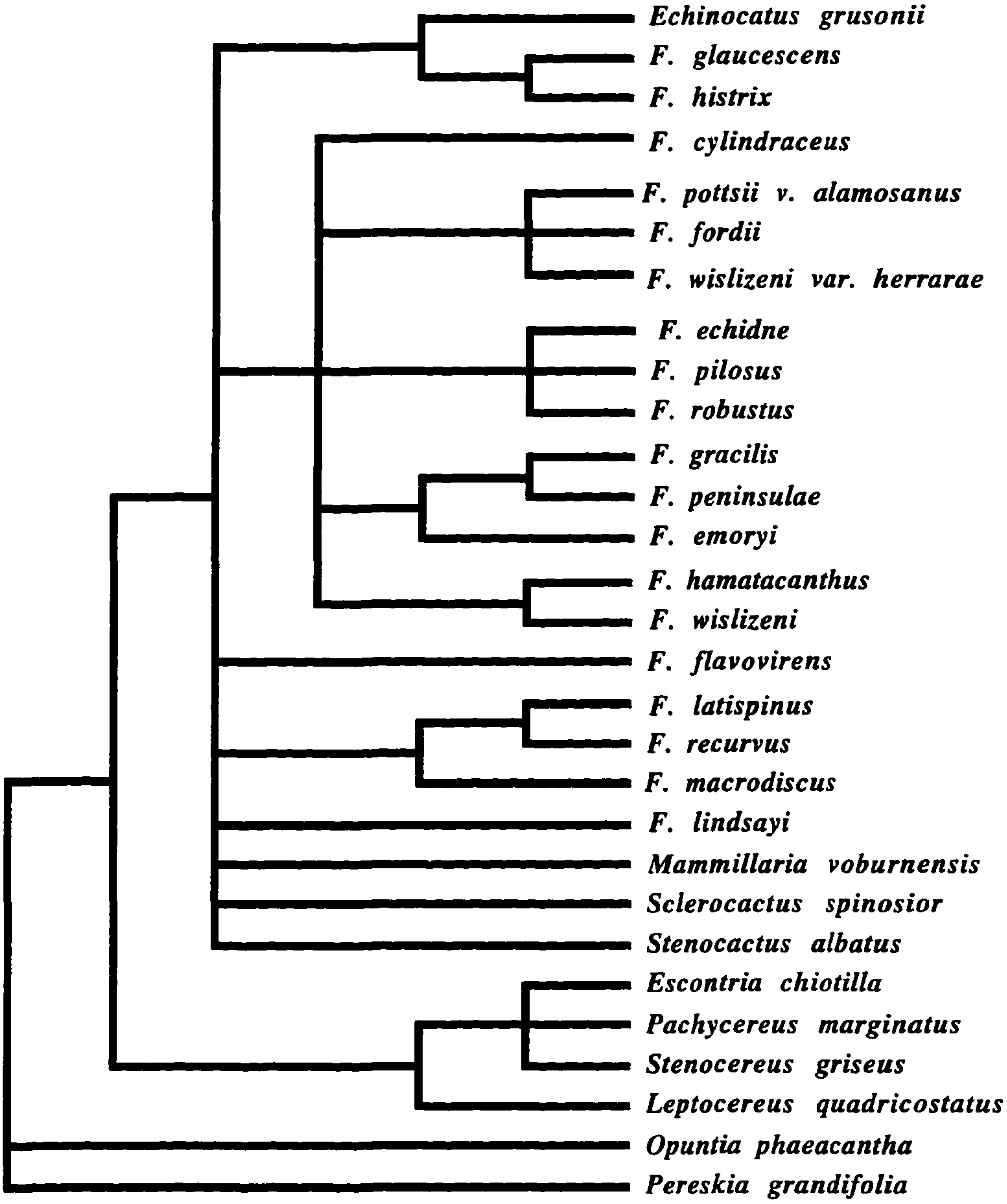

Figure 2. Strict consensus tree of 32,700 most parsimoniuos trees representing the phylogenetic relationships of *Ferocactus* based on DNA sequence analysis of the Intergenic Spacer between the *trn*L-*trn*F genes. Tree length = 157 steps; Consistency Index (CI) = 0.803; Retention Index (RI) = 0.767.

# APPENDIX I. Data matrix for *rpl*16 intron
DATATYPE = DNA; MISSING = N; GAP = -

TAXON

```
[                                     10        20        30        40      ]
[                                      .         .         .         .      ]

F. cylindraceus               GAATTTGGTTCT-TTTAGGGTTATAGTTGATGGTTTTTTCTTAAT
F. echidne                    ....C.......-................................
F. emoryi v. rectispinus      ............-................................
F. flavovirens                ....C.......-..............A.................
F. fordii                     ............-................................
F. glaucescens                ....C.......-................................
F. gracilis                   ............-................................
F. hamatacanthus              ....C.......-................................
F. histrix                    ....C.......-................................
F. latispinus                 ....C.......-................................
F. lindsayi                   ...A.CT.G.TC-................................
F. macrodiscus                ....C.......-................................
F. peninsulae                 ....C.......C................................
F. pilosus                    ....C.......-................................
F. pottsii v. alamosanus      ............-................................
F. rafaelensis                ....C.......-................................
F. recurvus                   ....C.......-................................
F. reppenhagenii              ............-................................
F. robustus                   ....C.......-................................
F. stainesii                  ....C.......-................................
F. wislizeni v. herrerae      ............-................................
F. wislizeni v. wislizeni     ............-................................
F. viridescens                ............-................................
F. sp. nov. ?                 ............-................................
Ancistrocactus uncinatus      ....C.......-................................
Aztekium ritterii             ....C.......-................................
Coryphantha pallida           .G.A.CT.G.T.T................................
Echinocactus grusonii         ....C.......-................................
E. horizonthalonius           ....C.......-................................
Geohintonia mexicana          ....C.......-................................
Glandulicactus crassipes      ....C.......-................................
Lophophora williamsii         ............-................................
Mammillaria haggeana          ....C.....T.-................................
Obregonia denegrii            ....C.......-................................
Sclerocactus spinosior        ....C.......-................................
Stenocactus lloydii           ....C.......-.......................G........
Strombocactus disciformis     ....C.......-..........................-.....
Pachycereus marginatus        ....C.......-...G............................
Stenocereus dumortieri        ....C.......-...G............................
Leptocereus quadricostatus    ....C.......-...G............................
Opuntia phaeacantha           ....C.......-...T............................
Pereskia aculeata             ....C.......-...G............................
```

APPENDIX I. Continued.

TAXON	
[	50 60 70 80 90]
[	. . . . .]
F. cylindraceus	TCCATCTCTATTACAGAACCGGACATGAGAGTTTCTTCTCATCCG
F. echidne	....C..
F. emoryi v. *rectispinus*	....
F. flavovirens	....
F. fordii	....
F. glaucescens	....
F. gracilis	....
F. hamatacanthus	....
F. histrix	....
F. latispinus	....A..
F. lindsayi	....
F. macrodiscus	....
F. peninsulae	....
F. pilosus	....
F. pottsii v. *alamosanus*	....
F. rafaelensis	....C..
F. recurvus	....
F. reppenhagenii	....
F. robustus	....
F. stainesii	....
F. wislizeni v. *herrerae*	....
F. wislizeni v. *wislizeni*	....
F. viridescens	....
F. sp. nov. ?	....
Ancistrocactus uncinatus	....
Aztekium ritterii	....
Coryphantha pallida	....
Echinocactus grusonii	....
E. horizonthalonius	....
Geohintonia mexicana	....
Glandulicactus crassipes	....
Lophophora williamsii	....
Mammillaria haggeana	....
Obregonia denegrii	....
Sclerocactus spinosior	....
Stenocactus lloydii	....
Strombocactus disciformis	....
Pachycereus marginatus	....
Stenocereus dumortieri	....
Leptocereus quadricostatus	....
Opuntia phaeacantha	....C.....C.............G....................
Pereskia aculeata	..........C..................................

APPENDIX I. Continued.

TAXON	
[	100 110 120 130]
[	. . . .]
F. cylindraceus	GCTCCTCGCGAATGAAAAAATTTCAAT-----TTTAATTGAATAT
F. echidne	...........................-----.............
F. emoryi v. *rectispinus*	...........................-----.............
F. flavovirens	...........................-----.............
F. fordii	............A..............-----.............
F. glaucescens	...........................-----.............
F. gracilis	...........................-----.............
F. hamatacanthus	...........................-----.............
F. histrix	.......................T...-----.............
F. latispinus	...........................-----.............
F. lindsayi	...........................-----.............
F. macrodiscus	...........................-----.............
F. peninsulae	...........................-----.............
F. pilosus	...........................-----.............
F. pottsii v. *alamosanus*	...........................-----.............
F. rafaelensis	.......................A...-----.............
F. recurvus	...........................-----.............
F. reppenhagenii	...........................-----.............
F. robustus	...........................-----.............
F. stainesii	...........................-----.............
F. wislizeni v. *herrerae*	...........................-----.............
F. wislizeni v. *wislizeni*	...........................-----.............
F. viridescens	...........................-----.............
F. sp. nov. ?	...........................-----.............
Ancistrocactus uncinatus	...........................-----.............
Aztekium ritterii	.......................TC..-----.............
Coryphantha pallida	.....................C.....-----...T.........
Echinocactus grusonii	...........................-----.............
E. horizonthalonius	...........................-----.............
Geohintonia mexicana	.......................TC..-----.............
Glandulicactus crassipes	...........................-----.............
Lophophora williamsii	........................G..-----.............
Mammillaria haggeana	...........................-----...T.........
Obregonia denegrii	........................G..-----..G..........
Sclerocactus spinosior	.............T............A-----.............
Stenocactus lloydii	...........................-----.............
Strombocactus disciformis	...........................-----.............
Pachycereus marginatus	..........................CTAGAA.......T.....
Stenocereus dumortieri	...........................TAGAA..C....T.....
Leptocereus quadricostatus	...........G...............-----.............
Opuntia phaeacantha	...........................-----.AA..........
Pereskia aculeata	..........................------.............

APPENDIX I. Continued.

TAXON	
[	140 150 160 170 180]
[	. . . . .]
F. cylindraceus	GAATATTTCTAAATTGAATTCTAATAAGAATAGAATTATAAATTA
F. echidne	.....................G...T...........C.......
F. emoryi v. *rectispinus*	.........................T...................
F. flavovirens	.........................T...........C.......
F. fordii	........A................T...................
F. glaucescens	.........................T...................
F. gracilis	.........................T...................
F. hamatacanthus	.........................T...........C.......
F. histrix	........A.....TT.........T...........C.T.....
F. latispinus	.........................T...........CC......
F. lindsayi	.........................T...........C.......
F. macrodiscus	.........................T...........CC......
F. peninsulae	.........A...............T...................
F. pilosus	.........................T...........C.......
F. pottsii v. *alamosanus*	.........................T...................
F. rafaelensis	.....................G...T...........C.......
F. recurvus	.........................T...........CC......
F. reppenhagenii	.........................T...................
F. robustus	.........................T...........C.......
F. stainesii	.........................T...........C.......
F. wislizeni v. *herrerae*	..........G..............T...................
F. wislizeni v. *wislizeni*	....
F. viridescens	.........................T...................
F. sp. nov. ?	.........................T...................
Ancistrocactus uncinatus	.........................T...........C.......
Aztekium ritterii	........AG...............T...........C.......
Coryphantha pallida	........AG...........G...T.....C.....CG.....C
Echinocactus grusonii	........A............C...T...........C.......
E. horizonthalonius	........A................T...........C...T..T
Geohintonia mexicana	........AG...............T...........C.......
Glandulicactus crassipes	.........................T...........C.....A.
Lophophora williamsii	TC............A..........T........G..C......T
Mammillaria haggeana	..-.....TG...............T.....C.....CG......
Obregonia denegrii	TC............A......A...T........G..C..C...T
Sclerocactus spinosior	........A...........A....T......T....C......T
Stenocactus lloydii	....---------------------T...........CG......
Strombocactus disciformis	........A................T...........C......T
Pachycereus marginatus	........AG..........A....T...................
Stenocereus dumortieri	........AG...............T...........CA......
Leptocereus quadricostatus	........A................T.....T.....C.......
Opuntia phaeacantha	........AA...........G...T...........C.......
Pereskia aculeata	........AA....A......G...T...................

APPENDIX I. Continued.

