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Systematics of Harrisia (Cactaceae)

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Systematics of *Harrisia* (Cactaceae)

by

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DEDICATION

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ABSTRACT

The genus *Harrisia* Britton (Cactaceae) comprises species of columnar cacti that are united by a unique seed morphology. The species range in form from prostrate shrubs to large trees and are native to South America and the Caribbean region. *Harrisia* is placed in an unresolved position within subtribe Trichocereinae of tribe Cereeae of subfamily Cactoideae. Relationships among the species within *Harrisia* are also poorly understood. In this study, several species of *Harrisia* were sequenced for as many as seven different regions of nuclear and plastid DNA. Species in the Caribbean were also examined with amplified fragment length polymorphisms. The morphology of *Harrisia* was characterized from herbarium specimens, live plants, and original descriptions. A biogeographic scenario was extrapolated from the molecular and morphological data. The flower morphology suggests a relationship between *Harrisia* and some species of *Echinopsis* s. l. However, DNA sequence analyses in this study do not clearly resolve generic relationships with *Harrisia*. Molecular and morphological data support recognition of two subgenera, four sections, and two series within *Harrisia*. It is proposed that *Harrisia* originated in the west-central Andes, ~3.5–6.5 Ma ago. Subgenus *Eriocereus* is composed of the species in the east Andes of Bolivia and the nearby species radiation in the Gran Chaco. Subgenus *Harrisia* originated by an early dispersal event into Brazil with subsequent dispersal into the Caribbean. In the last 500 Ka, *Harrisia*

colonized west Cuba and further diversified into other areas of the Caribbean. *Harrisia* is revised to contain 18 species.

PREFACE

Cactaceae comprises ca. 1800 species nearly endemic to the Americas, with only a few taxa of *Rhipsalis* Gaertn. native to the Old World tropics. In the Americas, the distribution of the family extends from southern Canada to southern Argentina and southern Chile. There are three main centers of diversity for cacti: the central Andes, Mexico and the southwest U.S.A., and eastern Brazil. The ca. 1780 species of core cacti (including all cacti except the basal leafy genus *Pereskia* Mill.) originated in the central Andes (Wallace & Gibson 2002; Edwards et al. 2005; Griffith & Porter 2009).

The delimitation of the Cactaceae has remained quite stable through time by the inclusion of taxa with areoles. Areoles are modified short-shoots which usually contain a cluster of spines or bristles. Intrafamilial classifications, however, have been revised several times by many researchers (for a review see Metzing & Kiesling 2008) such as de Candolle (1829), Labouret (1853), Schumann (1899), Berger (1905, 1929), Buxbaum (1958), Backeberg (1960, 1977), Barthlott (1988), Anderson (2001), Hunt et al. (2006), and Nyffeler & Eggli (2010). Recent revisions within the family have largely been the result of phylogenetic analyses of DNA sequences which have uncovered several examples of morphological homoplasy and non-monophyly. Many genera are in need of revisionary work to re-establish generic limits and clarify species delimitation.

The core cacti appear to be monophyletic (Nyffeler 2002; Edwards et al. 2005; Bárcenas et al. 2011) and all have succulent, photosynthetic stems and reduced leaves. The subfamily Cactoideae is monophyletic (Nyffeler 2002; Crozier 2005; Edwards et al. 2005; Korotkova et al. 2010; Bárcenas et al. 2011; Hernández-Hernández et al. 2011) and contains ca. 1500 species (Nyffeler & Eggli 2010) of columnar, globose, or vine-like cacti that have a vascularized pith and cortex as well as microscopic leaves (Mauseth 2007).

The monophyletic core Cactoideae (Nyffeler 2002; Crozier 2005; Korotkova et al. 2010; Bárcenas et al. 2011; Hernández-Hernández et al. 2011) comprises at least five tribes, all of which have flowers arising from the stem areoles. Tribe Cereeae (sensu Nyffeler & Eggli 2010) is a monophyletic group (Nyffeler 2002; Crozier 2005; Ritz et al. 2007; Korotkova et al. 2010; Bárcenas et al. 2011; Hernández-Hernández et al. 2011) mostly confined to South America. Within tribe Cereeae is subtribe Trichocereinae, containing ~250 species in 20 genera (Nyffeler & Eggli 2010). This subtribe has been revised by molecular work (Wallace 1995; Applequist & Wallace 2002; Nyffeler 2002; Crozier 2005; Ritz et al. 2007; Korotkova et al. 2010; Hernández-Hernández et al. 2011) to comprise taxa that have flowers with acuminate bracts, pilose areoles (Ritz et al. 2007), and an areolate pericarpel. The genus *Harrisia* Britton was only placed in the subtribe recently based on DNA evidence (Wallace 1995; Applequist & Wallace 2002).

The genus *Harrisia* comprises ~20 species (Nyffeler & Eggli 2010) ranging from south-central South America to northeast Brazil and into the Caribbean. Nearly all the species are narrow endemics and approximately 7,000 km separates the two most distant species. Seed morphology has been used to circumscribe the genus. Very few species of

Harrisia have been comparatively studied to determine evolutionary relationships through morphological and molecular analyses.

This study will use variation in DNA sequences to infer a phylogeny of *Harrisia*. Characterization of the morphology will help to interpret the taxonomy of the clades obtained in the phylogenies. The morphological and molecular analyses will clarify species relationships resulting in a biogeographic hypothesis concerning the evolution of the genus through time. Additionally, the analyses will help to reveal issues in need of further study.

MAIN OBJECTIVES

There were seven main objectives in this study of *Harrisia*:

1. Develop primers for variable nuclear and plastid regions for phylogenetic inference in *Harrisia* (Chapter 1).
2. Investigate relationships of *Harrisia* within the Cactaceae, subtribe Trichocereinae (Chapter 2).
3. Confirm the monophyly of *Harrisia* (Chapter 2).
4. Establish relationships among the four geographic groups of *Harrisia* (Bolivian Andes, Gran Chaco, northeast Brazil, and Caribbean) (Chapter 2).
5. Reveal relationships among the closely related species of the Caribbean (Chapter 3).
6. Specify a biogeographic scenario for the diversification of *Harrisia* (Chapters 2–3).
7. Monograph the genus with detailed morphological descriptions and a key to all taxa (Chapter 4).

Molecular phylogenies are extremely helpful in understanding evolutionary relationships within Cactaceae, although many studies rely on plastid sequences because of their convenience and simplicity. Plastid DNA represents only one chromosome that is predominantly maternally inherited in angiosperms. Plastid phylogenies are often incongruent with nuclear phylogenies and morphology, thus phylogenies solely composed of plastid sequences may not be reliable to infer relationships. Additionally, incongruent plastid phylogenies often follow geographic patterns (e.g. Rieseberg et al. 1996; McKinnon et al. 1999; Cristine Acosta & Premoli 2010; Wang et al. 2011; Escobar García et al. 2012; Jabaily et al. 2012). Several mechanisms have been proposed to explain anomalous plastid haplotypes (Azhagiri and Maliga 2007; Stegemann et al. 2012), including hybridization (Rieseberg & Soltis 1991; Tsitrone et al. 2003), which is common in Cactaceae (Friedrich 1974; Machado 2008). However, very few nuclear primers that amplify adequately variable regions are available in Cactaceae to reconstruct evolutionary relationships. To provide alternative DNA regions for phylogenetic analyses in Cactaceae, primers are here developed for low-copy nuclear regions spanning introns as well as for the highly variable *ycf1* plastid gene (Chapter 1).

Although molecular and morphological data have helped to define the subtribe Trichocereinae to include *Harrisia*, generic relationships are largely unknown. Flower morphology suggests that *Harrisia* is most closely related to some species of *Echinopsis* s. l. A previous study using the *rpl16* intron alleged that *Samaipaticereus* was sister to *Harrisia* (Wallace 1997b), though recent phylogenies do not indicate this. In Chapter 2, the generic level relationships of *Harrisia* were investigated by sequencing several

species within subtribe Trichocereinae for two low-copy nuclear regions and two plastid regions.

There is currently no detailed phylogeny for the species relationships within *Harrisia*. Previous molecular inquiries using the *rpl16* intron have been conducted on the genus to purport its monophyly and comment on infrageneric relationships (Wallace 1997a, 1997b). As several details from Wallace (1997a, 1997b) were not divulged, the genus is still in need of phylogenetic study. Traditionally, all the South American species have been recognized as subg. *Eriocereus* due to their splitting fruits. Twelve species of *Harrisia* from across its distributional range were included in the phylogenetic analyses to test the monophyly of the genus and elucidate infrageneric relationships (Chapter 2). From the molecular data, a biogeographic hypothesis and infrageneric classification is formulated for the genus (Chapter 2).

In the Caribbean region, at least 15 taxa have been proposed for *Harrisia*. Due to their morphological similarity and the geologic complexity of the Caribbean, species relationships and dispersal patterns are unknown. In Chapter 3, the species of *Harrisia* in the Caribbean region are investigated utilizing amplified fragment length polymorphisms and sequences from seven DNA regions. The evolution of the genus in the Caribbean is discussed. The phylogenetic results will also help to guide a taxonomic revision of *Harrisia* in the Caribbean.

The five species from the Gran Chaco area of South America and the one species from northeast Brazil have received considerable taxonomic effort. However, the one species endemic to the inter-Andean valleys of Bolivia and the species complex in the Caribbean have not received modern taxonomic study. The monograph of Britton & Rose

(1920) remains the only detailed revision of the genus in the Caribbean. Recent treatments have suggested treating all species in the Caribbean as one species (Hunt et al. 2006). Chapter 4 provides a detailed synthesis of the present knowledge of all the species of *Harrisia*. The morphology of each species is described and an identification key is provided. One new species is described from the Cayman Islands. Types are designated to stabilize nomenclature and synonyms listed for several species.

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CHAPTER ONE: LOW-COPY NUCLEAR PRIMERS AND *YCF1* PRIMERS IN CACTACEAE

INTRODUCTION

Note to Reader. Portions of this work have been accepted for publication (Franck et al. 2012) and are utilized with permission from the publisher.

For many plant groups, relatively few primers are available to amplify variable sequences for phylogenetic analysis. The plastid *ycf1* gene has been shown to be much more variable than other commonly used plastid markers in phylogenetic studies but primers are only available for certain groups (Gernandt et al. 2009; Neubig et al. 2009; Neubig & Abbott 2010). Low-copy nuclear genes also offer advantages over common plastid markers in that they are biparentally inherited and can occur on different chromosomes.

In an effort to increase the number of variable regions available for phylogenetic study in the Cactaceae, primer sets were designed to amplify portions of the nuclear genes *isi1* and *nhx1* and the plastid gene *ycf1*. The *isi1* gene encodes for the impaired sucrose induction protein (Rook et al. 2006). The *nhx1* gene encodes a Na^+/H^+ vacuolar antiporter (Gaxiola et al. 1999). The plastid *ycf1* gene encodes for a protein of unknown function that has been demonstrated to be essential for survival (Drescher et al. 2000).

The primers were tested for amplification on several families in the Caryophyllales (Table 1.1): Cactaceae (19 spp.), Portulacaceae (1 sp.), Didiereaceae (1 sp.), and Amaranthaceae (1 sp.). To assess sequence variation, sequences were obtained for regions of the *isi1*, *nhx1*, and *ycf1* genes from six species of *Harrisia* Britton. Sequence variation was compared to two other plastid regions, the *atpB-rbcL* intergenic spacer (IGS) and *rpl16* intron.

METHODS & RESULTS

Plant tissues (Table 1.1) were dried with silica gel at -20°C and stored at -20°C until further use. DNA was extracted with a modified CTAB protocol (Doyle and Doyle 1987). 10 mg of tissue were incubated at 55°C for 3 hr in 500 µL CTAB buffer (2% CTAB, 4% PVP-40,000, 100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 0.5 µL 2-mercaptoethanol, 25 µg proteinase-K, 10 µg Rnase A, pH 8.0). Samples were extracted twice with 24:1 chloroform:isoamyl alcohol. DNA was precipitated by adding 30 µL of 7.5 M ammonium acetate and 300 µL isopropanol at room temperature and immediately centrifuging without incubation (Shepherd & McLay 2011). The DNA pellet was washed with 70% and 100% ethanol. DNA was dissolved in 60 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

All newly designed primers are listed in Table 1.2. Primers for *ycf1* were designed from alignments of *Berberidopsis corallina* Hook. f. (GQ998001.1), *Beta vulgaris* L. (EF534108.1), and *S. oleracea* L. (AJ400848.1). Primers for *isi1* were designed from alignments of *Arabidopsis thaliana* (L.) Heynh. (NC_003075.7), *Medicago truncatula*

Gaertn. (AJ697743), and *Mesembryanthemum crystallinum* L. (AJ697742). Primers for *nhx1* were developed from alignments of *A. thaliana* (NC_003076.8), *M. crystallinum* (AM746985), *Salicornia europaea* L. (AY131235), and *Tetragonia tetragonioides* (Pall.) Kuntze (AF527625). PCR reactions were carried out in a T3 thermocycler (Biometra, Goettingen, Germany) with 20 µL volumes using 1 unit of ID*Proof*™ DNA polymerase, 2 µL 10X ID*Pol*™ polymerase reaction buffer (ID labs Inc., London, Ontario, Canada), 3 mM $MgCl_2$, 120 ng of each primer, 250 µM dNTPs, and 1 µL DNA (~40 ng). Primers used for amplification of the plastid locus *atpB-rbcL* intergenic spacer (IGS) were 2 and 10 from Savolainen et al. (1994). Primers for the *rpl16* intron were F71 and R1661 from Jordan et al. (1996) and R1516 from Kelchner and Clark (1997). All PCR amplifications had an initial denaturation of 94°C for 3 min, 40 cycles of 94°C for 45 s, annealing for 45 s, and extension at 72°C for 90–240 s, and a final extension of 72°C for 5 min. An annealing temperature of 42°C was used for the *atpB-rbcL* IGS, 50.5°C for *rpl16*, and 45°C for *ycf1*. The extension time was 4 min for both *rpl16* and *ycf1* and 1 min 30 s for *atpB-rbcL*. Annealing temperatures for the nuclear markers followed a touchdown protocol of 51.7–50°C for *nhx1* and 60.1–58.3°C for *isi1*, each with an extension time of 2 min 30 s. PCR products were analyzed on agarose gels stained with ethidium bromide.