TAXON	
[	190 200 210 220]
[	. . . .]
F. cylindraceus	ATTTATGGATTAATATATCATAAATTTCAAAA-TGAATA------
F. echidne	...........................T....-......------
F. emoryi v. *rectispinus*	................................-......------
F. flavovirens	................................-......------
F. fordii	................................-......------
F. glaucescens	...........................A....-......------
F. gracilis	................................-......------
F. hamatacanthus	............................T...-......------
F. histrix	................................-......------
F. latispinus	...........................A....-.T....------
F. lindsayi	...........................A....-......------
F. macrodiscus	.....................T.....A....-......------
F. peninsulae	.....................T..........-......------
F. pilosus	................................-......------
F. pottsii v. *alamosanus*	................................-......------
F. rafaelensis	...........................T....-......------
F. recurvus	...........................A....-.T....------
F. reppenhagenii	................................-......------
F. robustus	................................-......------
F. stainesii	................................-......------
F. wislizeni v. *herrerae*	................................-......------
F. wislizeni v. *wislizeni*	................................-......------
F. viridescens	................................-......------
F. sp. nov. ?	................................-......------
Ancistrocactus uncinatus	..........................GA....-......------
Aztekium ritterii	......A..............T..........-......------
Coryphantha pallida	................................-......------
Echinocactus grusonii	...........................A....-......------
E. horizonthalonius	......A..............T....AA....-C.....------
Geohintonia mexicana	......A..............T.....A....-......------
Glandulicactus crassipes	..........................GA....-......------
Lophophora williamsii	...............................G-......------
Mammillaria haggeana	...................--------------------------
Obregonia denegrii	.......T........G.........GA...G-......------
Sclerocactus spinosior	......A...................A-....T......------
Stenocactus lloydii	........................A.......-......------
Strombocactus disciformis	................................-......------
Pachycereus marginatus	......A...........TTG......TT...-......AA----
Stenocereus dumortieri	..................T........TTC..-......AA----
Leptocereus quadricostatus	......A...........T..T.....T....-.T....AA----
Opuntia phaeacantha	..A...A...........TC.......G....-......AA----
Pereskia aculeata	..A...A...........T........G....-......AAAATA

APPENDIX I. Continued.

TAXON	
[	230 240 250 260 270]
[	. . . . .]
F. cylindraceus	-----------------GAATAATAATATTGAATTAAGATATACA
F. echidne	-----------------............................
F. emoryi v. *rectispinus*	-----------------............................
F. flavovirens	-----------------......C.....................
F. fordii	-----------------............................
F. glaucescens	-----------------............................
F. gracilis	-----------------............................
F. hamatacanthus	-----------------............................
F. histrix	-----------------............................
F. latispinus	-----------------............................
F. lindsayi	-----------------............................
F. macrodiscus	-----------------............................
F. peninsulae	-----------------............................
F. pilosus	-----------------............................
F. pottsii v. *alamosanus*	-----------------.............T..............
F. rafaelensis	-----------------............................
F. recurvus	-----------------............................
F. reppenhagenii	-----------------............................
F. robustus	-----------------............................
F. stainesii	-----------------............................
F. wislizeni v. *herrerae*	-----------------.............T..............
F. wislizeni v. *wislizeni*	-----------------............................
F. viridescens	-----------------............................
F. sp. nov. ?	-----------------............................
Ancistrocactus uncinatus	-----------------.............T............T.
Aztekium ritterii	-----------------...................G......T.
Coryphantha pallida	-----------------A...........................
Echinocactus grusonii	-----------------............................
E. horizonthalonius	-----------------..............G.............
Geohintonia mexicana	-----------------..........................T.
Glandulicactus crassipes	-----------------.............T......A.....TG
Lophophora williamsii	-----------------.......T....................
Mammillaria haggeana	---
Obregonia denegrii	-----------------.......T....................
Sclerocactus spinosior	-----------------......................C.....
Stenocactus lloydii	-----------------.......T..................T.
Strombocactus disciformis	-----------------..........................T.
Pachycereus marginatus	----TAAAGA----ATA..........C..TC.............
Stenocereus dumortieri	-----AATGA----ATA..........C..TC.............
Leptocereus quadricostatus	-----AATGA----ATA..........C..TC.............
Opuntia phaeacantha	-----AATGA----ATA--....A...GAAT-.GA.TA...ATAT
Pereskia aculeata	AATAGAATTATAATATA............................

APPENDIX I. Continued.

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         TAXON
[                                     280       290       300       310   ]
[                                      .         .         .         .    ]

F. cylindraceus               TGTAATAATACACTAAATCAAAGG-------------------AT
F. echidne                    ..............G....C.T..-------------------..
F. emoryi v. rectispinus      ........................-------------------..
F. flavovirens                .....................T..-------------------..
F. fordii                     ........................-------------------..
F. glaucescens                .....................T..ATTCCTTGATATTCATCAG..
F. gracilis                   ........................-------------------..
F. hamatacanthus              .T...................T..CAAT-------------GG..
F. histrix                    .....................T..-------------------..
F. latispinus                 ........................-------------------..
F. lindsayi                   ........................-------------------..
F. macrodiscus                ........................-------------------..
F. peninsulae                 .....................T..-------------------..
F. pilosus                    .....................T..-------------------..
F. pottsii v. alamosanus      ........................-------------------..
F. rafaelensis                ..............G....C.T..-------------------..
F. recurvus                   ........................-------------------..
F. reppenhagenii              ...T....................-------------------..
F. robustus                   .....................T..-------------------..
F. stainesii                  .....................T..-------------------..
F. wislizeni v. herrerae      ........................-------------------..
F. wislizeni v. wislizeni     ........................-------------------..
F. viridescens                ........................-------------------..
F. sp. nov. ?                 ........................-------------------..
Ancistrocactus uncinatus      ..C.....G............T..-------------------..
Aztekium ritterii             .....................T..-------------------..
Coryphantha pallida           .....................TA.---------------------
Echinocactus grusonii         .....................T..-------------------..
E. horizonthalonius           .....................T..-------------------..
Geohintonia mexicana          .....................T..-------------------..
Glandulicactus crassipes      ..C.....G............T..---------------------
Lophophora williamsii         .....................T..-------------------..
Mammillaria haggeana          ---------------------------------------------
Obregonia denegrii            .....................T..-------------------..
Sclerocactus spinosior        .......................A-------------------..
Stenocactus lloydii           .....................TAT---------------------
Strombocactus disciformis     .....................T..-------------------..
Pachycereus marginatus        ...C.........A..----.T..-------------------..
Stenocereus dumortieri        ...C.........A.......T..-------------------..
Leptocereus quadricostatus    .............A.......T..-------------------..
Opuntia phaeacantha           ..A.T...G.T.TAC.T.T..TA--------------------..
Pereskia aculeata             .....................TA.-------------------..
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APPENDIX I. Continued.

TAXON	
[	320 330 340 350 360]
[	. . . . .]
F. cylindraceus	TCCTTGATATTCATCAGATTCTTTGATATTCATCTTAATTATTAG
F. echidne	...................................A.........
F. emoryi v. *rectispinus*	...................................A.........
F. flavovirens	...........T.......................A.........
F. fordii	...............T....T..............A.........
F. glaucescens	..T................................A.........
F. gracilis	...................................A.........
F. hamatacanthus	...............T...................A........T
F. histrix	....
F. latispinus	...................................A.........
F. lindsayi	...................................A.........
F. macrodiscus	...................................A.........
F. peninsulae	...................................A....T....
F. pilosus	...................................A.........
F. pottsii v. *alamosanus*	...............................T.............
F. rafaelensis	...................................A.........
F. recurvus	...................................A.........
F. reppenhagenii	...................................A.........
F. robustus	...................................A.........
F. stainesii	...................................A.........
F. wislizeni v. *herrerae*	....
F. wislizeni v. *wislizeni*	....
F. viridescens	...................................A.........
F. sp. nov. ?	...................................A.........
Ancistrocactus uncinatus	...................................A.........
Aztekium ritterii	...................................A.T.......
Coryphantha pallida	-----------------..................A.........
Echinocactus grusonii	...................................A.........
E. horizonthalonius	..T................................A.T.......
Geohintonia mexicana	...................................A.T.......
Glandulicactus crassipes	-----------------..................A.........
Lophophora williamsii	....A.G..T
Mammillaria haggeana	---
Obregonia denegrii	....T..................................A....T
Sclerocactus spinosior	....................T..............A.........
Stenocactus lloydii	-----------------..................A.........
Strombocactus disciformis	...................................A.........
Pachycereus marginatus	...................................A.T.......
Stenocereus dumortieri	........................T..........A.T.......
Leptocereus quadricostatus	...................................A.T.......
Opuntia phaeacantha	A.AC.--..A...AT....................A.T.......
Pereskia aculeata	..T..C..........T................A.A.T.......

APPENDIX I. Continued.

TAXON	
[	370 380 390 400]
[	. . . .]
F. cylindraceus	ATATTTTTTT-ATTTGGATTTAGAAGTTTATTTTCAATTAAAATT
F. echidne	..........-..................................
F. emoryi v. *rectispinus*	..........-..................................
F. flavovirens	..........-..................................
F. fordii	..........-.................A....C...........
F. glaucescens	..........-...........T......................
F. gracilis	..........-......................C...........
F. hamatacanthus	..........-..................................
F. histrix	..........-...........T......................
F. latispinus	..........T..................................
F. lindsayi	..........-..................................
F. macrodiscus	..........T..................................
F. peninsulae	..........-...........T......................
F. pilosus	..........-..................................
F. potsii v. *alamosanus*	..........-...........T......................
F. rafaelensis	..........-..................................
F. recurvus	..........T..................................
F. reppenhagenii	..........-..................................
F. robustus	..........-..................................
F. stainesii	..........-..................................
F. wislizeni v. *herrerae*	..........-..................................
F. wislizeni v. *wislizeni*	..........-..................................
F. viridescens	..........-......................C...........
F. sp. nov. ?	..........-..................................
Ancistrocactus uncinatus	.........G-..................................
Aztekium ritterii	..........-...C......CT..........AA...CT.....
Coryphantha pallida	..........-----------------------------------
Echinocactus grusonii	..........-..................................
E. horizonthalonius	..........-.......................A..........
Geohintonia mexicana	..........-...........T..........AA...A......
Glandulicactus crassipes	.........G-..................................
Lophophora williamsii	..........-..................................
Mammillaria haggeana	---
Obregonia denegrii	..........-..................................
Sclerocactus spinosior	..........-...........T...........A..........
Stenocactus lloydii	.........G-...........T................C.....
Strombocactus disciformis	..........-..................................
Pachycereus marginatus	........G.-...........T.....A....GA......TT..
Stenocereus dumortieri	........G.-...........T.....A....GA......TT..
Leptocereus quadricostatus	........G.-...........T.....A.....A......TT..
Opuntia phaeacantha	........A.-...A....A..T...GAA....GA...--.T...
Pereskia aculeata	........A.-........A..T...GAA...AAA...--.T...

APPENDIX I. Continued.

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TAXON
[                              410       420       430       440       450]
[                              .         .         .         .         .]

F. cylindraceus            TTATATAGTTTTTATTTTTTATTTTTTT-GTATAGATATATTTTA
F. echidne                 ...........G................-................
F. emoryi v. rectispinus   ...........G................-....C...........
F. flavovirens             ...........G................-................
F. fordii                  .A........GG.......A.......G-...A..........A.
F. glaucescens             ...........G................-................
F. gracilis                ...........G................-....C...........
F. hamatacanthus           .......C...G................-................
F. histrix                 ...........G................-................
F. latispinus              ...........G................-................
F. lindsayi                ...........G................-................
F. macrodiscus             ...........G................-................
F. peninsulae              ...........G................-................
F. pilosus                 ...........G................-.............A..
F. pottsii v. alamosanus   ...........G................-................
F. rafaelensis             ...........G................-................
F. recurvus                ...........G................-................
F. reppenhagenii           ...........G................-....C...........
F. robustus                ...........GC...............-................
F. stainesii               ...........G................-.............A..
F. wislizeni v. herrerae   ...........G................-................
F. wislizeni v. wislizeni  ............................-................
F. viridescens             ...........G.......A.......G-....C...........
F. sp. nov. ?              ...........G................-................
Ancistrocactus uncinatus   ...........G................-................
Aztekium ritterii          ...........G.......G........-................
Coryphantha pallida        -.G.T----------------------.-................
Echinocactus grusonii      ...........G................-................
E. horizonthalonius        ...........G................-T.........T.....
Geohintonia mexicana       ...........G................-................
Glandulicactus crassipes   ...........G................-................
Lophophora williamsii      ...........G...------.....C.----.............
Mammillaria haggeana       ----------------------------------------..CA.
Obregonia denegrii         ...........G...------.....C.----.............
Sclerocactus spinosior     ...........G................-..........T.....
Stenocactus lloydii        ...........G................-..............A.
Strombocactus disciformis  ...........G................T................
Pachycereus marginatus     ...........G...---..........-................
Stenocereus dumortieri     ...........G...---..........-................
Leptocereus quadricostatus ...........G...---..........-C...............
Opuntia phaeacantha        A..........G.C.---A.........-................
Pereskia aculeata          .......TAG...G.A.-A.........-................
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APPENDIX I. Continued.