To assess sequence variation, sequences were obtained from five species of *Harrisia* (Appendix A). Bands of the expected size were excised from agarose gels and DNA purified with QIAquick Gel Extraction Kit (QIAGEN, Valencia, California). Purified DNA was directly sequenced using amplification primers and additional internal sequencing primers (Table 1.2). Sequencing reactions were conducted at the DNA Laboratory at Arizona State University with a 3730 DNA Analyzer (Applied Biosystems,

Carlsbad, California). Electropherograms were analyzed with Sequence Scanner Software (Applied Biosystems). MEGA 5 (Tamura et al. 2011) was used for sequence editing, alignment, translation, and AT-content. Amplification success in shown in Table 1.1. The *ycf1* primers amplified a ~1100 bp fragment (bp 121,850–122,916 of the *S. oleracea* plastid genome, AJ400848.1, bp 4182–5248 of the *ycf1* gene). The *isi1* primers amplified a ~1100 bp fragment (bp 13,842,592–13,843,421, chromosome 4 of *A. thaliana*, NC_003075.7). The *isi1* portion of exon three amplified was 65 bp, the entirety of exon four was 74 bp, and the portion of exon five was 146 bp. The third intron of *isi1* was ~470 bp and the fourth ~420 bp. The *nhx1* primers amplified a ~570 bp fragment (bp 9,554,549–9,555,064, chromosome 5 of *A. thaliana*, NC_003076.8). The *nhx1* portion of exon two amplified was 58 bp and the portion of exon three was 37 bp. The second intron of *nhx1* was ~480 bp. The *atpB-rbcL* IGS primers amplified a ~400–800 bp fragment from the 5' end of the *atpB* gene to within the intergenic spacer (bp 53,017–53,649 of *S. oleracea*). The *rpl16* primers amplified a ~1,000 bp fragment from the 5' end of the *rpl16* exon to within its intron (bp 79,945–81,101 of *S. oleracea*). Sequence characterization of five species of *Harrisia* is presented in Table 1.3. Amino acid sequences of 94 codons of *isi1* amplified from *H. tetracantha* (JN166813) were 75.5% identical to *A. thaliana* (NM_118914) and 90.4% identical to *M. crystallinum* (AJ697742). Amino acid sequences of 31 codons of *nhx1* amplified from *H. earlei* (JN166839) were 100% identical to both *A. thaliana* (NC_003076) and *M. crystallinum* (AM746985). Amino acid sequences of 302 codons of *ycf1* amplified in *H. tetracantha* were 40.1% identical to *S. oleracea*.

CONCLUSIONS

Sequences from regions of the *isi1*, *nhx1*, and *ycf1* genes in *Harrisia* provide alternative informative markers (Table 1.3). Among the five sequenced regions, sequence variation in the *isi1* region was lowest, highest in the *ycf1* region, and second-highest in the *nhx1* region. Additionally, the three newly characterized markers (*isi1*, *nhx1*, and *ycf1*) revealed sequence variation among two closely-related species of *Harrisia* from the Caribbean region (*H. earlei* and *H. fragrans*), highlighting their possible application at low taxonomic levels. Results here demonstrate the potential utility of these loci in other taxa within Caryophyllales.

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TABLE 1.1. Amplification success of newly developed amplification primers in selected taxa of Caryophyllales.

Family	Taxon	Voucher	*isi*	*nhx*	*ycf1*
Amaranthaceae	*Blutaparon vermiculare* (L.) Mears	*A. R. Franck 1235* (USF)	+	–	+
Cactaceae	*Acanthocereus tetragonus* (L.) Hummelinck	*A. R. Franck 2967* (USF)	+	+	+
Cactaceae	*Arthrocereus spinosissimus* (Buining & Brederoo) F. Ritter	*A. R. Franck 1331* (USF)	+	+	+
Cactaceae	*Echinopsis atacamensis* (Phil.) Friedrich & G. D. Rowley subsp. *pasacana* (F. A. C. Weber ex Rümpler) G. Navarro	*A. R. Franck 2313* (USF)	+	+	+
Cactaceae	*Echinopsis bridgesii* Salm-Dyck subsp. *yungasensis* (F. Ritter) P. J. Braun & Esteves	*A. R. Franck 2639* (USF)	+	+	+
Cactaceae	*Echinopsis chiloensis* (Colla) Friedrich & G. D. Rowley subsp. *litoralis* (Johow) M. Lowry	*A. R. Franck 2498* (USF)	+	+	+
Cactaceae	*Echinopsis lageniformis* (Foerster) Friedrich & G. D. Rowley	*A. R. Franck 2635* (USF)	+	+	+
Cactaceae	*Haageocereus decumbens* (Vaupel) Backeb.	*M. Arakaki 1579* (USM)	+	+	+
Cactaceae	*Harrisia adscendens* (Gürke) Britton & Rose	*A. R. Franck 2641* (USF)	+	+	+
Cactaceae	*Harrisia earlei* Britton & Rose	*G. Ardisson 99.15* (USF)	+	+	+
Cactaceae	*Harrisia fragrans* Small ex Britton & Rose	*A. R. Franck 473* (USF)	+	+	+
Cactaceae	*Harrisia regelii* (Weing.) Borg	*A. R. Franck s.n. 23 Sep 08* (USF)	+	+	+
Cactaceae	*Harrisia tetracantha* (Labour.) D. R. Hunt	*A. R. Franck 2262* (USF)	+	+	+
Cactaceae	*Hylocereus undatus* (Haw.) Britton & Rose	*A. R. Franck 2968* (USF)	+	+	+
Cactaceae	*Monvillea cavendishii* (Monv.) Britton & Rose	*A. R. Franck 2631* (USF)	+	+	+
Cactaceae	*Pilosocereus pachycladus* F. Ritter	*A. R. Franck 2969* (USF)	+	+	+
Cactaceae	*Rhipsalis pilocarpa* Loefgr.	*A. R. Franck 1132* (USF)	+	+	+
Cactaceae	*Samaipaticereus corroanus* Cárdenas	*A. R. Franck 1785* (USF)	+	+	+
Cactaceae	*Selenicereus pteranthus* (Link & Otto) Britton & Rose	*A. R. Franck 1314* (USF)	+	+	+
Cactaceae	*Yungasocereus inquisivensis* (Cárdenas) F. Ritter ex Eggli	*A. R. Franck 2633* (USF)	+	+	+
Didiereaceae	*Alluaudia procera* (Drake) Drake	*A. R. Franck 2540* (USF)	+	–	+
Portulacaceae	*Portulaca amilis* Speg.	*A. R. Franck 1220* (USF)	+	–	+

TABLE 1.2. Newly developed primers used in this study. Asterisks indicate amplification primers. The primer prefix "int" denotes internal sequencing primers made for *Harrisia* and not necessarily appropriate for other taxa. Nuclear amplification primer names denote exon number of *A. thaliana*. Primer names of *ycf1* indicate the position on the *ycf1* gene of *S. oleracea*.

Region	Primer Name	PRIMER SEQUENCE (5′ to 3′)
isi1	isi-ex3F*	TWGAGCAARGWGCMTGYTTGGATGC
	isi-ex5R*	ARCCRAATTGACTVGCYGCCCATAT
	isi-int162F	TCTTGCAAACTCGATGCCTC
	isi-int498F	GTTAACATTACACTGCTGTGC
	isi-int565R	TGCGGCAACTTCCTCAATGC
	isi-int629R	ATAACTTSTGAGCTCGAGAAG
	isi-int636R	TCGAGAAGTAAACGTACCTG
nhx1	nhx-ex2F*	TCTTCAGTGAAGATCTYTTCTTYAT
	nhx-ex3R*	GAAGTTSCGGAARAATTGCTTCTT
	nhx-int230F	GGTAACTAACACTGTTTAGCATC
	nhx-int381R	GAACACCATTTATGCCTATGG
ycf1	ycf1-4182F*	AAATAYRRATAGAAAATATTTKGATT
	ycf1-5248R*	GAATTCTYAATTCTCTACGACG
	ycf1-int503F	CTTTGACCCACGATTTCATTTTC
	ycf1-int663R	AAGAAGAAAGCTAAACCTAAAACG
rpl16	rpl16-int446R	TGAACTTTGTTCTTGAGCCAACC
	rpl16-int621F	ATATGATTCCACTATGTAAGGTC

TABLE 1.3. Sequence characteristics of the five species of *Harrisia* listed in Appendix A.

	isi1	*nhx1*	*ycf1*	*atpB-rbcL* IGS	*rpl16* intron
Amplicon length	~1100	~570	~1100	~500–800	~1000
Length of fragments used in alignment	843–861	471–475	998–1022	448–659	771–776
Alignment length	861	475	1028	808	782
% variable sites	1.51	3.58	7.00	2.10	1.67
Indels	2	2	8	11	5
Avg. %A/T content	59.9	63.8	70.1	74.2	68.2

CHAPTER TWO: PHYLOGENY, BIOGEOGRAPHY, AND INFRAGENERIC CLASSIFICATION OF *HARRISIA* (CACTACEAE)

INTRODUCTION

Cactaceae is a predominantly neotropical family of succulents originating in the late Eocene-early Oligocene (Hershkovitz & Zimmer 1997; Arakaki et al. 2011) and contains about 1800 species (Nyffeler & Eggli 2010). The "core cacti," which includes all but the basal leafy *Pereskia* Mill. lineages (Edwards et al. 2005), likely originated in west-central South America along the present-day Andes (Wallace & Gibson 2002; Edwards et al. 2005; Griffith & Porter 2009) in the late Oligocene (Arakaki et al. 2011). All four subfamilies of Cactaceae occur along the Andes, with one (Maihuenioideae) endemic to the southern Andes. Many species that represent phylogenetically basal Cactaceae (Nyffeler 2002; Crozier 2005; Griffith & Porter 2009; Korotkova et al. 2010; Hernández-Hernández et al. 2011) are found along the Andes, also supporting the hypothesis of a west-central South America origin for the "core cacti." Additionally, several monotypic genera are restricted to the Andes.

The most species-rich subfamily is the Cactoideae, with about 1500 species in six tribes (Nyffeler & Eggli 2010). This subfamily is characterized by a seemingly leafless stem, as only microscopic leaves are evident at shoot apical meristems (Mauseth 2007). The core Cactoideae (Nyffeler 2002; Hernández-Hernández et al. 2011) is further

distinguished by long-shoot flowers arising from the areoles of the stem, as opposed to flowers often arising between the tubercles in the basal tribe Cacteae.

Core Cactoideae is divided into at least five tribes (Nyffeler & Eggli 2010). Tribe Cereeae, with about 600 species in 20 genera (Nyffeler & Eggli 2010), contains ribbed globose to columnar cacti and is predominantly South American with only three genera native to the Caribbean, Central America, and/or Mexico. Tribe Cereeae is further divided into three subtribes (Nyffeler & Eggli 2010). Subtribe Trichocereinae is characterized by flowers that have acuminate bracts, pilose areoles, and an areolate pericarpel (tissue surrounding the ovary). Molecular studies using plastid markers have helped define the subtribe (Wallace 1995; Applequist & Wallace 2002; Nyffeler 2002; Crozier 2005; Ritz et al. 2007; Hernández-Hernández et al. 2011) which contains approximately 250 species in 20 genera (Nyffeler & Eggli 2010). With the exception of *Harrisia* Britton in the Caribbean, Trichocereinae is confined to South America from Ecuador, Peru, and Brazil southward (Hunt et al. 2006). *Harrisia* was only recently placed in subtribe Trichocereinae based on molecular evidence (Wallace 1995; Wallace 1997a, 1997b; Applequist & Wallace 2002).

The generic-level relationships within subtribe Trichocereinae are unclear. The genera *Haageocereus* Backeb., *Matucana* Britton & Rose, *Mila* Britton & Rose, *Oreocereus* (A. Berger) Riccob., *Oroya* Britton & Rose, and *Pygmaeocereus* H. Johnson & Backeb. have received molecular support as a clade (Crozier 2005; Lendel et al. 2006; Ritz et al. 2007; Bárcenas et al. 2011; Hernández-Hernández et al. 2011), characterized by flowers being terminal on the stem (Lendel et al. 2006). *Echinopsis* Zucc. s. l. has perhaps the poorest taxonomy in the subtribe, with at least 11 segregate genera proposed

(Anderson 2001), many of them described and recognized only by Backeberg (Backeberg 1977; Anderson 2001). Current opinion favors retention of *Echinopsis* s. l. until more detailed studies can revise the group (Anderson 2001; Hunt et al. 2006) aided by seed morphology (Friedrich & Glaetzle 1983) and molecular data (Schlumpberger 2009).

Pollen morphology (Leuenberger 1976) and plastid phylogenies (Nyffeler 2002; Lendel et al. 2006; Korotkova et al. 2010; Bárcenas et al. 2011) have supported a relationship between *Echinopsis* s. l. and *Harrisia*. With increased sampling in these two genera, plastid phylogenies have specifically shown relationships between *Harrisia* and some species of *Echinopsis* s. s. and its segregate genus *Trichocereus* (A. Berger) Riccob. (Schlumpberger 2009; Hernández-Hernández et al. 2011). Flower morphology supports this relationship as they all have white, long-tubed nocturnal flowers in which the arrangement of the stamens is bilaterally symmetrical, with the majority concentrated in the lower portion, termed the dorsal stamen cluster (Schick 2011). The arborescent *H. tetracantha* (Labour.) D. R. Hunt had previously been placed in the genus *Trichocereus* (Britton & Rose 1920; Borg 1937) or allied with it in subfamilial classifications (Backeberg 1938; Backeberg 1977). The slender-stemmed *E. hahniana* (Backeb.) R. S. Wallace, also having dorsal stamen clusters, was once placed in *Harrisia* (Kimnach 1987). An analysis of unpublished *rpl16* intron sequences has suggested *Samaipaticereus* Cárdenas was sister to a monophyletic *Harrisia* (Wallace 1997b). Other phylogenetic studies have not recovered this sister-group relationship (Nyffeler 2002; Korotkova et al. 2010; Bárcenas et al. 2011; Hernández-Hernández et al. 2011).

The genus *Harrisia* is a columnar cactus comprising 18 species (Appendix B) native to South America and the Caribbean, found primarily in seasonally dry woodlands. Seed

morphology has been used to define *Harrisia* (Berger 1905) by the enlarged peripheral testa cells and cavernous hilum-micropylar region (Barthlott & Hunt 2000; Doweld 2001). *Harrisia* has been hypothesized to have an Andean origin (Wallace 1997a). One species, *H. tetracantha*, occurs in the eastern inter-Andean dry valleys of Bolivia at elevations of 1200–2600 m (Antezana & Navarro 2002). Five species of *Harrisia* are found in the Gran Chaco region (Prado 1993) of Argentina, Bolivia, Brazil, and Paraguay (Kiesling 1996; Eggli 2002). One species, *H. adscendens* (Gürke) Britton & Rose, occurs in the Caatinga of northeast Brazil (Taylor & Zappi 2004). Thirteen species are Caribbean, found mainly in wooded lowlands in the Greater Antilles, the Bahamas, and Florida, U.S.A. (Britton & Rose 1920). Some species of *Harrisia* have been naturalized in Australia (McFadyen 1986), Hawaii (Lorence et al. 1995), and South Africa (Henderson 2007). Two subgenera are often recognized in *Harrisia*. Subgenus *Eriocereus* (A. Berger) Britton & Rose ex R. Kiesling has included all the South American species because of their splitting fruits, while subg. *Harrisia* has been restricted to the Caribbean species which all have indehiscent fruits (Britton & Rose 1920; Kiesling 1996; Anderson 2001; Hunt et al. 2006).

The monophyly of *Harrisia* and the relationships among the widely disjunct species within *Harrisia* have not been clearly established. Previous work with unpublished sequences of the *rpl16* intron found subg. *Eriocereus* to be paraphyletic because the Brazilian *H. adscendens* was sister to the Caribbean species (Wallace 1997a, 1997b). Other molecular phylogenies have only included 1–3 species in family-level phylogenies (Nyffeler 2002; Bárcenas et al. 2011; Hernández-Hernández et al. 2011) or as an outgroup (Wallace 1995; Arias et al. 2005).

Here is presented a phylogenetic analysis of nuclear and plastid DNA sequences to elucidate infrageneric relationships within *Harrisia* and test the monophyly of *Harrisia*, especially against species of *Echinopsis* s. l. that have a similar flower morphology. As dates for the origin and diversification of many groups within Cactaceae have been estimated (Arakaki et al. 2011), divergence estimates were also applied to the phylogeny of *Harrisia*. Using the phylogeny and divergence estimates, a biogeographic scenario and infrageneric classification is proposed for *Harrisia*.

MATERIALS & METHODS

Taxon Sampling & DNA Extraction. The study included 40 species (Appendix C) from the core Cactoideae, representing seven genera of subtribe Trichocereinae. From *Harrisia* were included *H. tetracantha*, *H. adscendens*, four of five species from the Gran Chaco region, and six of 13 Caribbean species. Within *Echinopsis* s. l., 15 species with dorsal stamen clusters and three species with radially symmetrical stamen arrangements were included, together representing six segregate genera of *Echinopsis* s. l. (*Chamaecereus* Britton & Rose, *Helianthocereus* Backeb., *Hymenorebutia* Frič ex Buining, *Lobivia* Britton & Rose, *Soehrensia* Backeb., and *Trichocereus*). *Arthrocereus spinosissimus* (Buining & Brederoo) F. Ritter was sampled because it was originally described under *Eriocereus* (A. Berger) Riccob., a genus once segregated but currently included in *Harrisia*. *Samaipaticereus* was sampled to further investigate a putative relationship with *Harrisia* (Wallace 1997b). Three other genera (*Cleistocactus* Lem., *Haageocereus* Backeb., and *Yungasocereus* F. Ritter) were included to represent the

diversity of subtribe Trichocereinae. Outgroups included two genera from the closely related subtribe Cereinae (*Monvillea* Britton & Rose and *Pilosocereus* Byles & G.D. Rowley), one sample from tribe Rhipsalideae (*Rhipsalis* Gaertn.), and one sample from tribe Phyllocacteae (*Selenicereus* (A. Berger) Britton & Rose). Nomenclature and identification for *Harrisia* were verified from original descriptions. For other genera, nomenclature followed Tropicos (2012). Identification and taxonomy of other genera followed Hunt et al. (2006), with the exception of *Monvillea* (Kiesling 2011).

Vouchered specimens were obtained from field collections and botanical gardens (Appendix C). DNA was extracted with a modified CTAB protocol (Doyle and Doyle 1987; Shepherd & McLay 2011; Franck et al. 2012).

Markers & Primers. Four markers were sequenced for phylogenetic analyses including two plastid markers, the *atpB-rbcL* intergenic spacer (IGS) and *rpl16* intron, and two nuclear markers, parts of the *isi1* and *nhx1* genes. Primers for the *atpB-rbcL* IGS were from Savolainen et al.(1994) and amplified a ~400–800 bp fragment from the 5' end of the *atpB* gene to within the intergenic spacer. Primers for the *rpl16* intron were from Jordan et al. (1996), Kelchner & Clark (1997), and Franck et al. (in press) and amplify a ~1,000 bp fragment from the 5' end of the *rpl16* exon to within its intron. Primers for *isi1* were from Franck et al. (in press), anchored in two exons, and amplify a ~1000 bp fragment spanning two introns (~400 bp) and one exon (74 bp). Primers for *nhx1* were from Franck et al. (in press), anchored in two exons, and amplify a ~570 bp fragment consisting of a ~400 bp intron.

The plastid *trnS-trnG* IGS was sequenced for one sample of *Harrisia* to compare its similarity with a large insertion found in the *atpB-rbcL* IGS. The *trnS-trnG* IGS primers Caryo-trnG (5′ – TTTTACCACTAAACTATACCCGC – 3′) and Caryo-psbK (5′ – CAAGCTGCTGTAAGTTTTCGATGA – 3′) were developed for this study and were based on sequences from *Spinacia oleracea* L. (AJ400848.1) and *Fagopyrum esculentum* Moench (EU254477.1), amplifying a ~1500 bp fragment from *trnG* to *psbK*.

PCR & Sequencing. PCR conditions followed Franck et al. (in press). PCR products were gel-purified with the QIAquick Gel Extraction Kit (QIAGEN, Valenica, California). Sequencing analyses were carried out at the DNA Laboratory at Arizona State University with a 3730 DNA Analyzer (Applied Biosystems, Carlsbad, California). Electropherograms were analyzed with Sequence Scanner Software (Applied Biosystems). Sequences were deposited in GenBank (accession numbers JN166806–JN166933 and JQ889302–JQ889325; Appendix C).

Phylogeny. Bayesian and maximum parsimony analyses were performed on alignments of each individual marker and a combined four-marker alignment with all 40 species. MEGA 5 (Tamura et al. 2011) was used for alignment. Gaps were coded according to the simple indel coding method (Simmons & Ochoterena 2000). Alignments were deposited in TreeBASE (12596).

Bayesian analyses were performed with Mr. Bayes version 3.1 (Ronquist & Huelsenbeck 2003) with two simultaneous runs of four chains. Substitution models for each gene were determined with the Akaike information criterion (Akaike 1974) using

the likelihood score as estimated by PAUP (Posada and Crandall 1998). The GTR + I + G model was used for the *atpB-rbcL* IGS, *rpl16* intron, and *isi1* alignments. The GTR + G model was used for the *nhx1* alignment. Coded indels were partitioned as a restriction site data set with coding set to variable. Model parameters were unlinked between partitions. One million generations were run with sampling ever 100th generation and the first 25% of trees discarded as burn-in. Posterior probabilities (PP) 0.50 and above were reported.

Maximum parsimony analyses were performed with PAUP* version 4.0b10 (Swofford 2003) using a heuristic search with 1000 replicates, random sequence addition, tree-bisection-reconnection branch swapping, MAXTREES set to no limit, and MULTREES on. Consistency and retention indices were obtained from PAUP. Bootstrapping (BS) was performed with 1000 replicates under the same conditions with the exception that MAXTREES was set to 1000. Bootstrap values (BS) 50% and above were reported.

A neighbor-net network was produced from the four-marker alignment with SplitsTree4 (Huson & Bryant 2006) using the uncorrected P-distance with ambiguous states averaged. Coded gaps were treated as a fifth character and converted to bases. Bootstrapping was performed with 1000 replicates under the same conditions.

Estimates of Divergence Times. Arakaki et al. (2011) dated the origin of subtribe Trichocereinae at 6.5 (±2.0) Ma ago. Their dates are derived from a broad angiosperm phylogeny of 83 plastid genes (Jansen et al. 2007; Moore et al. 2010) supplemented with denser sampling in the Caryophyllales order (including Cactaceae). Their phylogeny was

constrained by 15 fossils, only one within Caryophyllales (Arakaki et al. 2011). There are no relevant fossils to date *Harrisia* or the Cactaceae.

The software r8s (Sanderson 2003) was used to date nodes from the combined four-marker Bayesian tree using a penalized likelihood analysis with a TN algorithm and an additive penalty function. The dating analysis was run using 6.5 Ma ago and the reported standard deviations of 4.5 and 8.5 Ma ago (Arakaki et al. 2011) as a fixed age for the split between subtribe Cereinae and Trichocereinae. *Rhipsalis* was pruned to remove the basal trichotomy. Optimal smoothing values were obtained by cross-validation using values from 10^{-3} to $10^{3.5}$ in increments of $10^{0.25}$. The smoothing value with the lowest Chi-square error was chosen. Smoothing values of 0.32 were used for the 6.5 and 8.5 Ma fixed ages and 0.01 for the 4.5 Ma fixed age.

RESULTS

Sequence Characteristics. The *atpB-rbcL* IGS alignment (Table 2.1) was composed entirely of the IGS. Two large, directly adjacent insertions within the *atpB-rbcL* IGS had high sequence similarity to the *trnS-trnG* IGS based on BLAST. The first insertion was 91–110 bp in size and was found in all samples of *Harrisia* as well as *Echinopsis aurea*, *E. haematantha*, *E. chiloensis* subsp. *litoralis*, *E. atacamensis* subsp. *pasacana*, and *E. terscheckii*. The second adjacent insertion was 232 bp and occurred only in *H. bonplandii*, *H. pomanensis*, *H. regelii*, *H. tetracantha*, and *H. tortuosa*. A 215 bp fragment of the *atpB-rbcL* IGS insertion (consisting of 80 bp of the first insertion and 135 bp of the second insertion) from *H. regelii* had 100% similarity with an aligned region in

its *trnS-trnG* IGS (JN166933) except for two 2 bp indels. Another insertion of 199 bp in the *atpB-rbcL* IGS unique to *E. chamaecereus* showed similarity to the *psbM-trnD* IGS by BLAST and had an 82.4% identity with an aligned region of *Beta vulgaris* L. (EF534108, unannotated).