TAXON	
[	460 470 480 490]
[	. . . .]
F. cylindraceus	TTAGA-TTG-CATTG-CTAAAATTAAATAAGAGCAATCCAATAAA
F. echidne	.....A...-.....G.............................
F. emoryi v. *rectispinus*	.....-...-.....-.............................
F. flavovirens	.....-...-.....-.............................
F. fordii	.A...-.G.-.....G.....T....T........T....-....
F. glaucescens	.....-...-.....-.............................
F. gracilis	.....-...-.....-.............................
F. hamatacanthus	.....-...-.....-.............................
F. histrix	.....-...-.....-.............................
F. latispinus	.....-...-.....-.....T...............T.......
F. lindsayi	.....-...-.....-.............................
F. macrodiscus	.....-...-.....-.....T.A.....................
F. peninsulae	.....-...-.....-.............................
F. pilosus	.....-...-.....G.............................
F. pottsii v. *alamosanus*	.....-...-T....-.......A.....................
F. rafaelensis	.....A.G.-.....G.............................
F. recurvus	.....-...-.....-.....T...............T.......
F. reppenhagenii	.....-...-.....-.............................
F. robustus	.....-...-A....-.............................
F. stainesii	.....-...-.....G.............................
F. wislizeni v. *herrerae*	..G..-...-.....-.......A...............N..T..
F. wislizeni v. *wislizeni*	.....-...-.....-...................T....T....
F. viridescens	.....-...-.....G.....T..................-....
F. sp. nov. ?	.....-...-.....-.............................
Ancistrocactus uncinatus	.....-...-.....-.....T.......................
Aztekium ritterii	.....-...-..G..-.......A.....................
Coryphantha pallida	.....-...-.....-.......G.....................
Echinocactus grusonii	.....-...-.....-.............................
E. horizonthalonius	.....-...-.....-.......A.....................
Geohintonia mexicana	.....-...-..G..-.......A.....................
Glandulicactus crassipes	.....-...-.....-.....T.......................
Lophophora williamsii	.....-...-.....-.....T.......................
Mammillaria haggeana	..---------------------------------------.-C.
Obregonia denegrii	.....-...-.....-.....T.A.....................
Sclerocactus spinosior	.....-...-....T-.......A.....................
Stenocactus lloydii	.....-...-.....-............T..............T.
Strombocactus disciformis	.....-...-.....-......AG................T....
Pachycereus marginatus	....G-...CTC..A-T.TT.T..T.GC..T-......T......
Stenocereus dumortieri	.....-...-.TC.T-A.TTT...TT.GC.AT......T......
Leptocereus quadricostatus	.....-...-.TC.T-A.TTT...TT.GC.AT......T......
Opuntia phaeacantha	.....-...-.....-.......A....GT.......TT......
Pereskia aculeata	..TAGA...-.....-.......A.....T........T......

APPENDIX I. Continued.

TAXON	
[	500 510 520 530 540]
[	. . . . .]
F. cylindraceus	AAAGGAATTTTCGCGGGCGAA-TATTTACTCTTTCTATATCTATT
F. echidne	.....................-.......................
F. emoryi v. *rectispinus*	.....................-.............C.........
F. flavovirens	.....................-.............A.........
F. fordii	....T.T...C..........-...................C...
F. glaucescens	.....................-.......................
F. gracilis	.....................-.............C.........
F. hamatacanthus	..........-..........-...........C...........
F. histrix	.....................-.......................
F. latispinus	.....................-.............G.........
F. lindsayi	.....................-.............G.........
F. macrodiscus	.....................-.............G.........
F. peninsulae	.....................-.......................
F. pilosus	.....................-.......................
F. pottsii v. *alamosanus*	.....................-.......................
F. rafaelensis	.....................-.......................
F. recurvus	.....................-.............G.........
F. reppenhagenii	.....................-.............C.........
F. robustus	.....................-.............A.........
F. stainesii	.....................-.......................
F. wislizeni v. *herrerae*	.....................-.......................
F. wislizeni v. *wislizeni*	.....................-.......................
F. viridescens	.....................-.............C.....C...
F. sp. nov. ?	.....................-.......................
Ancistrocactus uncinatus	.....................-.......................
Aztekium ritterii	...A.................-....................G..
Coryphantha pallida	.....................-..C....................
Echinocactus grusonii	.....................-.......................
E. horizonthalonius	.....................-.............A.........
Geohintonia mexicana	.....................-.......................
Glandulicactus crassipes	.....................-.......................
Lophophora williamsii	..G.........A........-.............C.........
Mammillaria haggeana	.....................-G.........G............
Obregonia denegrii	..G..................-.......................
Sclerocactus spinosior	.....................-.......................
Stenocactus lloydii	.....................-.......................
Strombocactus disciformis	.....................-.......................
Pachycereus marginatus	.....................-.............A.........
Stenocereus dumortieri	.....................A.............A.........
Leptocereus quadricostatus	.....................-.............A.........
Opuntia phaeacantha	.....................-.............A.........
Pereskia aculeata	.....................-.............A.........

APPENDIX I. Continued.

TAXON	
[	550 560 570 580]
[	. . . .]
F. cylindraceus	TCAGTTTTATAGG-ATTAGTTTATCACTTTTCAGAATAGA-----
F. echidne	.............-..........................-----
F. emoryi v. *rectispinus*	.............-.................A........-----
F. flavovirens	.............-..........................-----
F. fordii	.............-..........................-----
F. glaucescens	.............-.........................T-----
F. gracilis	.............-.................A........-----
F. hamatacanthus	.......A.....-..A....-...CAC............-----
F. histrix	.............-..........................-----
F. latispinus	.............-..........................-----
F. lindsayi	.............-..........................-----
F. macrodiscus	.............-..........................-----
F. peninsulae	.............-..........................-----
F. pilosus	.............-..........................-----
F. pottsii v. *alamosanus*	.............-..........................-----
F. rafaelensis	.............-..........................-----
F. recurvus	.............-..........................-----
F. reppenhagenii	.............-.................A........-----
F. robustus	.............-..........................-----
F. stainesii	.............-..........................-----
F. wislizeni v. *herrerae*	.............-..........................-----
F. wislizeni v. *wislizeni*	. ...-..........................-----
F. viridescens	.............-.................A........-----
F. sp. nov. ?	.............-..........................-----
Ancistrocactus uncinatus	.............-..........................-----
Aztekium ritterii	.............G..........................-----
Coryphantha pallida	.............-..........................-----
Echinocactus grusonii	.............-..........................-----
E. horizonthalonius	..........G..-..........................-----
Geohintonia mexicana	.............-..........................-----
Glandulicactus crassipes	.............-..........................ATAGA
Lophophora williamsii	.............-...................A......-----
Mammillaria haggeana	.............-..........................-----
Obregonia denegrii	.---.........-..........................-----
Sclerocactus spinosior	.............-..........................-----
Stenocactus lloydii	.......A.....-..........................-----
Strombocactus disciformis	.............-..........................-----
Pachycereus marginatus	...........T.-..........................-----
Stenocereus dumortieri	........CC.----------------------------------
Leptocereus quadricostatus	...........T.-..........................-----
Opuntia phaeacantha	.T...........-..........................-----
Pereskia aculeata	.............-G.........................-----

APPENDIX I. Continued.

TAXON	
[	590 600 610 620 630]
[	. . . . .]
F. cylindraceus	TAAATTGGTCTTTGGTTCGTTCCGCCATCCTTCCCAATGAATCAT
F. echidne	....
F. emoryi v. *rectispinus*	....
F. flavovirens	....
F. fordii	..................................-.G........
F. glaucescens	....
F. gracilis	....
F. hamatacanthus	AT........C......G..-....-........-.....-....
F. histrix	....
F. latispinus	....
F. lindsayi	....
F. macrodiscus	....
F. peninsulae	....
F. pilosus	....
F. pottsii v. *alamosanus*	..................................-.....-....
F. rafaelensis	A...........G...C...C.....T.......-.....-....
F. recurvus	..................................-..........
F. reppenhagenii	....
F. robustus	....
F. stainesii	............G...C.................-.....-....
F. wislizeni v. *herrerae*	..................................-.....-....
F. wislizeni v. *wislizeni*	....
F. viridescens	....
F. sp. nov. ?	............-.....................-.....-....
Ancistrocactus uncinatus	....
Aztekium ritterii	....
Coryphantha pallida	................................T............
Echinocactus grusonii	....
E. horizonthalonius	....
Geohintonia mexicana	....
Glandulicactus crassipes	....
Lophophora williamsii	................................T............
Mammillaria haggeana	................................T............
Obregonia denegrii	................................T............
Sclerocactus spinosior	................................T............
Stenocactus lloydii	....................................G.......G
Strombocactus disciformis	....
Pachycereus marginatus	.G.........C.................................
Stenocereus dumortieri	----------.A..A.CTAG.TTAGG...AC.TT.C.GA...A.A
Leptocereus quadricostatus	.G.........C.................................
Opuntia phaeacantha	.G.........C.................................
Pereskia aculeata	.G.........C.................................

APPENDIX I. Continued.

TAXON	
[	640 650 660 670]
[	. . . .]
F. cylindraceus	TAGGATCATTTT-CAATTGAATCTTC------------------CT
F. echidne	.....ATCA...T.............------------------T.
F. emoryi v. *rectispinus*	......TCA...-.............-------------------.
F. flavovirens	......TCA...T.............-------------------.
F. fordii	............-.............-------------------.
F. glaucescens	............-.............-------------------.
F. gracilis	......TCA...T.............-------------------.
F. hamatacanthus	............-.............-------------------G
F. histrix	-...........-.............-------------------.
F. latispinus	....GATCA...T.............-------------------.
F. lindsayi	.......CA...T.............------------------..
F. macrodiscus	......TCA...T.............-------------------.
F. peninsulae	......TC....T.............-------------------.
F. pilosus	............C.............------------------..
F. pottsii v. *alamosanus*	............-.............-------------------.
F. rafaelensis	-...........T.............-------------------.
F. recurvus	......TCA...T.............-------------------.
F. reppenhagenii	......TCA...T.............-------------------.
F. robustus	......TCA...T.............-------------------.
F. stainesii	-...........C.............------------------..
F. wislizeni v. *herrerae*	............-.............-------------------.
F. wislizeni v. *wislizeni*	......TCA...C.............-------------------.
F. viridescens	......TCA...-.............-------------------.
F. sp. nov. ?	............-.............-------------------.
Ancistrocactus uncinatus	......TCA.C.T.............-------------------.
Aztekium ritterii	......TCA...T.............-------------------.
Coryphantha pallida	.......T....-.............-------------------.
Echinocactus grusonii	............-.............-------------------.
E. horizonthalonius	......TCA...T.............-------------------.
Geohintonia mexicana	......TCA...T.............-------------------.
Glandulicactus crassipes	......TCA...T.............-------------------.
Lophophora williamsii	......TCA...T.............-------------------.
Mammillaria haggeana	......TC....T.............-------------------.
Obregonia denegrii	......TC....T.............-------------------.
Sclerocactus spinosior	......TTA...T.............TGTATTCATTGAATCTT..
Stenocactus lloydii	......TCA...T.............-------------------.
Strombocactus disciformis	......TCA...T.............-------------------.
Pachycereus marginatus	......TCA...T.............-------------------.
Stenocereus dumortieri	.--..---------.C...GCC..---------------------G
Leptocereus quadricostatus	......TCA...T.............-------------------.
Opuntia phaeacantha	......TC....T...A.........-------------------.
Pereskia aculeata	......TCG...T.............-------------------.

APPENDIX I. Continued.