The *rpl16* intron alignment (Table 2.1) was composed entirely of the intron. There was a mononucleic A/T microsatellite 9–14 bp long in *Harrisia* and 4–9 bp in all other taxa. The *isi1* alignment (Table 2.1) contained part of the third intron (343–347 bp), the entire fourth exon (74 bp), and part of the fourth intron (423–442 bp). The *nhx1* alignment (Table 2.1) consisted of a small part of the second exon (5 bp) and the second intron (434–470 bp). Codon position and exon numbers reported here are based on the genome of *Arabidopsis* (AGI 2000).

Phylogeny. *Harrisia* was supported as a monophyletic group in all phylogenetic analyses (Figs. 2.1 & 2.2) except the *nhx1* maximum parsimony tree (not shown). The monophyly of *Harrisia* in the *nhx1* dataset was supported only by the Bayesian tree (PP 0.58). The *atpB-rbcL* IGS Bayesian tree (Fig. 2.2) did not support any generic-level relationships for *Harrisia*. In the *atpB-rbcL* IGS majority rule consensus parsimony tree (not shown), *E. aurea* and *E. haematantha* were found to be sister to *Harrisia* (BS <50). Bayesian trees from the *rpl16* intron (Fig. 2.2) found *E. chiloensis* subsp. *litoralis*, *E. atacamensis* subsp. *pasacana*, and *E. terscheckii* as sister to *Harrisia* (PP 1.0), with *E. aurea* and *E. haematantha* as a basal to this clade (PP 1.0). The *rpl16* intron strict consensus parsimony tree (not shown) and four-marker Bayesian tree (Fig. 2.1) resolved a clade of three genera (*Cleistocactus*, *Samaipaticereus*, *Yungasocereus*) as sister to

Harrisia (BS <50; PP 0.78). The *isi1* majority rule consensus parsimony tree (not shown) supported a clade of *E. schickendantzii*, *E. thelegona*, and *Samaipaticereus* as sister to *Harrisia* (BS <50). Very low support was found for generic-level relationships with *Harrisia* in the *isi1* and *nhx1* Bayesian trees (Fig. 2.2). In the four-marker majority rule consensus parsimony tree (not shown), *Samaipaticereus* was placed as sister to *Harrisia* with *Cleistocactus* and *Yungasocereus* sister to *Samaipaticereus* and *Harrisia*. In the neighbor-net network (not shown), *Arthrocereus* formed the best-supported clade with *Harrisia* (BS 29). *Cleistocactus* formed a clade with the outgroups in the neighbor-net network (BS 44). The four-marker dataset resolved a monophyletic clade composed of the samples of *Echinopsis* and *Arthrocereus* in the Bayesian tree (PP 0.99) and strict consensus parsimony tree (BS <50, not shown).

All analyses except the *isi1* trees recovered the same two basal clades in *Harrisia* (Figs. 2.1 & 2.2). One clade consisted of *H. tetracantha* and the Gran Chaco species. The other clade was composed of *H. adscendens* and the Caribbean species. This relationship was supported by the *isi1* semi-strict parsimony tree (BS <50, not shown). All analyses except the *atpB-rbcL* IGS and *isi1* trees supported a Caribbean clade distinct from *H. adscendens* (Figs. 2.1 & 2.2). Only in the four-marker dataset was *H. earlei* supported as basal to the rest of the Caribbean species by the Bayesian tree (Fig 2.1.; PP 0.86) and the semi-strict parsimony tree (BS <50, not shown). The Gran Chaco species were recovered as a clade distinct from *H. tetracantha* in all plastid analyses (Fig. 2.2) and the four-marker analyses (Fig. 2.1).

Divergence Estimates. Using the four-marker Bayesian tree (Fig. 2.1), the r8s penalized likelihood analysis estimated the origin of *Harrisia* to be 3.45–6.53 Ma ago (Figs. 2.3 & 2.4). The split between the two basal clades of *Harrisia* was dated at 1.75–3.30 Ma ago. The divergence date between the Gran Chaco species and *H. tetracantha* was 0.69–1.30 Ma ago. Diversification of the Gran Chaco species was estimated at 0.20–0.38 Ma ago. The Caribbean species and *H. adscendens* had a divergence date of 0.81–1.53 Ma ago. The split between *H. earlei* and the rest of the Caribbean species was estimated at 0.24–0.45 Ma ago. The rest of the Caribbean species had a diversification date of 0.20–0.37 Ma ago (Figs. 2.3 & 2.4).

DISCUSSION

Monophyly of Harrisia & Generic Relationships. The molecular analyses presented here (Figs. 2.1 & 2.2) demonstrate that *Harrisia* is a monophyletic genus defined by its distinctive seed morphology (Berger 1905; Barthlott & Hunt 2000; Doweld 2001). The monophyly of *Harrisia* has been found previously in an analysis of unpublished *rpl16* intron sequences (Wallace 1997a, 1997b).

Generic-level relationships of *Harrisia* remain equivocal because of the low support and conflicting topologies obtained from the analyses (Figs. 2.1 & 2.2). *Samaipaticereus* has been alleged to be the sister-taxon to *Harrisia* in an *rpl16* intron phylogeny (Wallace 1997b) but this relationship was only recovered by the four-marker majority rule consensus parsimony tree in this study (BS <50, not shown). Other plastid-based phylogenies that included *Harrisia* and *Samaipaticereus* do not indicate a sister

relationship (Nyffeler 2002; Korotkova et al. 2010; Bárcenas et al. 2011; Hernández-Hernández et al. 2011). Support (PP 0.78) was found for a clade of *Cleistocactus*, *Samaipaticereus*, and *Yungasocereus* as sister to *Harrisia* in the four-marker Bayesian tree, though with a very short branch length (Fig. 2.1).

Within subtribe Trichocereinae, dorsal stamen clusters are found only in *Harrisia* and some species of *Echinopsis* s. l. Because of this potential synapomorphy, species of *Echinopsis* with dorsal stamen clusters were more densely sampled. No clear relationships between *Harrisia* and these species of *Echinopsis* were recovered. Only in the *rpl16* intron phylogeny were species of *Echinopsis* (*E. atacamensis* subsp. *pasacana*, *E. littoralis*, and *E. terscheckii*) with dorsal stamen clusters sister to *Harrisia*. These species of *Echinopsis* are arborescent and perhaps arborescence in the Bolivian *H. tetracantha* is plesiomorphic. *Harrisia* and the arborescent species of *Echinopsis* together were sister to dwarf, globose species of *Echinopsis* (*Echinopsis aurea* and *E. haematantha*) with a radially symmetrical stamen arrangement (Fig. 2.2). All of these species share a large insertion in the *atpB-rbcL* IGS (91–110 bp) apparently duplicated from the *trnS-trnG* IGS. The plastid relationship between *Harrisia* and these species of *Echinopsis* may be due to deep coalescence, reticulate evolution, or historic introgression (Rieseberg & Soltis 1991).

This study included nuclear markers to increase genomic sampling and mitigate potential bias from the plastid sequences, as the Cactaceae are well-noted for their ability to hybridize (Friedrich 1974; Machado 2008). One common cultivar, *Harrisia* ×*jusbertii* (Rebut ex K. Schum.) Frič, is thought to be an intergeneric hybrid between *Echinopsis* and *Harrisia* (Berger 1905). Ribosomal markers were not used due to the non-concerted

evolution revealed in several samples of *Harrisia* (data not shown), which has also been found in other members of Cactaceae (Hartmann et al. 2001; Harpke & Peterson 2006; Ritz et al. 2007; Harpke & Peterson 2008).

As the generic-level relationships are not well resolved, no conclusions can be made concerning generic relationships with *Harrisia*. These genera may have experienced a rapid radiation making relationships difficult to discern. It is noteworthy that the species of *Echinopsis* sampled here formed a monophyletic clade with *Arthrocereus* in the four-marker Bayesian (Fig. 2.1) and strict parsimony trees (not shown). Additional sampling within subtribe Trichocereinae is needed to establish generic-level relationships with *Harrisia* and investigate monophyly among species of *Echinopsis* s. l.

Infrageneric Relationships within Harrisia. The analyses supported two early-diverging clades in *Harrisia* (Fig. 2.1). One clade was South American and contained the Bolivian *H. tetracantha* and the species of the Gran Chaco region. The other clade contained all Caribbean species and the Brazilian *H. adscendens* as the basal member, a relationship also found by Wallace (1997b). While seed morphology is consistent with the two basal clades (Fig. 2.4), other characters such as growth form, rib number, and fruit color appear to be homoplasious or plesiomorphic. *Harrisia tetracantha* is arborescent whereas all other species are generally shrubby. Ribs of 8–10 are found in *H. tetracantha*, *H. adscendens*, and all but one of the Caribbean species. Ribs of 5–7 are found in one Caribbean species (*H. earlei*) and some of the Gran Chaco species. Red fruits are found in all four clades although most Caribbean species have yellow fruits.

Morphological homoplasy is widespread and common in Cactaceae (Terrazas & Arias 2003; Ritz et al. 2007; Ogburn & Edwards 2009; Hernández-Hernández et al. 2011).

The Gran Chaco species were recovered as a clade in the plastid analyses (Fig. 2.2) and the four-marker phylogeny (Fig. 2.1). Species within the strictly Caribbean clade (excluding the Brazilian *H. adscendens*) formed a well-supported clade (Figs. 2.1 & 2.2). The four-marker phylogeny supported *H. earlei* as basal to the rest of the Caribbean species (PP 0.86, Fig. 2.1), also hypothesized by Britton & Rose (1920). Among the Caribbean species, *H. earlei* is the most distinct, having 5–7 ribs and a prostrate to pendent habit along elevated, inland limestone outcrops. All other Caribbean species have 8–14 ribs and are generally erect shrubs found in lowland coastal habitats. With the exception of *H. earlei*, the Caribbean species are morphologically very similar to each other and perhaps only recently diverged (Figs. 2.3 & 2.4).

Biogeography of Harrisia. A central Andean origin of *Harrisia* (Wallace 1997a) is supported by the high endemism found in the central Andes (López 2003), and more specifically by the numerous cacti of tribe Cereeae endemic to the eastern Andes of Bolivia, including *H. tetracantha* (Navarro 1996). The estimate of a late-Miocene to early-Pliocene origin of *Harrisia* (Figs. 2.3 & 2.4) follows the earlier development of arid habitat in the east-central Andes in the late Miocene, such as its recent uplift in the last 10 Ma (Gregory-Wodzicki 2000), near completion of upper-crustal shortening and deformation along the inter-Andean region 9–11 Ma ago (Mosolf et al. 2010), a climate-affecting orographic barrier around 8–14 Ma ago (Hulka & Huebeck 2010), and a change to seasonal precipitation and increased aridity 8–9 Ma ago (Mulch et al. 2010).

The divergence of the two basal clades in *Harrisia* (Fig. 2.1) was dated to the early-Pleistocene to late-Pliocene (Figs. 2.3 & 2.4). This possibly coincides with an early dispersal event giving rise to the clade of Caribbean and Brazilian species. The distribution patterns of other cacti suggest *Harrisia* dispersed into northeast Brazil from the Andes (Wallace 1997a). Subtribe Trichocereinae is largely confined to the Andean region of west-central South America and southward. *Harrisia* is one of only four genera from subtribe Trichocereinae found in Brazil (the other thee being *Arthrocereus* A. Berger, *Cleistocactus*, and *Echinopsis*). Other cactus genera also have similar distributions from western South America to Brazil, such as *Brasiliopuntia* (K. Schum.) A. Berger, *Frailea* Britton & Rose, *Quiabentia* Britton & Rose, and *Parodia* Speg. The fire-prone Cerrado, where very few cacti occur (Eiten 1972), separates suitable cactus habitat between northeast Brazil and southwest South America.

It is hypothesized that *Harrisia* dispersed into the Caatinga of Brazil, where *H. adscendens* occurs, after the development of the Cerrado in the late-Miocene to early-Pliocene (Simon et al. 2009). Other plants from the Caatinga of northeast Brazil have origins estimated in the mid-Miocene to Pliocene (Pennington et al. 2004; Queiroz & Lavin 2011). Manfrin et al. (2001) dated the origin of a clade of northeastern Brazilian flies which feed on cacti as 3–6 Ma ago. The monophyletic Caatinga species, *Drosophila borborema*, had a much more recent origin in the Pleistocene (Manfrin et al. 2001).

The species of the Gran Chaco possibly diverged from the Bolivian *H. tetracantha* (Figs. 2.3 & 2.4) during the middle Pleistocene transition (Clark et al. 2006). The present location of the Gran Chaco was an inundated sea, the Paranese Sea, 10 Ma ago (Marshall et al. 1993) with the Bolivian Chaco foreland basin experiencing marine transgression

until 6 Ma ago (Hulka et al. 2006). Studies of Gran Chaco legumes (Fabaceae) have been estimated to originate in the late Miocene (*Prosopis* L., Andrés Catalano et al. 2008; *Macroptilium* (Benth.) Urb., Espert & Burghardt 2010). The Gran Chaco toad, *Rhinella arenarum* (Hensel), diverged from its sister group around 5.17 Ma ago (Medeiros Maciel et al. 2010). The diversification of the Gran Chaco species of *Harrisia* was dated to the late Pleistocene (Figs. 2.3 & 2.4).