TAXON	
[	680 690 700 710 720]
[	. . . . .]
F. cylindraceus	GTATTC-ACGGATTCC-ATCGTTCCCATCGC-TTCTTGATTAA-T
F. echidne	......-.........-..............T.C.........-.
F. emoryi v. *rectispinus*	......-.........-..............-...........-.
F. flavovirens	......-.........-..............-...........-.
F. fordii	......-.........-..............-...........-.
F. glaucescens	......-.........-..............T.C.........-.
F. gracilis	......-.........-..............-...........-.
F. hamatacanthus	......-.........-..............-...........-.
F. histrix	......-.........-..............T.C.........-.
F. latispinus	......-.........-..............T.C.........-.
F. lindsayi	......-.........-..............-...........-.
F. macrodiscus	......-.........-..............-...........-.
F. peninsulae	......-.........-..............-...........-.
F. pilosus	......-.........-..............-.C.........-.
F. pottsii v. *alamosanus*	......-.........-..............-...........-.
F. rafaelensis	......-.........-..............-...........-.
F. recurvus	......-.........-..............-...........-.
F. reppenhagenii	......-.........-..............-...........-.
F. robustus	......-.........-..............-.CT.G......-.
F. stainesii	......-.........-..............-...........-.
F. wislizeni v. *herrerae*	......-.........-..............-...........-.
F. wislizeni v. *wislizeni*	......-.........-..............-...........-.
F. viridescens	......-.........-..............-...........-.
F. sp. nov. ?	......-.........-..............-...........-.
Ancistrocactus uncinatus	......-.........-.........C....-...........-.
Aztekium ritterii	......ACA.AT.C..-..............-...........-.
Coryphantha pallida	......-.........-C.............-...........-.
Echinocactus grusonii	......-........T-..............T.C....GA.T.-.
E. horizonthalonius	......-.........-T.............T.CT.G......-.
Geohintonia mexicana	......-..A......-..............-...........-.
Glandulicactus crassipes	......-.........-.........C....-...........-.
Lophophora williamsii	......-.........C..............-...........-.
Mammillaria haggeana	......-.........-C.............-...........-.
Obregonia denegrii	......-.........C..............-...........-.
Sclerocactus spinosior	......-.........-..............-...........-.
Stenocactus lloydii	......-.........-..............-...........G.
Strombocactus disciformis	......-.........-..............-...........-.
Pachycereus marginatus	......-.........-..............-...........-.
Stenocereus dumortieri	......-.........-GC.A..........-...........-.
Leptocereus quadricostatus	......-.........-..............-...........-.
Opuntia phaeacantha	......-.T.....A.-..............-...........TG
Pereskia aculeata	......-.T.......-..............-...........-.

APPENDIX I. Continued.

TAXON	
[	730 740 750 760]
[	. . . .]
F. cylindraceus	GGTTAGGTCTTAATTCT-ACAATGGAGCTCTT-AATGAACTTTGG
F. echidne	.................-..............-......-C..T.
F. emoryi v. *rectispinus*	.................-..............-.......C..T.
F. flavovirens	.................-..............-......-C..T.
F. fordii	.................-....C.........-.......C..T.
F. glaucescens	.................-..............-...A..-C..T.
F. gracilis	.................-..............-.......C..T.
F. hamatacanthus	.................-..............-......-C..T.
F. histrix	.................-..............-......-C..T.
F. latispinus	.................-..............-......-C..T.
F. lindsayi	.................-..............-......-C..T.
F. macrodiscus	................G-..............-......-C..T.
F. peninsulae	.................-..............-.......C..T.
F. pilosus	.................-..............-.......C....
F. pottsii v. *alamosanus*	.................-..............-.......C..T.
F. rafaelensis	.................-..............-......-C..T.
F. recurvus	.................-..............-......-C..T.
F. reppenhagenii	.................-..............-......-C..T.
F. robustus	.................-..............-......-C..T.
F. stainesii	.................-..............-......-C..T.
F. wislizeni v. *herrerae*	.................-..............-.......C..T.
F. wislizeni v. *wislizeni*	.................-..............-.......C..T.
F. viridescens	.................-..............-.......C..T.
F. sp. nov. ?	.................-..............-.......C..T.
Ancistrocactus uncinatus	.................-..............-......-C..T.
Aztekium ritterii	.................-..............-......-C..T.
Coryphantha pallida	.................-..............-......-...T.
Echinocactus grusonii	.................-..............-......-C..T.
E. horizonthalonius	................C-..............-......-C..T.
Geohintonia mexicana	.................-..............-......-C..T.
Glandulicactus crassipes	................CT..............-......-C..A.
Lophophora williamsii	.................-..............-......-C..T.
Mammillaria haggeana	.................-..............-......-C..T.
Obregonia denegrii	.................-..............-......-C..T.
Sclerocactus spinosior	.C...............-..............-......-C..T.
Stenocactus lloydii	.................-..............G......TC..T.
Strombocactus disciformis	.................-..............-......-C..T.
Pachycereus marginatus	..........CC.....-..............-......-C..T.
Stenocereus dumortieri	..........CC.....-..............-......-C..T.
Leptocereus quadricostatus	..........CC.....-..............-......-C..T.
Opuntia phaeacantha	AA........G......-..........C...-......-C..T.
Pereskia aculeata	..........G......-..............-......-C..T.

APPENDIX I. Continued.

```
           TAXON
[                             770       780       790       800      810]
[                             .         .         .         .         .]

F. cylindraceus            TTCTTTGAGCCAACCTTCTTTAGTCTTTAT-TGGCCCGAGGCTCT
F. echidne                 ...-.GAGA.........-...........-....T.........
F. emoryi v. rectispinus   ...-..............-...........-....T.........
F. flavovirens             ...-..............-...........-....T.........
F. fordii                  ...-..............-...........-....T.........
F. glaucescens             ...-..............-...........-....T.........
F. gracilis                ..................-...........-....T.........
F. hamatacanthus           ...-..............-...........-....T.........
F. histrix                 ...-..............-...........-....T.........
F. latispinus              ...-..............-...........-....T.........
F. lindsayi                ...-..............-...........-....T.........
F. macrodiscus             ...-....A.........-...........-....T.........
F. peninsulae              ...............C..-...........-....T.........
F. pilosus                 ...-.GAGA.........-...........-....T........C
F. pottsii v. alamosanus   ...-..A...........-...........-..............
F. rafaelensis             ...-..............-...........-....T.........
F. recurvus                ...-..............-...........-....T.........
F. reppenhagenii           ...-..............-...........-....T........C
F. robustus                ...-..............-...........-....T.........
F. stainesii               ...-.G............-...........-....T.........
F. wislizeni v. herrerae   ...-..............-...........-..............
F. wislizeni v. wislizeni  ......AGA...........AT........-..............
F. viridescens             ...-..............-...........-....T.........
F. sp. nov. ?              ...-..............-...........-..............
Ancistrocactus uncinatus   ...-..............-...........-....T.CGA.GCTC
Aztekium ritterii          ...-..............-...........-....T.........
Coryphantha pallida        ...-..............-...........-....T.........
Echinocactus grusonii      ...-..............-...........-....T.........
E. horizonthalonius        ...-.........T....-...........-....T...A.....
Geohintonia mexicana       ...-..............-...........-....T.........
Glandulicactus crassipes   ...-..............-...........-....T.........
Lophophora williamsii      ...C..............-...........-....T.........
Mammillaria haggeana       ...-..............-...........-....T.........
Obregonia denegrii         ...-..............-...........-....T.........
Sclerocactus spinosior     ...-..............-...........-...TT.........
Stenocactus lloydii        ...C..............-...........-....T.........
Strombocactus disciformis  ...-..............-...........-....T.........
Pachycereus marginatus     ...-..............-...........-....TT........
Stenocereus dumortieri     ...-..............-...........-....TT........
Leptocereus quadricostatus ...-..............-...........-....TT........
Opuntia phaeacantha        ...-..........TC..-...........-.....G........
Pereskia aculeata          ...-..............-...........A....T.........
```

APPENDIX I. Continued.

TAXON	
[	820 830 840 850]
[	. . . .]
F. cylindraceus	TTACTTC-TTTCTTTTTTCTATGAATAGATTCATATCTGATAATT
F. echidne	-......T..-..................................
F. emoryi v. *rectispinus*	-......-.....................................
F. flavovirens	-......T..-..................................
F. fordii	-......T.....................................
F. glaucescens	-......T..-..................................
F. gracilis	.......-.....................................
F. hamatacanthus	-......T..-....................T......T......
F. histrix	-......T..-..................................
F. latispinus	-......T..C.......A..........................
F. lindsayi	-......T..-..................................
F. macrodiscus	-......T..-.......A..........................
F. peninsulae	-......-..........A..........................
F. pilosus	-......T..-..................................
F. pottsii v. *alamosanus*	-......T.....................................
F. rafaelensis	-......T..-..................................
F. recurvus	-......T..-.......A..........................
F. reppenhagenii	.......C..-..................................
F. robustus	-......T..-..................................
F. stainesii	-......T..-..................................
F. wislizeni v. *herrerae*	-......T.....................................
F. wislizeni v. *wislizeni*	....A.TC.....................................
F. viridescens	-......-.....................................
F. sp. nov. ?	-......T.....................................
Ancistrocactus uncinatus	-.TAC.TC-.-..................................
Aztekium ritterii	-......T..-..................................
Coryphantha pallida	-......T..-..................................
Echinocactus grusonii	-......T..-..................................
E. horizonthalonius	-......T..-..................................
Geohintonia mexicana	-......T..-..................................
Glandulicactus crassipes	-......T..-..................................
Lophophora williamsii	-......T..-..................................
Mammillaria haggeana	-......T..-..................................
Obregonia denegrii	-......T..-...........................T......
Sclerocactus spinosior	-......T..-..................................
Stenocactus lloydii	GC.T...T..C....A.....................A.......
Strombocactus disciformis	-......-.....................................
Pachycereus marginatus	-......T..-..........................A.......
Stenocereus dumortieri	-......T..-..........................A.......
Leptocereus quadricostatus	-......T..-A........................AA.......
Opuntia phaeacantha	-.....TT..-..........................A.......
Pereskia aculeata	-.....TC..-..........................A.......

APPENDIX I. Continued.

TAXON

```
[                                860       870       880       890       900]
[                                 .         .         .         .         .]

F. cylindraceus               ATGT-----GTGAATCTGTATTCATGCTT-TATTACATTGTCTTT
F. echidne                    ....-----..............C.....-...............
F. emoryi v. rectispinus      ....-----....................-...............
F. flavovirens                ....-----....................T...............
F. fordii                     ....-----....................-...............
F. glaucescens                ....-----.C..................-............A..
F. gracilis                   ....-----....................-...............
F. hamatacanthus              ....-----....................-...............
F. histrix                    ....-----....................-...A...........
F. latispinus                 ....-----....................-.......C.......
F. lindsayi                   ....-----....................-...............
F. macrodiscus                ....-----....................-...............
F. peninsulae                 ....-----....................-...............
F. pilosus                    ....-----..............C.....-...............
F. pottsii v. alamosanus      ....-----..............G.....-...............
F. rafaelensis                ....-----..............C.....-...............
F. recurvus                   ....-----....................-.......C.......
F. reppenhagenii              ....-----....................-...............
F. robustus                   ....-----..............C.....T...............
F. stainesii                  ....-----..............C.....-...............
F. wislizeni v. herrerae      ....-----..............G.....-...............
F. wislizeni v. wislizeni     ....-----....................-...............
F. viridescens                ....-----....................-...............
F. sp. nov. ?                 ....-----....................-...............
Ancistrocactus uncinatus      ....-----....................-...............
Aztekium ritterii             ....-----T...................-...............
Coryphantha pallida           ....-----....................-...............
Echinocactus grusonii         ....-----....................-...............
E. horizonthalonius           ....-----......A.............-...............
Geohintonia mexicana          ....-----.............T......-...............
Glandulicactus crassipes      ....-----....................-...............
Lophophora williamsii         ....TATGT......T.............-...............
Mammillaria haggeana          ....-----.......C............-...............
Obregonia denegrii            ....-----....G.T...........C.-...............
Sclerocactus spinosior        ....-----....................-...............
Stenocactus lloydii           ....-----.............T......-...............
Strombocactus disciformis     ....-----....................-...............
Pachycereus marginatus        ....-----....................-...............
Stenocereus dumortieri        ....-----....................-...............
Leptocereus quadricostatus    ....-----....................-...............
Opuntia phaeacantha           ....-----....................-...............
Pereskia aculeata             ....-----.......C............A........C....A.
```

APPENDIX I. Continued.

TAXON	
[	910 920 930 940]
[	. . . .]
F. cylindraceus	TATGATATGATTCAAAGAC-CTTACATAGTGGAATCAGATATCAT
F. echidne	...................-................G........
F. emoryi v. *rectispinus*	...................-.........................
F. flavovirens	...................-.........................
F. fordii	...................T.........................
F. glaucescens	...................-.........................
F. gracilis	...................T.........................
F. hamatacanthus	...................-.........................
F. histrix	...................-.........................
F. latispinus	...................-.........................
F. lindsayi	...................-.........................
F. macrodiscus	...................-.........................
F. peninsulae	...................-.........................
F. pilosus	...................-.........................
F. pottsii v. *alamosanus*	...................-.........................
F. rafaelensis	...................-................G........
F. recurvus	...................-.........................
F. reppenhagenii	...................-.........................
F. robustus	...................-.........................
F. stainesii	...................-.........................
F. wislizeni v. *herrerae*	...................-.........................
F. wislizeni v. *wislizeni*	...................-.........................
F. viridescens	...................T.........................
F. sp. nov. ?	...................-.........................
Ancistrocactus uncinatus	...A...............-.........................
Aztekium ritterii	...................-.................T.......
Coryphantha pallida	...................-.........................
Echinocactus grusonii	...................-.........................
E. horizonthalonius	...................-.................T.......
Geohintonia mexicana	...................-.................T.......
Glandulicactus crassipes	...................-.......G.................
Lophophora williamsii	...................-.........................
Mammillaria haggeana	...................-..............--GATA.....
Obregonia denegrii	...................-.........................
Sclerocactus spinosior	...................-................T........
Stenocactus lloydii	...................-.........................
Strombocactus disciformis	...................-.........................
Pachycereus marginatus	..........--------.-................TT.......
Stenocereus dumortieri	..........--------.-................TT.......
Leptocereus quadricostatus	...T......--------.-................TT.......
Opuntia phaeacantha	...................-................GT.......
Pereskia aculeata	G....G.......G.....-...T.............TTC.....

APPENDIX I. Continued.

```
TAXON
[                              950       960       970       980       990]
[                               .         .         .         .         .]

F. cylindraceus             TTATATTCATTTTTTT--CTTTCTTTCGCCTTT-CCATTTACCCG
F. echidne                  ................--...............-...........
F. emoryi v. rectispinus    ................--...............-...........
F. flavovirens              ................--...............-.T.........
F. fordii                   ................--...............-...........
F. glaucescens              ................-T...............-...........
F. gracilis                 ................--...............-...........
F. hamatacanthus            ................--...............-...........
F. histrix                  ................--...............-...........
F. latispinus               ................--...............-.........T.
F. lindsayi                 ................--...............-.........T.
F. macrodiscus              ................--...............-.T.........
F. peninsulae               ................--...............-...........
F. pilosus                  ................--...............-...........
F. pottsii v. alamosanus    ................--...............-...........
F. rafaelensis              ................--...............-...........
F. recurvus                 ................--...............-...........
F. reppenhagenii            ................--...............-...........
F. robustus                 ................--...............-...........
F. stainesii                ................--...............-...........
F. wislizeni v. herrerae    ................--...............-...........
F. wislizeni v. wislizeni   ................--...............-...........
F. viridescens              ................--...............-...........
F. sp. nov. ?               ................--...............-...........
Ancistrocactus uncinatus    ..C.............--...............-...........
Aztekium ritterii           ................--...............-.......T...
Coryphantha pallida         ..C.............--.........C.....-...........
Echinocactus grusonii       ................--...............-...........
E. horizonthalonius         ................--...............-...........
Geohintonia mexicana        ................--...............-.......T...
Glandulicactus crassipes    ..C.............--...............-...........
Lophophora williamsii       ...G............--...............-...........
Mammillaria haggeana        ..C...........G.TCT..C...........T...........
Obregonia denegrii          ...G............--...............-...........
Sclerocactus spinosior      ................--...............-...........
Stenocactus lloydii         .......T........--...............-...........
Strombocactus disciformis   ................--...............-...........
Pachycereus marginatus      ..C.............--...............-..T....T...
Stenocereus dumortieri      ..T.............--...............-..T....T...
Leptocereus quadricostatus  ..C............---...............-..T....T...
Opuntia phaeacantha         ...G............TT...............-.......T...
Pereskia aculeata           A..C...........---...............-.......T...
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APPENDIX I. Continued.

TAXON

```
[                                      1000      1010      1020      1030   ]
[                                      .         .         .         .      ]

F. cylindraceus               CATCCCCTTCTTT----------------TAATGTATATT----T
F. echidne                    .............----------------...........----.
F. emoryi v. rectispinus      .............----------------....T......----.
F. flavovirens                .............T---------------...........----.
F. fordii                     .............----------------...........----.
F. glaucescens                .............----------------...........TTTA.
F. gracilis                   .............----------------....T......----.
F. hamatacanthus              .............----------------...........----.
F. histrix                    .............----------------...........----.
F. latispinus                 .............A---------------...........----.
F. lindsayi                   .............----------------...........----.
F. macrodiscus                .............A---------------...........----.
F. peninsulae                 ..C..........----------------...........----.
F. pilosus                    .............----------------...........----.
F. pottsii v. alamosanus      .............----------------...........----.
F. rafaelensis                .............----------------...........----.
F. recurvus                   .............A---------------...........----.
F. reppenhagenii              .............----------------....T......----.
F. robustus                   .............T---------------...........----.
F. stainesii                  .............----------------...........----.
F. wislizeni v. herrerae      .............----------------...........----.
F. wislizeni v. wislizeni     .............----------------...........----.
F. viridescens                .............----------------....T......----.
F. sp. nov. ?                 .............----------------...........----.
Ancistrocactus uncinatus      .....T.......----------------...........----.
Aztekium ritterii             ......AC.....----------------...........----.
Coryphantha pallida           .............A---------------...........----.
Echinocactus grusonii         .............----------------...........----.
E. horizonthalonius           T............----------------...G.......----.
Geohintonia mexicana          .......C.....----------------...........----.
Glandulicactus crassipes      .....T.......A---------------...........----.
Lophophora williamsii         .............----------------...........----.
Mammillaria haggeana          .....T.C.T-------------------.........A.----.
Obregonia denegrii            G............TT--------------...........----.
Sclerocactus spinosior        ........C....----------------...........----.
Stenocactus lloydii           .............----------------A..........----.
Strombocactus disciformis     .............T---------------...........----.
Pachycereus marginatus        ..........C..----------------...........----.
Stenocereus dumortieri        ..........C..----------------...........----.
Leptocereus quadricostatus    ..........C..----------------...........-----
Opuntia phaeacantha           .......C..C..----------------...........----.
Pereskia aculeata             ..........C..TAATGTAGATAAAATTAG..A......----.
```

APPENDIX I. Continued.

```
TAXON
[                          1040      1050      1060      1070      1080]
[                             .         .         .         .         .]

F. cylindraceus           TTCTTTTTTTTTT----------AACAACTCA-TTCGA-TTTCTT
F. echidne                .............T---------.........-.....-......
F. emoryi v. rectispinus  .............TTT-------.........-...A.-......
F. flavovirens            .............T---------.........-.....-......
F. fordii                 .-..........-----------.........-.....-......
F. glaucescens            ..A..........T---------.........-.....-......
F. gracilis               .............TTT-------.........-...A.-......
F. hamatacanthus          ............-----------.........-..G..-......
F. histrix                ..A.........-----------.........-.....-......
F. latispinus             ............-------------.......-.....-......
F. lindsayi               ............-----------.........-.....-......
F. macrodiscus            .............T-----------......C-...C.-..C...
F. peninsulae             .............TTA-------.........-...A.-......
F. pilosus                .............T---------.........-.....-......
F. pottsii v. alamosanus  .............TTT-------.........-...A.-......
F. rafaelensis            .............----------.........-.....-......
F. recurvus               ............-------------.......-.....-......
F. reppenhagenii          .............TTT-------........T-...A.-......
F. robustus               .............----------.........-.....-......
F. stainesii              .............T---------.........-...A.-......
F. wislizeni v. herrerae  .............T---------.........-.....-......
F. wislizeni v. wislizeni .............T---------.........-.....-......
F. viridescens            .............T---------.........-...A.-......
F. sp. nov. ?             .............----------.........-.....-......
Ancistrocactus uncinatus  .............TT--------.........-...T.-......
Aztekium ritterii         .............----------G........-.....-...A..
Coryphantha pallida       A............----------.........-.....-......
Echinocactus grusonii     ..A..........----------.........-.....-......
E. horizonthalonius       ............-----------.........-.....-......
Geohintonia mexicana      .............T-----G---G........-.....-......
Glandulicactus crassipes  ............-----------.........-.....-......
Lophophora williamsii     .............TTTTTTA---.........-.....-......
Mammillaria haggeana      ..TC.........TTT-------.........C..T..A......
Obregonia denegrii        .............T---------.........-.....-......
Sclerocactus spinosior    .............T---------.........-.....-......
Stenocactus lloydii       ............-----------.........-.....-......
Strombocactus disciformis ............-----------.........-.....-......
Pachycereus marginatus    ............-------G---G.......T-...A.-.....G
Stenocereus dumortieri    ............-----------G......ATT.....-..-...
Leptocereus quadricostatus..AA.........T--------TG.......T-.....-......
Opuntia phaeacantha       .............CCTTTGGCTTG........-..T..-......
Pereskia aculeata         ..T...G....G--CT------TG........-.....-..-..G
```

APPENDIX I. Continued.

TAXON	
[	1090 1100 1110 1120]
[	. . . .]
F. cylindraceus	GTTTATGCAAAAAAAAAA-------TTCATTTGCTGCAATGA-TA
F. echidne	..................-------....G............-..
F. emoryi v. *rectispinus*	T.................A-----T.C....G..G...T.A.-.G
F. flavovirens	.................--------....G............-..
F. fordii	.................--------....G............-..
F. glaucescens	..................A------....G............-.G
F. gracilis	T.................-------...T..G.TG.....A.-.G
F. hamatacanthus	..................A------.................-..
F. histrix	..................-------....G............-..
F. latispinus	..................-------....G............A..
F. lindsayi	..................A------....G............-..
F. macrodiscus	..................-------.............T...A.G
F. peninsulae	T................-------T.C.T..G.TG...T.A.-.G
F. pilosus	..................A------.C....G......T...-.T
F. pottsii v. *alamosanus*	..................AAAAAA-.........G.....A.TAG
F. rafaelensis	..................A------.................-..
F. recurvus	..................A------....G............A..
F. reppenhagenii	T.................-------.C....G..G...T.A.-.G
F. robustus	.................--------....G............-..
F. stainesii	..................A------.................-..
F. wislizeni v. *herrerae*	..................AA-----....G............-..
F. wislizeni v. *wislizeni*	..................-------....G............-..
F. viridescens	T.................A------.C.T..G..G...T.A.-.G
F. sp. nov. ?	..................-------....G............-..
Ancistrocactus uncinatus	..................A-----T...T..G..G....AA.-.G
Aztekium ritterii	..................AA-----....G............-..
Coryphantha pallida	..................-------.................-..
Echinocactus grusonii	.......T..........A------....G............-..
E. horizonthalonius	..................-------....G............TAG
Geohintonia mexicana	..................A------....G............-..
Glandulicactus crassipes	..................A-----T............C.A..-..
Lophophora williamsii	..................------T.........G.......-.G
Mammillaria haggeana	..................-------.................-..
Obregonia denegrii	..................AAAAA---...G............-..
Sclerocactus spinosior	.................--------....G.........A..-..
Stenocactus lloydii	..................-------....G............-..
Strombocactus disciformis	..................A------...............A.-.G
Pachycereus marginatus	..................-------.C..G.G..G.......-..
Stenocereus dumortieri	..................AAA----.................-..
Leptocereus quadricostatus	.G................AAA----.C..G.G..G.......-.G
Opuntia phaeacantha	.......-..........-------.................-..
Pereskia aculeata	.....A...........--------..-.G............-..

APPENDIX I. Continued.

```
TAXON
[                            1130      1140      1150      1160     1170]
[                              .         .         .         .         .]