Harrisia adscendens diverged from the Caribbean species (Figs. 2.3 & 2.4), also around the middle Pleistocene transition (Clark et al. 2006). The progenitor of the Caribbean species perhaps dispersed from northeast Brazil into northern South America, Central America, and Mexico before reaching the Caribbean (Fig. 2.3). This dispersal route is reflected in the distribution patterns of other cacti in the closely related subtribe Cereinae, with its greatest diversity found in northeast Brazil. Subtribe Cereinae contains two species-rich genera, *Melocactus* Link & Otto and *Pilosocereus*, found in Brazil (Taylor & Zappi 2004), northern South America (Leuenberger 1987; Ruiz et al. 2002; Gröger & Huber 2007), Central America (Hunt et al. 2006), and Mexico (Hunt et al. 2006). The cactus genus *Tacinga* Britton & Rose (subfamily Opuntioideae) also likely dispersed into northern South America from northeast Brazil (Majure et al. 2012). Floristic affinities are common between the Caatinga and the northern coast of South America (Sarmiento 1975; López et al. 2006); between South America, Central America, and the Greater Antilles (Smidt et al. 2007; Acevedo-Rodríguez & Strong 2008); and between nuclear Central America and the Greater Antilles (Chiappy-Jhones et al. 2001; Trejo-Torres and Ackerman 2001; Anderberg and Zhang 2002; Stanzel 2004; Graham 2010; Jardón-Barbolla et al. 2011). The phylogenetically basal *Harrisia earlei*, found

prostrate to pendent on dry forest rock outcrops from west Cuba, may have once been sympatric with some species of *Hylocereus* (A. Berger) Britton & Rose and *Strophocactus* Britton & Rose which share a similar growth habit and habitat preference in Central America and southeast Mexico (Pérez-García et al. 2001; Sánchez-Sánchez and Islebe 2002; Bridgewater et al. 2006; Francisco Morales 2006).

Harrisia either became extinct, remains undiscovered, or was never present in northern South America, Central America, and Mexico. Its appearance in the Caribbean could be due to long-distance dispersal from Brazil. Many taxonomic disjunctions occur between South America and the Greater Antilles, especially Cuba (Acevedo-Rodríguez & Strong 2008). The fruits of *Harrisia* are well-suited for long-distance animal dispersal as they are sweet, edible, and contain ~1000 tiny seeds (Rojas-Sandoval & Meléndez-Ackerman 2009). Oceanic dispersal could have played a role because prevailing surface currents flow northwestward from the northern coast of Brazil to Central America and the western end of Cuba as indicated by deployed buoys (Brucks 1971; Molinari et al. 1979).

Cuba contains the greatest diversity (5 species) of the Caribbean species of *Harrisia*. The diversity of *Harrisia* in Cuba and its absence in the Lesser Antilles suggest initial colonization of the Caribbean in Cuba. *Harrisia earlei*, endemic to west Cuba, has been regarded as intermediate between the South American species and the rest of the Caribbean species (Britton & Rose 1920) and was basal in the phylogeny of the Caribbean clade (Fig. 2.1). *Harrisia* likely first colonized western Cuba where *H. earlei* is endemic before dispersing into other regions of the Caribbean in the late-Pleistocene (Fig. 2.3). Recent species radiations in the Caribbean during the Pleistocene have been postulated for species of *Opuntia* Mill. (Majure et al. 2012), *Pinus* L. (Jardón-Barbolla et

al. 2011) and ants (Formicidae: *Platythyrea* Roger, Seal et al. 2011). Other studies also indicate plant radiations originating in Cuba and dispersing into the rest of the Caribbean (Liu et al. 2004; Stanzel 2004; Namoff et al. 2007; Francisco-Ortega et al. 2008; Jardón-Barbolla et al. 2011).

The origin of *Harrisia* in the central Andes with subsequent dispersal to the Caribbean from Brazil and into the Gran Chaco from the Andes is consistent with the phylogenetic data and other floristic patterns (López et al. 2003; López et al. 2006), specifically in the Cactaceae (Sarmiento 1975). The progenitor of the Caribbean species of *Harrisia* may have once inhabited dry forest habitat in nuclear Central America and northern South America. Dry forest habitat was likely more widespread historically in the Pleistocene (Bonatti & Gartner 1973; Leyden 1984; Leyden 1985; Leyden et al. 1994; Hodell et al. 2008; Correa-Metrio et al. 2011; Werneck et al. 2011).

Taxonomy of Harrisia. The phylogeny supports a monophyletic *Harrisia* as presently circumscribed. Two segregate genera, *Eriocereus* (A. Berger) Riccob. and *Roseocereus* (Backeb.) Backeb., had once been proposed for *Harrisia*. This analysis (Fig. 2.1) supports their inclusion under one genus. All of the South America species had been recognized as the distinct genus *Eriocereus* (Berger 1905) until Britton & Rose (1920) united them with the Caribbean genus *Harrisia*. Britton & Rose (1920) still recognized an unranked group *Eriocereus* within *Harrisia* to encompass all the South America species within the genus. The phylogeny (Fig. 2.1) and seed morphology support defining a subgenus *Eriocereus* which includes all extant South American species except *H. adscendens*. The subgenus *Eriocereus* can be recognized by its semi-matte seeds that are

as long as wide. The subgenus *Harrisia* then includes *H. adscendens* from NE Brazil and the Caribbean species. This subgenus is characterized by oblong, glossy seeds.

Two sections within each subgenus are proposed, congruent with the phylogeny (Fig. 2.1). *Harrisia tetracantha* is the sole representative of sect. *Roseocereus* characterized by a arborescent habit with erect, 8–11-ribbed stems. The five species of the Gran Chaco form sect. *Eriocereus* having a scrambling to clambering habit with curvaceous 3–8-ribbed stems. Section *Adscendens* is monotypic and confined to the Caatinga, characterized by long stigma lobes and a splitting fruit. Section *Harrisia* comprises 13 Caribbean species which have short stigma lobes and indehiscent fruits. Two series within sect. *Harrisia* are recognized here. Series *Earlei* is monotypic, having 5–7 ribs and a prostrate to pendent habit. Series *Harrisia* contains 12 Caribbean species with 8–14 ribs and an erect, shrubby habit.

Conclusions. *Harrisia* is a monophyletic genus that likely originated in the Andes in the late-Miocene to early-Pliocene (Figs. 2.3 & 2.4). Within subtribe Trichocereinae, the generic affinities with *Harrisia* are unclear. *Harrisia* is composed of two subgenera, each divided into two sections. The phylogeny suggests that *Harrisia* dispersed to Brazil in the early-Pleistocene to late-Pliocene, before speciation in the Gran Chaco or Caribbean (Figs. 2.1 & 2.3). In the Pleistocene, *Harrisia* probably invaded the Gran Chaco from the Andes and reached the Caribbean from Brazil. In the late-Pleistocene, further speciation of *Harrisia* occurred in the Gran Chaco and the Caribbean.

TAXONOMY

HARRISIA Britton, Bull. Torrey Bot. Club 35: 561. 1908. *Cereus* Mill. [unranked] *Attenuati* Labour., Monogr. Cact.: 335. 1858. *Cereus* Mill. series *Attenuati* (Labour.) K. Schum., Gesamtbeschr. Kakt. 54, 95. 1899.—TYPE: *Harrisia gracilis* (Mill.) Britton.

1. Subgenus ***Harrisia***.

A. Section ***Harrisia***.

i. Series ***Harrisia***.

12 species; Greater Antilles, Bahamas, and Florida, U.S.A.

ii. Series ***Earlei*** A. R. Franck, series nov.—TYPE: *Harrisia earlei* Britton & Rose.

Caulibus prostratis; costae 5–7.

Stems prostrate, ribs 5–7.

1 species; Pinar del Río Province, Cuba.

B. Section ***Adscendens*** A. R. Franck, sect. nov.—TYPE: *Harrisia adscendens* (Gürke) Britton & Rose.

Caulibus curvus aut erigere; costae 6–10.

Stems curvaceous or erect; ribs 6–10.

1 species; Caatinga of NE Brazil.

2. Subgenus ***Eriocereus*** (A. Berger.) Britton & Rose ex R. Kiesling, Darwiniana 34: 390. 1996. *Cereus* Mill. series *Tortuosi* K. Schum., Gesamtbeschr. Kakt. 54, 135. 1899. *Cereus* Mill. subg. *Eriocereus* A. Berger, Rep. (Annual) Missouri Bot. Gard. 16:

74. 1905. *Eriocereus* (A. Berger) Riccob., Boll. Reale Orto Bot. Palermo 8: 238. 1909. *Harrisia* Britton [unranked] *Eriocereus* (A. Berger) Britton & Rose. 1920. Cact. 2: 148. —TYPE: *Harrisia tortuosa* (J. Forbes ex Otto & A. Dietr.) Britton & Rose.

A. Section ***Eriocereus***.

5 species; Gran Chaco region of Argentina, Bolivia, Brazil, and Paraguay.

B. Section ***Roseocereus*** (Backeb.) A. R. Franck, comb. et stat. nov. *Eriocereus* (A. Berger) Riccob. subg. *Roseocereus* Backeb., Blätt. Kakteenf., 1936–6. 1936. *Roseocereus* (Backeb.) Backeb., Blätt. Kakteenf. 1938–6. 1938.—TYPE: *Harrisia tetracantha* (Labour.) D. R. Hunt.

1 species; Eastern Cordillera of Bolivia.

INFRAGENERIC KEY FOR HARRISIA

1. Seeds oblong, >0.5 mm longer than wide, glossy; subg. *Harrisia*
   2. Stigma lobes 0.7–1.0 mm; fruits indehiscent; Caribbean; sect. *Harrisia*
      3. Ribs 8–14; stems erect to clambering; young spines yellow-brownish .....series *Harrisia*
      3. Ribs 5–7; stems prostrate to pendent; young spines bright reddish.. series *Earlei*
   2. Stigma lobes 1.2–2 mm; fruits dehiscent; Caatinga of Brazil ..... sect. *Adscendens*

1. Seed length & width subequal, differing by <0.5 mm, semi-matte; subg. *Eriocereus*
   4. Shrubs; stems curvaceous, arching, scrambling or clambering; ribs 3–8; Gran Chaco and adjacent areas .....sect. *Eriocereus*
   4. Trees; stems erect; ribs 7–9; dry inter-Andean valleys of Bolivia.. sect. *Roseocereus*

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TABLE 2.1. Statistics for the alignments of the four markers used in the phylogenetic analyses. Tree length, consistency index (CI), and retention index (RI) are reported from the maximum parsimony analyses.

	atpB-rbcL IGS	rpl16 intron	isi1	nhx1	Combined 4-marker
No. of species	39	40	35	35	40
Alignment length (bp)	1250	869	863	484	3466
% missing data	0	3.8	14.4	4.2	10.2
Avg. %AT content	74.2	68.4	59.6	63.7	66.2
Parsimony informative sites (bp)	56	58	51	55	220
Parsimony uninformative sites (bp)	70	46	50	48	214
Parsimony informative gaps	16	12	2	8	38
Parsimony uninformative gaps	20	14	10	7	51
Tree Length	237	177	164	167	819
CI/RI	0.78/0.85	0.83/0.91	0.74/0.86	0.83/0.85	0.72/0.81