F. cylindraceus            GGACCAAAATATCATATCTTGACTGCTTCTTTGG-ATCCAGATAA
F. echidne                 ..................................-..........
F. emoryi v. rectispinus   ........T.T..T.T...GA..G.......G..-.C...A...T
F. flavovirens             ..................................-..........
F. fordii                  ..................................-..........
F. glaucescens             ..................................-..........
F. gracilis                ........T.TC.T.C...GA..G.......G..-.C...A.A.T
F. hamatacanthus           ..................................-.....A....
F. histrix                 ..................................-..........
F. latispinus              ..................................-..........
F. lindsayi                ..................................-..........
F. macrodiscus             ........T.T....T...G...G..........-.....A....
F. peninsulae              ........T.T..T.T...GA..G.......G..-.....A....
F. pilosus                 ...A.........T.T...GA.G....C...G..-....GA....
F. pottsii v. alamosanus   ..........T....T.......G..........-.....A...T
F. rafaelensis             ..................................-.....A....
F. recurvus                ..................................-..........
F. reppenhagenii           ........T.T..T.T...GA..G.......G..-.C...A.A.T
F. robustus                ...............................G..-..........
F. stainesii               ...............T..................-.....A....
F. wislizeni v. herrerae   ..................................-.....A....
F. wislizeni v. wislizeni  ..................................-..........
F. viridescens             ........T.T..T.T...GA..G.......G..-.C...A.A.T
F. sp. nov. ?              ..................................-..........
Ancistrocactus uncinatus   ........T.T..T.T...GA..G.......G..-.C...A...T
Aztekium ritterii          ...............T.......G..........-.....A....
Coryphantha pallida        .........................A........-..........
Echinocactus grusonii      ..................................-..........
E. horizonthalonius        ..................................G..........
Geohintonia mexicana       ...............T.......G..........-..........
Glandulicactus crassipes   ...A......T..T.T..................-..........
Lophophora williamsii      ..........T..T.T.......N.......N..-....G.....
Mammillaria haggeana       ..................................-..........
Obregonia denegrii         ...A..............................-....G.....
Sclerocactus spinosior     ..................................-..........
Stenocactus lloydii        ...A..............................-..........
Strombocactus disciformis  .............T.T..................-.....A....
Pachycereus marginatus     .....C.............G...G...C...G..-.....A....
Stenocereus dumortieri     ......C........T..................-.....A....
Leptocereus quadricostatus...................G...G...C...G..-.....A....
Opuntia phaeacantha        .............C.................G..-.....A....
Pereskia aculeata          .T................................-.....A....
```

APPENDIX I. Continued.

TAXON	
[	1180 1190 1200 1210]
[	. . . .]
F. cylindraceus	TGTAATGCGATGA-GTTGGTTATTAGTTCTAT-AGTCATTAGTTC
F. echidne	...G.........-..................-...T........
F. emoryi v. *rectispinus*	.T......A.AA.-T.G......A.T.C...A-.T....A.T.C.
F. flavovirens	...G....A..A.-T..........T......-............
F. fordii	...G.........-..................-............
F. glaucescens	...G.........A..................-............
F. gracilis	.T......A.AA.-T.G...A....T.C.A.A-.T....A.T.C.
F. hamatacanthus	...G.........-..................-............
F. histrix	...G.........-..................-............
F. latispinus	...G......A..-A.................-............
F. lindsayi	...G.........-..................-............
F. macrodiscus	...G....A....-..G......A...C....-..........C.
F. peninsulae	.T...G..A.AA.-T.G......A.T.C.A.A-.T....A...C.
F. pilosus	...G.A..A.A..AT.G......A.T.C.A.A--A........C.
F. pottsii v. *alamosanus*	G..GTA....A..AT.G........T.C...A-......A.T.C.
F. rafaelensis	...G.........-..................-...T........
F. recurvus	...G.........-..G...............-............
F. reppenhagenii	.T......A.AA.-T.G......A.T.C...A-.T....A.T.C.
F. robustus	...G....A..A.-..................-..........C.
F. stainesii	...G.........A..................-............
F. wislizeni v. *herrerae*	...G.........-..G...............-............
F. wislizeni v. *wislizeni*	........A..A.-..................-............
F. viridescens	.T......A.AA.-T.G......A.T.C...A-.T....A.T.C.
F. sp. nov. ?	...G.........-..G...............-............
Ancistrocactus uncinatus	.T......A.AA.-T.G........T.C.A.A-.T....A.T.C.
Aztekium ritterii	.T.G....A..A.-T..........T......-......A.....
Coryphantha pallida	...G.........-.............----------------..
Echinocactus grusonii	...G.........-..................-............
E. horizonthalonius	...G.........-..................-............
Geohintonia mexicana	...G.......A.-T..........T......-......A.....
Glandulicactus crassipes	...G.........-..................-............
Lophophora williamsii	.T.G....A.GA.-T.N......A.T.....A-......N.....
Mammillaria haggeana	...G.........-AG................C............
Obregonia denegrii	.T.G.........AT.G........T......-..C...A.....
Sclerocactus spinosior	.N.G.........-..............A...-............
Stenocactus lloydii	...G.........-.........G........-............
Strombocactus disciformis	.T.G....A..A.-T........A........-......A...C.
Pachycereus marginatus	G..G....A..A.-..G..........C....-............
Stenocereus dumortieri	...G....A..A.-T..........T......-............
Leptocereus quadricostatus	...G....A.AA.-..G........N.C....-......A...C.
Opuntia phaeacantha	...G....A..A.-T.................-...T........
Pereskia aculeata	...G.........-..................-...T........

APPENDIX I. Continued.

TAXON	
[	1220 1230 1240 1250 1260]
[	. . . . .]
F. cylindraceus	ATA-CTTCATACTATGGGCTCTTATCCCCCC--TTTTTTTTCGTC
F. echidne	...-..........................---............
F. emoryi v. *rectispinus*	.A.-..C.......G.........C......---........C.T
F. flavovirens	...-..........................---C...........
F. fordii	...-...........................--............
F. glaucescens	...-..........................---.......C....
F. gracilis	.A.-..C.......G.......A.C.....----.......TC..
F. hamatacanthus	...-..........................---............
F. histrix	...-...........................-T............
F. latispinus	...-......................T..----............
F. lindsayi	...-..........................---............
F. macrodiscus	...-..........................----...........
F. peninsulae	.A.-..C..A....G....C.C..C.....----........C.T
F. pilosus	...-..........................---.......C....
F. pottsii v. *alamosanus*	.A.-..C.......G....C....N.....---.......C....
F. rafaelensis	...-..........................---............
F. recurvus	...-.........................----............
F. reppenhagenii	.A.-..C..A.AAGG.........C.....----........C.T
F. robustus	...-..........................---C...........
F. stainesii	...-...........................---...........
F. wislizeni v. *herrerae*	...-..........................---............
F. wislizeni v. *wislizeni*	...-...........................--............
F. viridescens	...-..C..A...GG.........C......---........C..
F. sp. nov. ?	...-..........................---............
Ancistrocactus uncinatus	.A.-.....A.....AAC......C......---......C....
Aztekium ritterii	...-...............-....C..-----T............
Coryphantha pallida	...-..........................----.C......CGT
Echinocactus grusonii	...-.........................---T.C..........
E. horizonthalonius	...-....................C.....----...........
Geohintonia mexicana	...-....................C..-TTTTT.......C....
Glandulicactus crassipes	...-...........A......ATC.....----...........
Lophophora williamsii	.A.-...............N..........--T.......C....
Mammillaria haggeana	...A...................TC.....----......C...T
Obregonia denegrii	...-..........................TTT............
Sclerocactus spinosior	...-...................TCTTT...CCC...........
Stenocactus lloydii	...-..........................-----....A.....
Strombocactus disciformis	...-.........................----............
Pachycereus marginatus	...-..C.......G.........C.-------.......C....
Stenocereus dumortieri	...-....................C.-------............
Leptocereus quadricostatus	...-..C.......G.........C.-------.......C.C.
Opuntia phaeacantha	...-....................C---------..........A
Pereskia aculeata	...-..........C.........C-------T............

APPENDIX I. Continued.

TAXON	
[	1270 1280]
[	. .]
F. cylindraceus	TTAATTCTAACAAAAACCAACGA
F. echidne	....
F. emoryi v. *rectispinus*	C......CT..C......C....
F. flavovirens	.....................A.
F. fordii	....
F. glaucescens	..................-....
F. gracilis	C.....TCT..C......-.G..
F. hamatacanthus	......A................
F. histrix	....
F. latispinus	....
F. lindsayi	......A................
F. macrodiscus	....
F. peninsulae	C.....TCT..C......C.A..
F. pilosus	...T.C..........A.C....
F. pottsii v. *alamosanus*	.....C.C...........G.N.
F. rafaelensis	....
F. recurvus	....
F. reppenhagenii	C......CT..C......C.NN.
F. robustus	.......C-..............
F. stainesii	....
F. wislizeni v. *herrerae*	....
F. wislizeni v. *wislizeni*	....
F. viridescens	C......CC.A.....A.C.AC.
F. sp. nov. ?	....
Ancistrocactus uncinatus	.A.T...........C.....A.
Aztekium ritterii	...................G...
Coryphantha pallida	C......C..........-....
Echinocactus grusonii	....
E. horizonthalonius	....
Geohintonia mexicana	.A.T...C.......C...GA..
Glandulicactus crassipes	...T..TC...........GAAG
Lophophora williamsii	...-..T-...C.......NGA.
Mammillaria haggeana	......T...........TC...
Obregonia denegrii	..................-..A.
Sclerocactus spinosior	....
Stenocactus lloydii	....
Strombocactus disciformis	.A.T..T..............A.
Pachycereus marginatus	.....C...............A.
Stenocereus dumortieri	....
Leptocereus quadricostatus	.A...C...C.....-..C....
Opuntia phaeacantha	.....................A.
Pereskia aculeata	.....C.................

APPENDIX II. Data matrix for the intergenic spacer between the *trn*L-*trn*F genes

MISSING DATA = N; GAP = -

TAXON	
	[1 1 2 3 4]
	0 0 0 0]
	. . . . .
Echinocactus grusonii	CACTCaCGAACTAG-TTATCCTTG-TTTTATTAACAGTTTG
Escontria chiotilla	CACTCCCTAACTAG-TTATCCTCT-TTTTATTAACAGTTTG
Ferocactus cylindraceus	CACTCGCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. pottsii var. *alamosanus*	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. echidne	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. flavovirens	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTCG
F. fordii	CACTC-CGAACTAG-TTATCCTTTATTTTATTAACAGTTTG
F. glaucescens	CACTCACGAACTAG-TTATACTTT-TTTTATTAACAGTTTG
F. gracilis	CACTC-CGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. hamatacanthus	CACTC-CGAACTAG-TTATCCTTTATTTTATTAACAGTTTG
F. wislizeni var. *herrerae*	CACTCCCGAACTAG-TTATCCTTA-TTCTATTAACAGTTTG
F. histrix	CAATCaCGGCCTAAGTTATCCTTT-TTTTATTAACAGTTTG
F. latispinus	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. lindsayi	CACTCCCGAACTAG-TTATCTTTT-TTTTATTAACAGTTTG
F. macrodiscus	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTAG
F. peninsulae	CACTC-CGAACTAG-TTATCCTTTATCTTATTAACAGTTTG
F. pilosus	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. rafaelensis	CACTCCCGAACTCG-TTATCCTTT-TTTTATTAACAGTTTG
F. rectispinus	CACTC-CGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. recurvus	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. robustus	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
F. wislizeni	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
Leptocereus quadricostatus	CACTCCCTAACTAG-TTATCCTCT-TTTTATTAACAGTTTG
Mammillaria voburnensis	CACTCCCGAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
Opuntia phaeacantha	CACTCCCTAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
Pachycereus marginatus	CACTCCCTAACTAG-TTATCCTCT-TTTTATTAACAGTTTG
Pereskia grandifolia	CACTCCCTAACTAG-TTATCCTTT-TTTTATTAACAGTTTG
Sclerocactus spinosior	CACTC-CGAACTAG-TTATCCTTTATTTTATTAAGAGTTTG
Stenocactus albatus	CACTC-CGAACTAG-TTATCCTTTATTTTATTAACAGTTTG
Stenocereus griseus	CACTCCCTAACTAG-TTATCCTCT-TTTTATTAACAGTTTG

APPENDIX II. Continued.