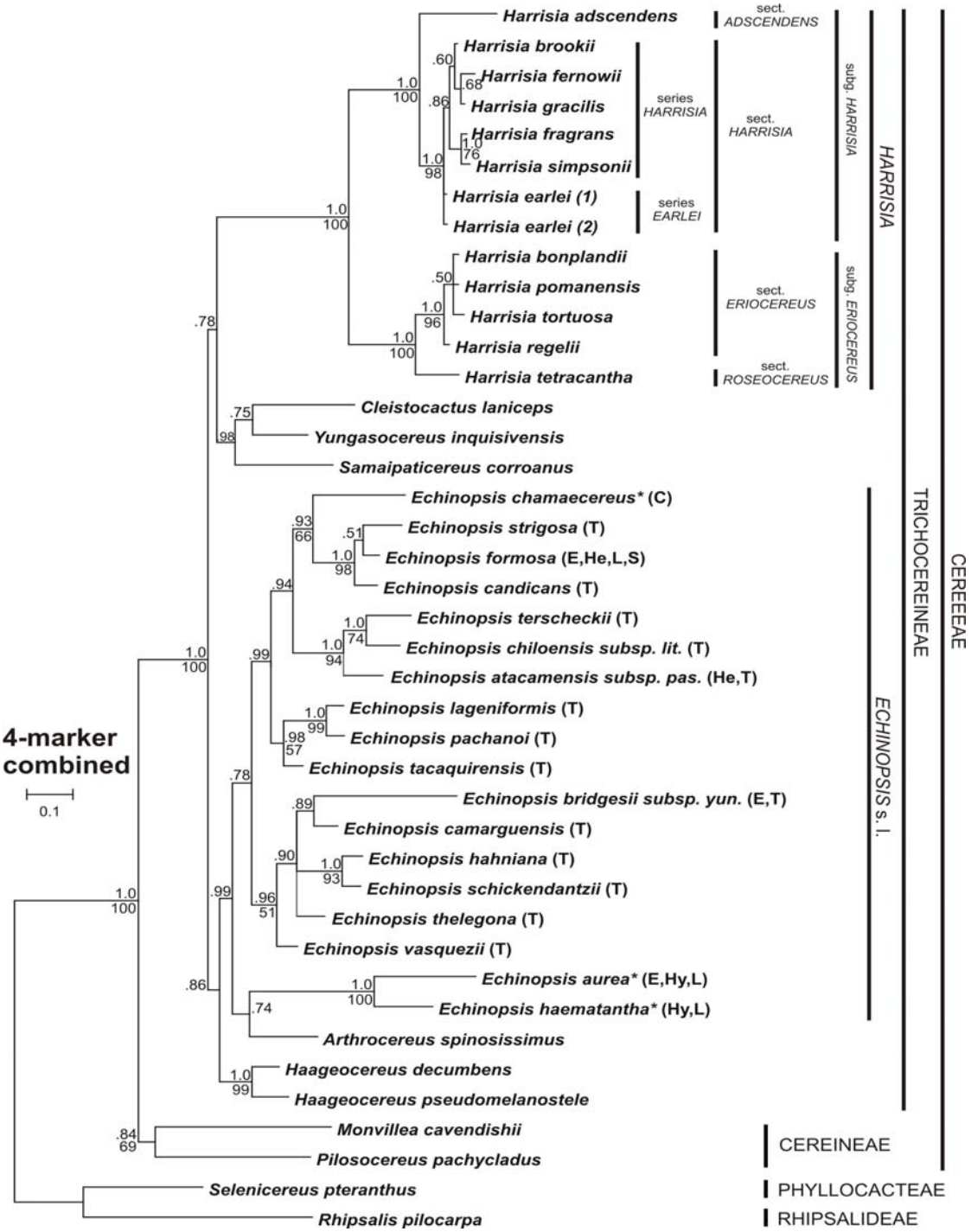

Figure 2.1. Bayesian tree obtained from the combined four-marker alignment. Bayesian posterior probabilities and PAUP bootstrap percentages above 50 are above and below branches, respectively. Asterisks indicate species of *Echinopsis* with radial stamen arrangement. Abbreviations following samples of *Echinopsis* represent classifications or synonyms in segregate genera (Britton & Rose 1920; Backeberg 1977; Anderson 2001; Hunt et al. 2006): C=*Chamaecereus*, E=*Echinopsis* s. s., He=*Helianthocereus*, Hy=*Hymenorebutia*, L=*Lobivia*, S=*Soehrensia*, and T=*Trichocereus*.

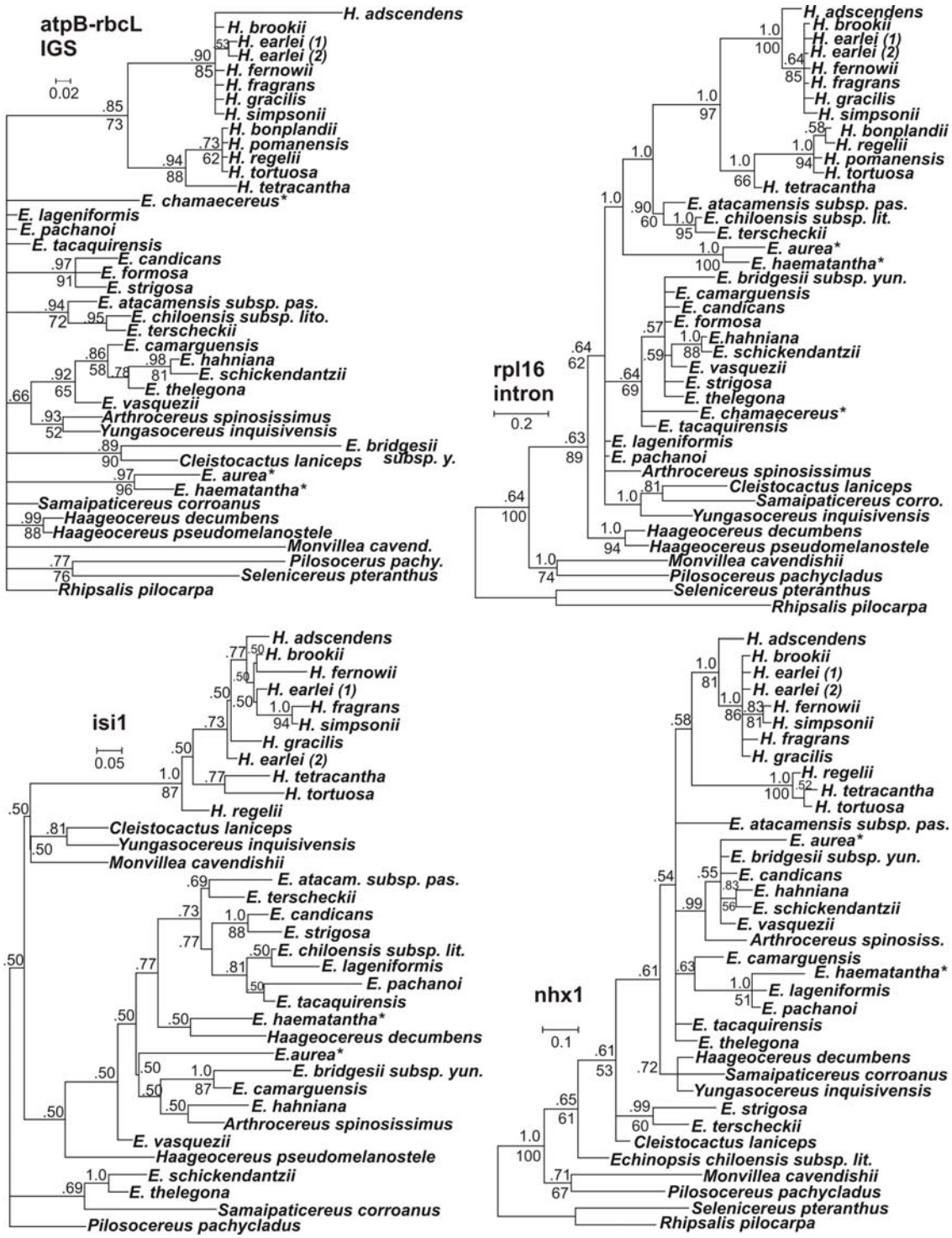

Figure 2.2. Bayesian tree obtained from the each of the four markers. Bayesian posterior probabilities and PAUP bootstrap percentages above 50 are above and below branches, respectively. Asterisks indicate species of *Echinopsis* with radial stamen arrangement. Generic abbreviations are *E*. for *Echinopsis* and *H*. for *Harrisia*.

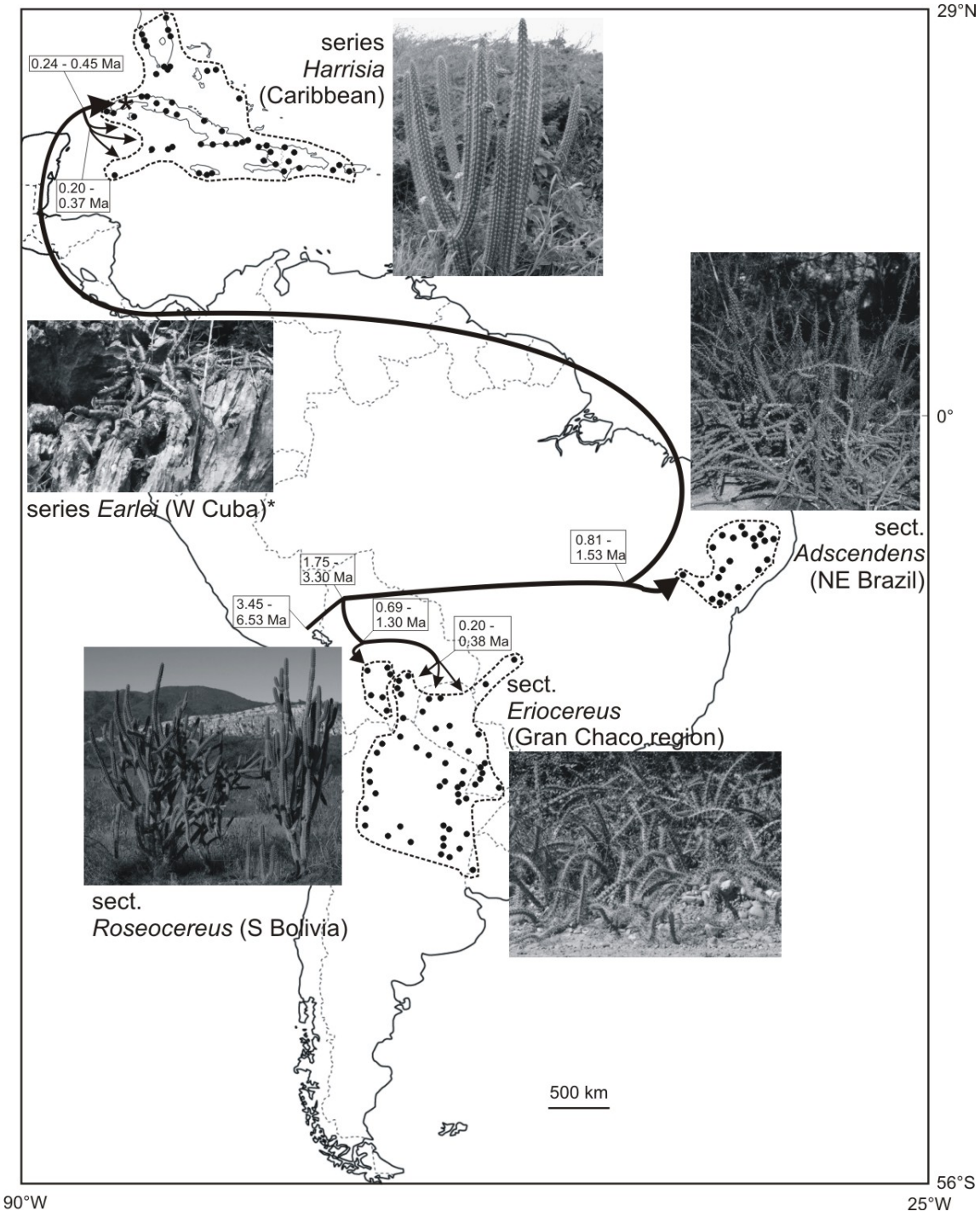

Figure 2.3. Distribution, phylogenetically imposed biogeographic scenario, and divergence estimates of *Harrisia*. Distributions are based on Britton & Rose (1920), Kiesling (1996), Antezana & Navarro (2002), Eggli (2002), Taylor & Zappi (2004), and herbarium specimens cited in Chapter 4. The range of each infrageneric taxon is enclosed in a dashed line except for series *Earlei* which is indicated by an asterisk. Photo of series *Earlei* by G. Ardisson; photo of sect. *Adscendens* by Nigel P. Taylor (K); and photos of sect. *Eriocereus* and sect. *Roseocereus* by Michael Nee (NY).

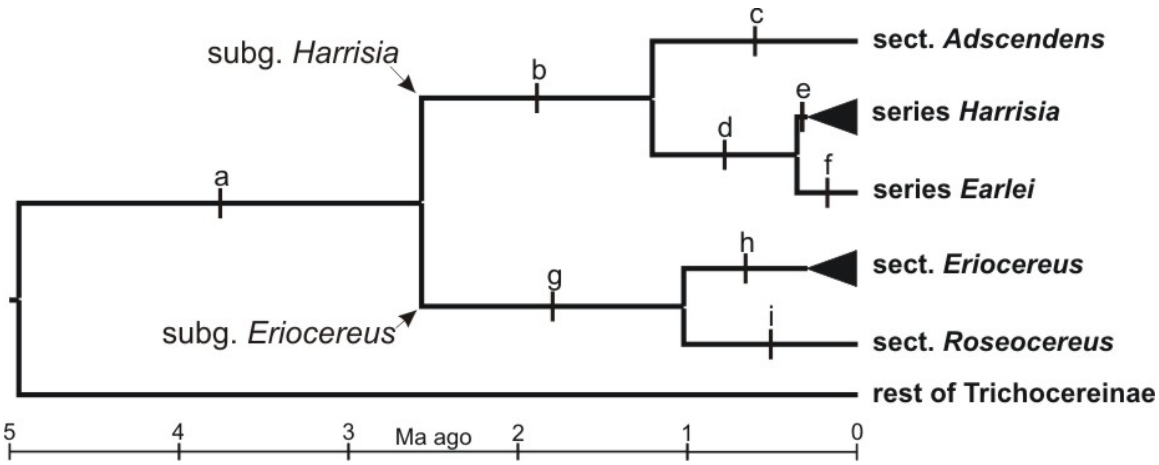

Figure 2.4. Overall phylogeny of *Harrisia*. The scale bar represents divergence estimates from the 6.5 Ma calibration and vertical bars are mapped for morphological clade synapomorphies: a - cavernous HMR, b - seeds oblong, c - stems flexible and glaucous, d - stems green, relatively rigid, styles relatively short, and fruit indehiscent, e - habit erect and ribs 8–14, f - habit prostrate and ribs 5–7, g - seed length and width subequal, h - habit shrubby, stems flexible, and ribs 3–8, i - habit arborescent, stems relatively rigid, and ribs 7–9.