```
TAXON

                           4       5         6         7         8         9         1 ]
                           2       0         0         0         0         0         0 ]
                                                                                     0 ]
                           .       .         .         .         .         .         .
E. grusonii                ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
E. chiotilla               ----AAGGTGGTTATGTTCCTTATTTTTTTT-GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. cylindraceus            ----AAGGTGGTTATGTTCCTCATTTTTTTT--GTTTTCAATTTCAAAAAAGGCTAAAGG
F. pottsii var. alamosanus ----AaGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. echidne                 ----A-GGTGGTTATGTTCCTCATTTTTTTT--GTTTTCAATTTCAAAAA-GGCTAAAGG
F. flavovirens             ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. fordii                  ---CACGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. glaucescens             ----AAGGTGGTTATGTTCCTCATTTTTTTT-GGTTTCCAATTTCAAAAA-GGCTAAAGG
F. gracilis                ---CAAGGTGGTTATGTTCCTCATTTTTTTT--GTTTTCAATTTCAAAAAAGGCTAAAGG
F. hamatacanthus           ----AAGGTGGTTATGTTCCTCATTTTTTTTTGGTTTCCAATTTCAAAAA-GGTTAAAGG
F. wislizeni var. herrerae ----AaGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAGGG
F. histrix                 ----AAGGTGGTTATGTTCCTCATTTTTTTT-GGTTTCCAATTTCAAAAA-GGCTAAAGG
F. latispinus              ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. lindsayi                ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. macrodiscus             TTTGAAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. peninsulae              ----ACGGTGGTTATGTTCCTCATTTTTTTT--GTTTTCAATTTCAAAAAAGGCTAAAGG
F. pilosus                 ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. rafaelensis             ----AAGGTGGTTATGTTCCTCATTTTTTTT--GTTTTCAATTTCAAAAA-GGCTAAAGG
F. rectispinus             ----AAGGTGGTTATGTTCCTCATTTTTTTT--GTTTTCAATTTCAAAAAAGGCTAAAGG
F. recurvus                ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. robustus                ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
F. wislizeni               ----AAGGTGGTTATGTTCCTCATTTTTTTTT-GTTTTCAATTTCAAAAAAGGCTAAAGG
L. quadricostatuS          ----AAGGTGGTTATGTTCCTTATTTTTTTT-GGTTTTCAATTTCAAAAA-GCCTAAAGG
M. voburnensis             -----AG-------TGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
O. phaeacantha             ----AAGGTGGCTATGCTCCTCATTTTTT---GGTTTTCAATTTCAAAAG-GGCTAAAGG
P. marginatus              ----AAGGTGGTTATGTTCCTTATTTTTTTT-GGTTTTCAATTTCAAAAA-GGCTAAAGG
P. grandifolia             ----AAGGTGGTTATGTTCCTCATTTTTTT---GTTTTCAATTTCAAAAA-GGCTAAAGG
S. spinosior               ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTCAAAAA-GGCTAAAGG
S. albatus                 ----AAGGTGGTTATGTTCCTCATTTTTTT--GGTTTTCAATTTAAAAAA-GGCTAAAGG
S. griseus                 ----AAGGTGGTTATGTTCCTTATTTTTTTT-GGTTTTCAATTTCAAAAA-GGCTAAAGG
```

APPENDIX II. Continued.

TAXON	
	1 1 1 1 1 1 1]
	0 1 2 3 4 5 5]
	2 0 0 0 0 0 8]
	. . . .
E. grusonii	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
E. chiotilla	CTCCGGACGG-AAAT----TTCCCTTATAAAAAGTCTTGTGAT---ATACGTAAAAA
F. cylindraceus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. pottsii var. *alamosanus*	CTCCGGACGGCAAATGCTTAACCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. echidne	CTCCGGACGGCAAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. flavovirens	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. fordii	CTCCGGACGG-AAATGCTTACCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. glaucescens	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGGT---ATACGTAAAAA
F. gracilis	CTCCGGACGGTAAATGCTATTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. hamatacanthus	TTCCGGACGG-AAATGCTTTCCCCTTATCAAAAGTCTTGTGAT---TTACGTAAAAT
F. wislizeni var. *herrerae*	CTCCGGACGG-AAATGCTTATCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. histrix	TTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. latispinus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGATATAATACGTAAAAA
F. lindsayi	CTCCGGACGG-AAATGCTTTTCCCCTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. macrodiscus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. peninsulae	CTCCGGACGG-AAATGCTATTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. pilosus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. rafaelensis	CTCCGGACGG-AAATGCTTAACCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. rectispinus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. recurvus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGATATAATACGTAAAAA
F. robustus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
F. wislizeni	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
L. quadricostatus	CTCCGGACGG-AAATGCTTTTCCCTTATAAAAAGTCTTGTCAT---ATATGTAAAAA
M. voburnensis	CTCCGG----------CTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
O. phaeacantha	CTCCGGACGG-AAATGCTTTTCCCCTATCACAAGTCTTGTGAT---ATACGTACAAA
P. marginatus	CTCCGGACGG-AAATGCTTTTCCCTTATAAAAAGTCTTGTGAT---ATACGTAAAAA
P. grandifolia	CTCCGGACGG-AAATGCTTTTCCCTTATCACAAGTCTTGTGAT---ATACGTACAAA
S. spinosior	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
S. albatus	CTCCGGACGG-AAATGCTTTTCCCTTATCAAAAGTCTTGTGAT---ATACGTAAAAA
S. griseus	CTCCGGACGG-AAAT----TTCCCTTATAAAAAGTCTTGTGAT---ATACGTAAAAA

APPENDIX II. Continued.

TAXON	
	1 1 1 1 2 2 2]
	6 7 8 9 0 1 1]
	0 0 0 0 0 0 8]
	. . . .
E. grusonii	TGAATATCTTTGAGCAAGG-AATAATCTTTGG---AGTGATTCACAATCAATATCATTAC
E. chiotilla	TGAATATCTTGGAGCAAGG-AATAATCATTTG---AGTGATTCACAATCAATATCATTAC
F. cylindraceus	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
F. pottsii var.*alamosanus*	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
F. echidne	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
F. flavovirens	TGAATATCTTTGAGCAAGG-AATAATCTTTTG---AGTGATTCACAATCAATATCATTAC
F. fordii	TGAATATCTTTGAGCAAGG-AATAATCCCTTG---AGTGATTCACAATCAATATCATTAC
F. glaucescens	TGAATATCTTGGAGCAAGG-AATAATCTTTGG---AGTGATTCACAATCAATATCATTAC
F. gracilis	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTTATTCACAATCAATATCATTAC
F. hamatacanthus	TGATTTTCTTGGAGCAAGG-ATTAATCCTTGG---AGTGATTCACAATCAATTTCATTAC
F. wislizeni var.*herrerae*	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
F. histrix	TGAATATCTTGGAGCAAGG-AATAATCTTTGG---AGTGATTCACAATCAATATCATTAC
F. latispinus	TGAATATCTTGGAGCAAGG-AATAATCTTTGG---AGTGATTCACAATCAATATCATTAC
F. lindsayi	TGAATATCTTGGAGCAAGG-AATAATCTTTGG---AGTGATTCACAATCAATATCATTAC
F. macrodiscus	TGAATATCTTTGAGCAAGG-AATAATCTTTTG---AGTGATTCACAATCAATATCATTAC
F. peninsulae	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTTATTCACAATCAATATCATTAC
F. pilosus	TAAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
F. rafaelensis	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
F. rectispinus	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTTATTCACAATCAATATCATTAC
F. recurvus	TGAATATCTTTGAGCAAGG-AATAATCTTTTG---AGTGATTCACAATCAATATCATTAC
F. robustus	TGAATATCTTTGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
F. wislizeni	TGAATATCTTGGAGCAAGG-AATAATCCTTTG---AGTGATTCACAATCAATATCATTAC
L. quadricostatus	TGAATATCTTGGAGCAAGG-AAGAATCATTTG---AGTGATTCACAATCAATATCATTAC
M. voburnensis	TGAATATCTTTGAGCAAGA-AATAATCTTTTG---AGTGATTCACAATCAATATCATTAC
O. phaeacantha	TGAATATCTTTGAGCAAGG-AATAATCATTTG---AGTGATTCACAATCAATATCATTAC
P. marginatus	TGAATATCTTGGAGCAAGG-AATAATCATTTG---AGTGATTCACAATCAATATCATTAC
P. grandifolia	TGAATATCTTTGAGCAAGG-AATAATCATTTGATGAGTGATTCACAATCAATATCATTAC
S. spinosior	TGAATATCTTTTAGCAAGG-AATAATCTTTTG---AGTGATTCACAATCAATATAATTAA
S. albatus	TGAATATCTTTGAGCAAGG-AATAATCTTTTG---AGTGATTCACAATCAATATCATTAC
S. griseus	TGAATATCTTGGAGCAAGG-AATAATCATTTG---AGTGATTCACAATCAATATCATTAC

APPENDIX II. Continued.

TAXON	
	2 2 2 2 2 2 2]
	2 3 4 5 6 7 7]
	0 0 0 0 0 0 5]
	. . . . . .
E. grusonii	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
E. chiotilla	ACGTACTAAAACTTAAATAAACTTAGAG---------ACAAAGTCCTCCTTTTGGAA
F. cylindraceus	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. pottsii var. *alamosanus*	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. echidne	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTTGAA
F. flavovirens	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. fordii	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. glaucescens	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTCCTTTTGGAA
F. gracilis	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. hamatacanthus	ACGTCCTAAAACTTAATTAAACTTAGAGACAAAGGAGACAAAGTCCTCCTTTTGGAA
F. wislizeni var. *herrerae*	ACGTACTAAAACTAAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. histrix	ACGTACTAAAACTTAATTAAACTTAGAGACAAAGGAGACAAAGTCCTCCTTTTGGAA
F. latispinus	ACGTACTAAAACTTAAATAAACTTAGAG---------ACAAAGCACTTCTTTTGGAA
F. lindsayi	ACGTACTAAAACTTAAATAAACTTAGAG---------GCAAAGTCCTTCTTTTGGAA
F. macrodiscus	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGCCCTTCTTTTGGAA
F. peninsulae	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. pilosus	ACGTACTAAAACTAAAATAAACTTAGATACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. rafaelensis	ACGTACTAAAACTTAATTAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. rectispinus	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. recurvus	ACGTACTAAAACTTAAATAAACTTAGAG---------ACAAAGCACTTCTTTTGGAA
F. robustus	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
F. wislizeni	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTCCTTTTGGAA
L. quadricostatus	ACGTACTAAAACTTAAATAAACTTGGAG---------ACAAAGTCCTCCTTTTGGAA
M. voburnensis	ACGTACTAAAACTTAAATAAACTTAGAGACAAGGGAGACAAAGTCCTTCTTTTGGAA
O. phaeacantha	GCGTACTAAACCTTAAATAAACTTATAGACAAAGGAAACAAAGTCCTTCTTTTGGAA
P. marginatus	ACGTACTAAAACTTAAATAAACTTAGAG---------ACAAAGTCCTCCTTTTGGAA
P. grandifolia	GCGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTGGAA
S. spinosior	ACGTACTAAAACTTAAATAAACTTAGAGACAAAGGAGACAAAGTCCTTCTTTTTGAA
S. albatus	ACGTACTAAAACTTAAATAAACTTAGAG---------ACAAAGTCCTTCTTTTGGAA
S. griseus	ACGTACTAAAACTTAAATAAACTTAGAG---------ACAAAGTCCTCCTTTTGGAA

APPENDIX II. Continued.

TAXON	
	2 2 2 3 3 3 3]
	7 8 9 0 1 2 3]
	6 0 0 0 0 0 0]
	. . . . . .
E. grusonii	GACCAAAGAATTTGCGGTACCTAGATAAGACTTTGTTATACTTTTCGcCCTTTTA
E. chiotilla	GACCAAAGAAATTGCGGTACCTAGATAAGACTTGGTTATACTTTCCGTCCTTTTA
F. cylindraceus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACCTTTCGTCCTTTTA
F. pottsii var. *alamosanus*	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACCTTTCGTCCTTTTA
F. echidne	GACCAAAGAAATTGCGGTACCTAGATCAGACTTTGTTATACCTTTCGTCCTTTTA
F. flavovirens	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACTTTTCGTCCTTTTA
F. fordii	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACCTTTCGTCCTTTTA
F. glaucescens	GACCAAAGAATTTGCGGTACCTAGATAAGACTT-GTTATACTTTCCGCCCTTTTA
F. gracilis	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACCTTTCGTCCTTTTA
F. hamatacanthus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTGGTTATACCTTTCGTCCTTTTA
F. wislizeni var. *herrerae*	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACCTTTCGTCCTTTTA
F. histrix	GACCAAAGAATTTGCGGTACCTAGATAAGACTTGGTTATACTTTCCGCCCTTTTA
F. latispinus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACTTTTCGTCCTTTTA
F. lindsayi	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACTTTCCGTCCTTTTA
F. macrodiscus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACTTTTCGTCCTTTTA
F. peninsulae	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACCTTTCGTCCTTTTA
F. pilosus	GACCAAAGAAATTGCGGTACCTAGATCAGACTTTGTTATACCTTTCGTCCTTTTA
F. rafaelensis	GACCAAAGAAATTGCGGTACCTAGATCAGACTTTGTTATACCTTTCGTCCTTTTA
F. rectispinus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACCTTTCGTCCTTTTA
F. recurvus	GACTAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACTTTTCGTCCTTTTA
F. robustus	GACCAAAGAAATTGCGGTACCTAGATCAGACTTTGTTATACCTTTCGTCCTTTTA
F. wislizeni	GACCAAAGAAATTGCGGTACCTAGATAAGACTTGGTTATACCTTTCGTCCTTTTA
L. quadricostatus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTGGTTATACTTTTCGTCCTTTTA
M. voburnensis	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTAGACTTTTCGTCCTTTTA
O. phaeacantha	GACCAAAGAAATTAAGGCACCTAGATAAGCCTTTGTAAGACCTTTCGTCCTTTTA
P. marginatus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTGGTTATACTTTCCGTCCTTTTA
P. grandifolia	GACCAAAGAAATTACGGTACCTAGATAAGACTTTGTAATACTTTTCGTCCTTTTA
S. spinosior	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGGTATACTTTTCGTCCTTTTA
S. albatus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTTGTTATACTTTTCGTCCTTTTA