CHAPTER 3: RELATIONSHIPS AMONG THE CARIBBEAN SPECIES OF *HARRISIA* (SECT. *HARRISIA*) USING AFLP AND SEVEN DNA REGIONS

INTRODUCTION

The Caribbean region (cf. Myers et al. 2000) contains a complex assembly of islands, for which permanently emergent land has only been available since the middle Eocene (Iturralde-Vinent 2006), consistent with the age of the oldest palynofloras (Graham 2003a) and the arrival of the mangrove genus *Rhizophora* L. in the Caribbean (Graham 2006). Because of the diverse geology and geographic isolation, approximately half of the vascular flora of the Caribbean is endemic (Takhtajan 1986; Santiago-Valentin & Olmstead 2004).

The native cactus flora (Cactaceae) of the Caribbean region includes ~75 species in 17 genera. Most of the species, as well as three of the genera, are endemic to the region. The most species rich cactus genera in the Caribbean are *Consolea* Lem., *Harrisia* Britton, *Leptocereus* (A. Berger) Britton & Rose, *Melocactus* Link & Otto, *Opuntia* Mill., and *Pilosocereus* Byles & G. D. Rowley, together comprising ~60 species.

Based on phylogenetic context several cactus genera appear to have radiated from Mexico into the Caribbean, such as *Acanthocereus* (Engelm. ex A. Berger) Britton & Rose (Korotkova et al. 2010; Bárcenas et al. 2011; Hernández-Hernández et al. 2011), *Cylindropuntia* (Engelm.) F. M. Knuth (Griffith & Porter 2009), *Mammillaria* Haw. (Crozier 2005; Bárcenas et al. 2011), *Opuntia* (Majure et al. 2012), and *Stenocereus* (A.

Berger) Riccob. (Bárcenas et al. 2011). *Dendrocereus* Britton & Rose and *Leptocereus* may have been derived from northern Andean taxa (Hernández-Hernández et al. 2011). Three genera (*Harrisia*, *Melocactus*, and *Pilosocereus*) likely radiated from northeast Brazil to reach the Caribbean, possibly by dispersing along northern South America and Central America (Chapter 2).

Little is known about the phylogenetic relationships and dispersal patterns of cacti within the Caribbean. *Harrisia* sect. *Harrisia* comprises 11 species endemic to the Greater Antilles, the Bahamas, and Florida. The species are closely related and estimated to have speciated in the last 370 Ka (Chapter 2). Relationships among the species of *Harrisia* in the Caribbean are poorly known, and, consequently, dispersal routes also cannot be proposed. Most of the species were described or evaluated by Britton & Rose (1920), which remains the most recent in-depth study of the group.

Britton & Rose (1920) relied heavily on three characters to separate the Caribbean species of *Harrisia*—color of the flower hairs, flower bud shape, and petal margins. The utility of these characters is likely dubious (see Chapter 4) and thus uninformative of species relationships. Perhaps because of this, Hunt et al. (2006) proposed that only one species should be recognized in the Caribbean.

This work was initiated to investigate the morphological and molecular diversity of *Harrisia* in the Caribbean in order to reveal species relationships and infer dispersal routes. Morphology was characterized from herbarium specimens, live plant photos, and field studies. DNA sequences were obtained from three plastid regions and four nuclear regions. Amplified fragment length polymorphisms (AFLP) from ten different primer sets were also examined. The results of the analyses are used to invoke a biogeographic

scenario and comment on species delimitation. All species of sect. *Harrisia* recognized here were sampled, though not for all analyses and DNA regions.

MATERIALS & METHODS

Morphology. Approximately 200 herbarium specimens of *Harrisia* from the Caribbean were examined (Chapter 4). Field studies were carried out in the Cayman Islands, Dominican Republic, Florida, and Jamaica. Photos of live plants were also consulted (K. A. Bradley, pers. comm.; E. Guerrero, pers. comm.; Z. Rigerski, pers. comm.; N. Taylor, pers. comm.).

Datasets. Phylogenetic analyses were performed on DNA sequences from a seven-marker alignment (*at103*, *isi1*, *nhx1*, *xdh1*, *atpB-rbcL* IGS, *rpl16* intron, *ycf1*), four-nuclear marker alignment (*at103*, *isi1*, *nhx1*, *xdh1*), and a three-plastid marker alignment (*atpB-rbcL* IGS, *rpl16* intron, *ycf1*). The ingroup consisted of 21 samples from 12 species of sect. *Harrisia* (Appendix D). The outgroups included one species each from sect. *Adscendens*, sect. *Eriocereus*, and sect. *Roseocereus*, and two species from *Echinopsis* s. l. (Appendix D) that exhibited a close relationship with *Harrisia* in previous plastid phylogenetic analyses (Chapter 2). A few herbarium specimens were also sequenced for the *ycf1* marker. AFLP data from ten primer sets were scored for 21 samples from eight species of sect. *Harrisia*.

DNA Extraction. The DNA extraction and PCR reagent concentrations followed Chapter 1 (Doyle & Doyle 1987; Shepherd & McLay 2011). For DNA extraction of herbarium specimens, 5 mM of N-phenacylthiazolium bromide (PTB) was added to the CTAB buffer and the incubation with the CTAB buffer was extended to 6 hours.

PCR. All amplifications had an initial denaturation of 94°C for 3 min., 40 PCR cycles (94°C for 45 s; annealing for 45 s; and extension at 72°C), and a final extension of 72°C for 5 min. Detailed PCR conditions for *isi1, nhx1, atpB-rbcL* IGS, *rpl16* intron, and *ycf1* follow Chapter 1. For *xdh1* and *at103*, the annealing temperature was 56°C for 10 cycles, 54°C for 10 cycles, and 52°C for 20 cycles. Their extension time was 1 min 20 s.

Markers & Primers. All newly designed primers are listed in Table 3.1. The *at103* primers used were from Li et al. (2008). The amplicon is from bp 21,077,501–21,077,860 of chromosome 3 of *Arabidopsis thaliana* (L.) Heynh. (CP002686.1), spanning part of exon 3, the entire third intron, and part of exon 4. Primers for *xdh1* (Table 3.1) were developed from alignments of *Pereskia portulacifolia* (L.) DC. (EU264349.1), *Portulaca grandiflora* Pfeiff. (EU264350.1), and *Talinum paniculatum* (Jacq.) Gaertn. (EU264348.1). The amplicon is from bp 16,622,894–16,623,943 of chromosome 4 of *A. thaliana* (CP002687.1) and is entirely within exon 4 of *xdh1*.

Primers used for the *atpB-rbcL* intergenic spacer (IGS) were from Savolainen et al. (1994). Primers for the *rpl16* intron were from Jordan et al. (1996), Kelchner & Clark (1997), and Chapter 1. Primers for *isi1*, *nhx1*, and *ycf1* were from Chapter 1. Additional

primers were used for *isi1* and *ycf1* (Table 3.1). All primers were obtained from Integrated DNA Technologies, Inc., Iowa, U.S.A.

Sequencing & Alignment. PCR products were analyzed on agarose gels stained with ethidium bromide. Bands were excised and DNA extracted with QIAquick Gel Extraction Kit (QIAGEN, Valenica, California). Sequencing analyses were carried out at Arizona State University with a 3730 DNA Analyzer (Applied Biosystems, Carlsbad, California). Electropherograms were analyzed with Sequence Scanner Software (Applied Biosystems). MEGA 5 (Tamura et al. 2011) was used for sequence editing, alignment, translation, AT-content. Gaps were coded according to the simple indel coding method (Simmons & Ochoterena 2000). Alignments were deposited in TreeBASE (12820). Sequences were deposited in GenBank (Appendix D).

Bayesian Inference. Bayesian inference was performed with Mr. Bayes version 3.1 (Ronquist & Huelsenbeck 2003) with two simultaneous runs and four chains. Nucleotide substitution models were selected using the Akaike information criterion (Akaike 1974) by applying the likelihood score estimated in PAUP (Posada and Crandall 1998). The models selected were GTR+G for the *atpB-rbcL* IGS, GTR+I for the *rpl16* intron, F81+I for *ycf1*, GTR for *nhx1*, and HKY for *at103*, *isi1*, and *xdh1*. Indels were partitioned as a restriction site data set with coding set to variable. Model parameters were unlinked between partitions. One millions generations were run with sampling ever 100th generation. The first 25% of trees were discarded as burn-in. Posterior probabilities (PP)

0.50 and above were reported. PP values of 0.50–0.89 were considered as weak support, 0.90–0.94 as moderate support, and 0.95–1.0 as strong support.

Parsimony Analysis. Maximum parsimony analyses were performed with PAUP* version 4.0b10 (Swofford 2003) using a heuristic search with 1000 replicates, random sequence addition, tree-bisection-reconnection branch swapping, MAXTREES set to no limit, and MULTREES on. Consistency and retention indices were obtained from PAUP. Bootstrapping (BS) was performed with 1000 replicates under the same conditions with the exception that MAXTREES was set to 1000. Bootstrap values (BS) 50% and above were reported. BS values of 50–74% were interpreted as weak support, 75–89% as moderate support, and 90–100% as strong support.

Neighbor-net Networks. Neighbor-net networks of the seven-marker alignment of sect. *Harrisia* were visualized using SplitsTree4 (Huson & Bryant 2006) with support estimated by 1000 bootstrap replicates. Coded indels were treated as a fifth character state. Insertions were arbitrarily scored as T and deletions as C. The AFLP dataset was also visualized in SplitsTree 4 with 1000 bootstrap replicates.

Haplotype Network. The program TCS version 1.21 (Clement et al. 2000) was used to infer a haplotype network with a 95% probability of parsimony. Haplotype inference followed Clark (1990). Coded indels were treated as a fifth character state. Insertions were arbitrarily scored as T and deletions as C. The indel regions were then deleted to

remove gaps. The analysis was performed on the seven-marker, four-nuclear marker, and three-plastid marker alignments of sect. *Harrisia*.

AFLP – Gel Extraction. To isolate high-quality genomic DNA, samples for AFLP analysis were further purified on a 1.3% low-melting point agarose (Promega, Wisconsin, U.S.A.) gel for 1 hour at 70 volts. Only samples using healthy, fresh stem tissue were included. Samples from diseased tissue, roots, or herbarium specimens were not included in the AFLP analysis.

Genomic bands co-migrating near (~20 kbp) the 23 kbp band of *HindIII* digested Lambda phage were excised and incubated in 200 µL of TE buffer (10 mM Tris-base, 1 mM EDTA, ph 8) at 65°C for 5 min. Then 200 µL of phenol (10 mM Tris-base, 1% 8-hydroxyquinolone, pH 7.9) were added and the tube centrifuged. The supernatant was pipetted off into a new tube. Another 125 µL of TE buffer was added to the phenol and centrifuged. Both supernatants were combined and extracted with 500 µL 24:1 chloroform:isoamyl alcohol. The DNA extract was then incubated with 30 µL of 7.5 M ammonium acetate and 1 mL of ethanol overnight at -20°C. After centrifugation, the DNA extract was washed with 700 µL of 70% and 100% ethanol. The DNA extract was suspended in 12 µL of water.

AFLP – Digestion & Ligation. Approximately 40 ng of gel-purified genomic DNA extract was digested using 10 units each of *EcoRI* and *MseI* with 2 µL of NEBuffer 4 (New England Biolabs, Massachusetts, U.S.A.) in a 20 µL reaction. Digestion occurred at 37°C for 3 hours. The *EcoRI* linkers (5′ – CTCGTAGACTGCGTACC – 3′ and 5′ –

AATTGGTACGCAGTCTAC – 3′) and *MseI* linkers (5′ – GACGATGAGTCCTGAG – 3′ and 5′ – TACTCAGGACTCAT – 3′) were each annealed in a 20 µL reaction by combining the two sticky-ended complementary linker strands in TE buffer at a final concentration of 33 µM. The linker reactions were heated to 95°C for 5 min and incrementally cooled to 10°C over 15 min. To the 20 µL digestion reaction, 4 µL of ligation buffer, 75 pM of each linker, and 1 unit of T4 DNA ligase were added immediately for a final volume of 40 µL and incubated for 12 hours at 16°C.

AFLP – Preselective PCR Amplification. The digestion-ligation reaction was diluted with 160 µL of water. Five µL of the diluted digestion-ligation reaction were added to a 20 µL volume PCR reaction, with same reagent concentrations as (Chapter 1). Initial denaturation occurred at 94°C for 2 min followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min 30 s, and a final extension of 5 min. The 20 µL reaction was then diluted with 80 µL of water. The preselective PCR primers were EcoRI+A (5′ – GACTGCGTACCAATTC<u>A</u> – 3′) and Mse+C (5′ – GATGAGTCCTGAGTAA<u>C</u> – 3′). The underlined part of the primers are after the restriction site and thus selective for a subset of the restricted fragments.