S. griseus	GACCAAAGAAATTGCGGTACCTAGATAAGACTTGGTTATACTTTCCGTCCTTTTA

APPENDIX II. Continued.

TAXON	
	3 3 3 3 3 3 3] 3 4 5 6 7 8 8] 1 0 0 0 0 0 6] . . . . .
E. grusonii	ATTGACATAGACCcGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
E. chiotilla	ATTGACATAGACC-GAGTTCTCCATTAAAATGAGTAGATGATGCCCCAGAAGGGGG
F. cylindraceus	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. pottsii var. *alamosanus*	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. echidne	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. flavovirens	ATTGACATAGACCCGAGTTCTCTATAAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. fordii	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. glaucescens	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGACGCGCCAGAGGGGGG
F. gracilis	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. hamatacanthus	ATTGACATAGACC-GAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. wislizeni var. *herrerae*	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. histrix	ATTGACATAGACCCGAGTCCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. latispinus	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. lindsayi	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. macrodiscus	ATTGACATAGACCCGAGTCCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. peninsulae	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. pilosus	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. rafaelensis	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. rectispinus	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. recurvus	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. robustus	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
F. wislizeni	ATTGACATAGACCCGAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
L. quadricostatus	ATTGACATAGACC-GAGTTCTCCATTAAAATGAGTAGATGATGCCCCAGAAGGGGC
M. voburnensis	ATTGACATAGACC-GAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
O. phaeacantha	ATTGACATAGACC-GAGTTCTCTATTAAAATGAGTAGATGTTGCGCCAGAAGGGGG
P. marginatus	ATTGACAT---------TTCTCCATTAAAATGAGTAGATGATGCCCCAGAAGGGGG
P. grandifolia	ATTGACATAGACC-GAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
S. spinosior	ATTGACATAGACC-GAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
S. albatus	ATTGACATAGACC-GAGTTCTCTATTAAAATGAGTAGATGATGCGCCAGAAGGGGG
S. griseus	ATTGACATAGACC-GAGTTCTCCATTAAAATGAGTAGATGATGCCCCAGAAGGGGG

APPENDIX II. Continued.

TAXON	
	3 4 4 4 4 4] 9 0 1 2 3 3] 0 0 0 0 0 5] . . . . . .
E. grusonii	AATGCCCAGG---ATAGCTCAGCTGGTAGAGCAaGAGGACTGAAAATCC
E. chiotilla	AATGGTCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. cylindraceus	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAA-TCC
F. pottsii var. *alamosanus*	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. echidne	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. flavovirens	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-G-GG-CTGATCG-CC
F. fordii	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. glaucescens	AATGCCCAGTT--ATNGCTCAGTTGG-GGAGAGAAGGGGTTTTN---CC
F. gracilis	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. hamatacanthus	AATGGCCGG----ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. wislizeni var. *herrerae*	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. histrix	AATGCCCGGG---ATAGCTCAGCTGGTAGAGAAAGAGGACTGAAAATTC
F. latispinus	AATGGCCGGT---ATAGCTCAGGGGGTAGAGCA-GAGGACTGAATTCCC
F. lindsayi	AATGGCCGGG---ATAGCTCAGCCGGTAGAGCA-GAGGACTGAAAATCC
F. macrodiscus	AATGGCCGGT---ATAGCTCAGGTGGTAGAGCA-GAGGACTGAACCTCC
F. peninsulae	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. pilosus	AATGGCCGG----ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAA-TCC
F. rafaelensis	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. rectispinus	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. recurvus	AATGGCCGGG---ATAGCTCAGGGGGTAGAGCA-GAGGACTGAAAATCC
F. robustus	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
F. wislizeni	AATGGCCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGA---TCC
L. quadricostatus	AATGGTCGGG---ATAGCTCAGCAGGTAGAGCA-GAGGACTGAAA-TCC
M. voburnensis	AATGGCGGGCGGGATAGCTCAGCAGGTAGAGCA-GAGGACTGAAAATCC
O. phaeacantha	AATGGTCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAA-TCC
P. marginatus	AATGGTCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
P. grandifolia	AATGGTCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAA-TCC
S. spinosior	AATGG-CAGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAAAATCC
S. albatus	AATGG-CGGG---ATAGCTCAGCTGG-AGAGCA-GAGGACTGAAAATCC
S. griseus	AATGGTCGGG---ATAGCTCAGCTGGTAGAGCA-GAGGACTGAA--TCC

CHAPTER SIX

GENERAL CONCLUSIONS

The different approaches in which the research project of this dissertation was conducted provided significant results about the evolutionary history of *Ferocactus*, and they are presented as cytological, phylogenetic and taxonomic conclusions.

Cytological Conclusions

The data from the cytological analyses confirmed the presence of a basic chromosome number ($x = 11$) for *Ferocactus*, and chromosome numbers for seven species were reported for the first time. In addition, the results do not support the issue of hybridization at least for the taxa and populations examined. Nonetheless, hybridization is not ruled out until further cytological analyses are performed in particular in conflictive populations of some Baja Californian species for which cases of morphological intermediacy are relatively common. It appears, therefore, that chromosome evolution in the genus is taking place at the molecular level as evidenced by the homogeneity of chromosomes and the lost of the *rpo*C1 intron of the chloroplast genome in the subfamily Cactoideae, thus chromosome rearrangements remain cryptic.

Phylogenetic Conclusions

The molecular data from the restriction site analysis and the comparative sequence analyses of DNA from non-coding regions failed to support monophyly for *Ferocactus*. Instead, independent analyses of data sets have rendered *Ferocactus* as a paraphyletic assemblage, with *Echinocactus grusonii* sister to *F. histrix* and *F. glaucescens*. This clade was consistently found in the three molecular phylogenies further suggesting the likely origin of *Ferocactus* from an *Echinocactus*-like ancestor.

It remains unclear whether the two putatively "transitional lineages" (*F. flavovirens* and *F. robustus*) as proposed by Taylor (1984) represent the basal species within the genus. These two species appear in different subclades probably indicating evolutionary independence and different patterns of radiation. Also, the different placement of these taxa in the molecular phylogenies might suggests that other biological processes such as hybridization and lineage sorting are playing a major role in the evolution *Ferocactus*. A comparative nuclear phylogeny would certainly provide insight into the processes of evolution in the genus. The limited phylogenetic resolution at the base of the nodes in the *rpl*16 and intergenic spacer of the *trn*L-*trn*F genes is probably due to a rapid radiation event which was accompanied by major morphological changes but involved little genetic divergence, which translated in a few molecular synapomorphies to define major lineages. It may be the case in which many mutations characterize single species, contributing to long branch attraction.

Similarly, the phylogenetically basal position in which *Ferocactus* has been placed within the tribe Cacteae by Buxbaum (1958) and Barthlott and Hunt (1993) is not supported by the molecular phylogenies. The relative small generic taxonomic sampling within the tribe Cacteae has limited the phylogenetic resolution in this study.

The hypothesis about the phylogenetic relationship of the North American columnar cactus *Escontria chiotilla* (tribe Pachycereeae) and *F. flavovirens* based superficially on the presence of chartaceous scales which accumulate calcium oxalate, was not supported by any of the molecular phylogenies. The lack of evidence to support this relationship is another example of parallelism in the family, in which similar structures which have evolved in distantly related lineages may be misleading morphological characters to assess degree of phylogenetic relationship.

For the North American columnar cacti investigated, the study provided evidence of monophyly for the tribe Pachycereeae and its two subtribes (Pachycereinae and Stenocereinae) as suggested by Gibson (1986). Also, the presence of unique restriction site changes in *Stenocereus dumortieri* place it basal to the rest of the Stenocereinae suggesting that this lineage evolved separately early in the divergence of the Stenocereinae.

Taxonomic Conclusions

Several studies have demonstrated that the sole use of morphological characters in the taxonomy of the Cactaceae is constrained by cases of parallelism (see above), and makes the classification more difficult. My study, as indicated above, is not an exception to this rule. Ideally, we should combine data sets to infer phylogeny, particularly when morphological characters do not provide sufficient taxonomic resolution. In this study, molecular phylogenies have proved to be effective in targeting some conflictive species (e.g., relationship between *Echinocactus* and *F. histrix* and *F. glaucescens*, and taxonomic transfer of *S. dumortieri*) by enhancing the divergence or degree of similarity, resolution which morphological traits, in some cases, are less likely to provide.

The issue of paraphyly as indicated by the close phylogenetic relationship between *Ferocactus* and *Echinocactus* has certainly added the consideration of future taxonomic work for *Ferocactus*. Although additional taxonomic sampling is necessary from within *Echinocactus*, in particular the inclusion of the type species *E. platyacanthus*, to verify the findings of these molecular studies, the preliminary evidence indicates that some taxonomic changes such and further reevaluation of the taxonomic circumscriptions might be needed if subsequent studies confirm the paraphyletic origin of *Ferocactus*. Thus, if the type species *E. platyacanthus* appears within the same clade, i.e., together with *F. histrix* and *F. glaucescens*, the best way to get around the taxonomic changes will be to segregate both *F. histrix* and *F. glaucescens* from *Ferocactus*. By doing this, both generic names, *Echinocactus* and

Ferocactus are maintained and at the same time, this type of taxonomic reevaluation would minimize the number of taxonomic transfers.

The taxonomic transfer of *Stenocereus dumortieri* to *Isolatocereus dumortieri* is guaranteed. Morphologically, this species has retained also plesiomorphic features of stem, flower and fruit, in addition to the presence an unique triterpene, and unique restriction site changes, set of characters that support the resurrection of former generic name for which it was first described: *Isolatocereus dumortieri*. Because these taxonomic implications appeared as the data was being analyzed, the paper dealing with nomenclature will be published separately.

Overall, my study has answered most of the questions for which the project was originally designed, and most importantly, the pieces of this evolutionary puzzle are starting to come together in order to reconstruct the phylogeny and evolutionary history of *Ferocactus*. It is evident that increasing terminal taxa more likely clarify those aspects which remained unresolved, and that future studies are needed to fully understand *Ferocactus*, which I consider as a controversial taxonomic complex and evolutionary unit.

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BIOGRAPHICAL SKETCH

J. Hugo Cota was born in the west coast of Mexico in the city of Los Mochis, state of Sinaloa, May 19 of 1956. He earned his Bachelor degree in Biology at the Escuela Nacional de Ciencias Biológicas (ENCB) in 1984 and then worked for the Mexican Government inventorying classifying plant communities. From 1986-1989 he worked for ENCB herbarium and taught plant taxonomy at the same institution. Hugo earned his Master's degree at The Claremont Graduate School/Rancho Santa Ana Botanic Garden, Claremont, California, in 1991 studying chromosome evolution in genus *Echinocereus*, a North American columnar cacti. His interest in cacti dates from his childhood and over the years he has investigated the biology, taxonomy and evolution of several cacti of North American.

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