AFLP – Selective PCR Amplification. Selective PCR was carried out using 5 µL of the diluted preselective reaction in a 20 µL volume. Initial denaturation at 94°C for 2 min was followed by 35 cycles of denaturation at 94°C for 45 s, touchdown annealing at 65–56°C for 10 cycles and at 56°C for 25 cycles for 45 s, extension at 72°C for 1 min for 10 cycles and 1 min 30 s for 25 cycles, and a final extension of 10 min. Selective PCR

primers for the *EcoRI* ends were fluorescently labeled with 6-FAM™ at the 5′ end. The selective primers EcoRI+ACT (5′ – GACTGCGTACCAATTCACT – 3′) and EcoRI+ATAG (5′ – GACTGCGTACCAATTCATAG – 3′) were used. Selective PCR primers for the *MseI* ends were unlabeled and were 5′ – GATGAGTCCTGAGTACN – 3′. The N site represents 3–4 selective nucleotides that varied among the ten *MseI* primers: Five *MseI* amplification primers were used with EcoRI+ACT: Mse+CAAG, Mse+CGAT, Mse+CGC, Mse+CTCG, and Mse+CTTC. Five *MseI* amplification primers were used with EcoRI+ATAG: Mse+CAC, Mse+CATAG, Mse+CCG, Mse+CGGC, and Mse+CTG.

AFLP Analysis. AFLP error rates were calculated by counting the number of differing allele calls divided by the total number of alleles for the entire dataset (Bonin et al. 2004; Holland et al. 2008), with one duplicate per primer set using the *H. caymanensis* sample. The raw data from the ten-primer pair dataset was analyzed with three different settings in GeneMapper (Applied Biosystems): the default settings (bin width 1, rfu >50, alleles >50bp, polynomial degree 3), the 0.5 bin width setting (default settings except bin width changed to 0.5), and the optimized settings (bin width 0.5, rfu >100, alleles >100bp, polynomial degree 2).

RESULTS

Alignment. The *at103* alignment consisted of 198 bp of exon 3, the entire third intron (179 bp), and nine bp of exon 4. The *isi1* alignment consisted of 96 bp of the third intron,

the entire fourth exon (74 bp), and 155 bp of the fourth intron. The *nhx1* alignment was 466 bp, entirely within the second intron. The *xdh1* alignment was 466 bp, entirely within exon 4. Exon and intron numbers are based on *A. thaliana* (AGI 2000). The *atpB-rbcL* alignment was entirely within the IGS. The *rpl16* alignment was entirely within the intron. The *ycf1* alignment was 792 bp of the coding region. The combined alignment of all seven markers was 3623 bp in length (Table 3.2).

Sequence Characteristics. The 26-sample seven-marker alignment had 3.6% variable nucleotides (Table 3.2). Within sect. *Harrisia*, the most variable markers were *isi1* and *ycf1* (Table. 3.3). Within sect. *Harrisia*, the two samples of *H. earlei* (=series *Earlei*) had the shortest pairwise genetic distance with *H. adscendens* (=sect. *Adscendens*).

With respect to subg. *Harrisia*, only two substitutions were observed in *H. earlei*. One substitution in the nuclear *at103* gene was recovered from one of two samples of *H. earlei*. This substitution was also observed in two samples of *H. gracilis*. Both samples of *H. earlei* uniquely shared a substitution with *H. adscendens* in the plastid *atpB-rbcL* IGS. This was the only substitution found in the *atpB-rbcL* IGS of sect. *Harrisia*. All other informative sites shared between *H. adscendens* and sect. *Harrisia* were found in all samples of sect. *Harrisia*. Only two mutations were observed in the Cuban species *H. taylorii* as well. All other samples of series *Harrisia* had three or more substitutions with respect to the most frequent state.

All Cuban samples of series *Harrisia* (*H. fernowii*, *H. taylorii*, and *H. taetra*) had the same plastid sequences. The ~95-year-old herbarium specimen of *H. eriophora* from Havana Prov. (*León 7179* (NY)) had the same *ycf1* sequence as the Cuban samples.

Within Florida, all samples of *H. fragrans* and *H. simpsonii* shared the same plastid haplotype except for samples of *H. fragrans* from St Lucie Co. (*Franck 472*). This sample alone shared the same plastid haplotype as all samples of *H. aboriginum*. Multiple samples of *H. fragrans* from St. Lucie Co. (*Franck 472*) and Volusia Co. (*Franck 473*) were sequenced to verify this pattern but not included in the analyses. All samples from the Dominican Republic (*H. divaricata*) and Puerto Rico (*H. portoricensis*) shared the same nucleotide haplotype. The sample *H. divaricata (3)* and the ~25-year-old herbarium specimen from Azua Prov. (*Zanoni 31122* (USF)) had the same *ycf1* sequence. The ~45-year-old herbarium specimen of *H. portoricensis* from Santiago Prov. (*Liogier 11241* (NY)) had the same *ycf1* sequence as the other two samples of *H. divaricata (1,2)*. One substitution in the *isi1* sequences was found in all samples from the Bahamas (*H. brookii*), southeast Cuba (*H. fernowii*, *H. taylorii*), the Dominican Republic (*H. divaricata*), Jamaica (*H. gracilis*), and Puerto Rico (*H. portoricensis*). Samples from the Bahamas (*H. brookii*) and the Cayman Islands (*H. caymanensis*) shared the same *xdh1* allele characterized by three substitutions.

Bayesian & Parsimony Analyses. The seven-marker alignment strongly supported the genus *Harrisia*, its two subgenera, and the sect. *Harrisia* as monophyletic (Figs. 3.1 & 3.2). *Harrisia earlei* was recovered as sister to series *Harrisia* with low support (PP 0.69) in the Bayesian tree and in the majority-rule consensus parsimony tree. Within series *Harrisia*, three clades were recovered with low support—one clade (PP 0.77) consisted of three Cuban species (*H. fernowii*, *H. taetra*, *H. taylorii*), one clade (PP 0.75) contained two Florida species (*H. fragrans* and *H. simpsonii*), and one clade (PP 0.75)

contained samples from Florida (*H. aboriginum*, *H. fragrans*), the Bahamas (*H. brookii*), the Cayman Islands (*H. caymanensis*), Jamaica (*H. gracilis*), Dominican Republic (*H. divaricata*), and Puerto Rico (*H. portoricensis*). Samples from Jamaica (*H. gracilis*), the Dominican Republic (*H. divaricata*), and Puerto Rico (*H. portoricensis*) were moderately supported as one clade (PP 0.83, BS 86). The samples from the Dominican Republic (*H. divaricata*) and Puerto Rico (*H. portoricensis*) formed a clade (PP 1.0). Samples from the Bahamas (*H. brookii*) and the Cayman Islands (*H. caymanensis*) formed a clade (PP 1.0, BS 87).

The three-plastid marker alignment and four-nuclear marker alignment both produced similar but less resolved topologies to the seven-marker alignment with few notable differences. In the plastid trees, *H. caymanensis* and *H. gracilis* formed a clade (PP 0.96). In the nuclear trees, two samples of *H. simpsonii* formed a clade with *H. fernowii* (PP 0.99).

DNA Sequence Neighbor-net Networks. In the seven-marker alignment, *H. earlei* formed an isolated lineage (BS 76) (Fig. 3.3). Within series *Harrisia*, the Cuban species (*H. fernowii*, *H. taylorii*, and *H. taetra*) were recovered as a clade (BS 57). Two southeast Cuban species (*H. fernowii*, *H. taylorii*) received support as a clade (BS 56). The Florida species (*H. aboriginum*, *H. fragrans*, *H. simpsonii*) formed a clade (BS 61). One sample of *H. fragrans* claded with *H. aboriginum* (BS 75). The other two samples of *H. fragrans* formed a clade with *H. simpsonii* (BS 56). The samples from the Dominican Republic (*H. divaricata*) and Puerto Rico (*H. portoricensis*) formed a clade (BS 89). Samples from the Bahamas and the Cayman Islands formed a clade (BS 90).

The three-plastid marker alignment and four-nuclear marker alignment both produced similar but less resolved topologies to the seven-marker alignment with few notable differences. In the plastid tree, *H. caymanensis* and *H. gracilis* formed a clade (BS 53) and all the Cuban species formed a clade with *H. fragrans* and *H. simpsonii* (BS 70). In the nuclear tree, two samples of *H. simpsonii* formed a clade with *H. fernowii* and *H. taetra* (BS 64).

Haplotype Network. There were not enough polymorphisms to recover an informative network using either the four-nuclear marker alignment or the three-plastid marker alignment. Only the haplotype network from the seven-marker alignment (Fig. 3.4) is further reported.

The two samples of *H. earlei* formed an isolated lineage (Fig. 3.4). The Florida species (*H. aboriginum*, *H. fragrans*, *H. simpsonii*) separated into an isolated lineage. The Cuban species of series *Harrisia* (*H. fernowii*, *H. taylorii*, and *H. taetra*) were joined as one lineage. The samples from Jamaica (*H. gracilis*), Dominican Republic (*H. divaricata*), the Bahamas (*H. brookii*), and Cayman Islands (*H. caymanensis*) were isolated into one lineage. Within this lineage, were three more lineages, one of Jamaica (*H. gracilis*), one of the Dominican Republic (*H. divaricata*) and Puerto Rico (*H. portoricensis*), and one of the Bahamas (*H. brookii*) and the Cayman Islands (*H. caymanensis*). Two ambiguities (closed circles) were found in the network.

AFLP Error Rates. AFLP percentage error rates averaged 4.89±2.08 for the optimized settings, 5.64±2.03 for the 0.5 bin width setting, and 6.89±2.43 for the default

settings. The error rates were lowest for the EcoRI+ATAG/Mse+CATAG primer set and highest for the EcoRI+ACT/Mse+CGC primer set. Only the error rate of the optimized settings was within the range of reported AFLP error rates of 2–5% (Bonin et al. 2004; Meudt & Clarke 2007; Holland et al. 2008).

AFLP Neighbor-net Network. All three analyses (default settings, 0.5 bin width, and optimized settings) produced a neighbor-net network with a similar topology. However, the analysis using the optimized settings had higher overall bootstrap values and lower error rates. Only the neighbor-net network from the optimized settings are further reported.

All species with multiple samples were well-supported except for *H. aboriginum*, which had one sample unplaced within a clade including *H. fragrans* (Fig. 3.5). The species *H. aboriginum* and *H. fragrans* formed a well-supported clade (BS 88). Moderate support (BS 56) was found for *H. simpsonii* and two of three samples of *H. aboriginum*. Samples of *H. gracilis*, *H. divaricata*, *H. taetra*, *and H. caymanensis* formed a clade (BS 59). Support was also found for *H. gracilis* and 3 of 4 samples of *H. divaricata* (BS 78). The two samples of *H. earlei* (series *Earlei*) formed the only clade with BS 100 and were isolated, having no other supported relationships with other samples.

DISCUSSION

Species Relationships of Harrisia in the Caribbean. The molecular and morphological data support *H. earlei* (=series *Earlei*) as a lineage distinct from and sister

to the rest of the species of *Harrisia* in the Caribbean (series *Harrisia*). The neighbor-net and haplotype networks (Figs. 3.3–3.5) showed *H. earlei* as an isolated lineage. The prostrate habit, 5–7 ribs, and bright red young spines of *H. earlei* are unique within the Caribbean species of *Harrisia*.

Harrisia earlei has been interpreted as morphologically intermediate between the South American species and series *Harrisia* of the Caribbean (Britton & Rose 1920). *Harrisia adscendens* (= sect. *Adscendens*) from northeast Brazil is the most closely related species to the Caribbean species (sect. *Harrisia*) (Fig. 3.1). *Harrisia adscendens* and *H. earlei* overlap in rib number (6–10 and 5–7, respectively) and both have spines that are bright red when first emerging. Additionally, both have mature stems that become rounded due to the shallow sulci and low ribs.

The sister relationship between *H. earlei* and series *Harrisia* was weakly supported in the Bayesian tree (PP 0.69, Fig. 3.2). Additional sampling from the other west Cuban species, *H. eriophora* and *H. taetra*, would be ideal to corroborate the basal position of *H. earlei*, also from west Cuba.

Within series *Harrisia,* three main groups arose from the molecular phylogenies (Figs. 3.1–3.4): a Florida group, a Cuban group, and a southern/eastern Greater Antilles-Bahamas (SEGAB) group. These three groups were recovered as monophyletic in some analyses but not all (Figs. 3.2–3.5). The non-monophyly may be due to poor resolution but admixture or incomplete lineage sorting could also be responsible..

The Cuban group consists of *H. fernowii*, *H. taetra* and *H. taylorii*, but probably includes *H. eriophora* (as indicated by the *ycf1* sequence of *León 7179* (NY)). The Cuban group received monophyletic support in the Bayesian tree and neighbor-net network

(Figs. 3.2–3.3) but not in the haplotype network (Fig. 3.4) The neighbor-net networks (Fig. 3.3 & 3.5) placed the Cuban group nearest to *H. earlei*. However, the placement of the Cuban group in the haplotype network was ambiguous (Fig. 3.4). All of the Cuban samples of series *Harrisia* had the same plastid sequences. There is no apparent morphological apomorphy for the Cuban group. The longer spines on the lower parts of the stem in the Cuban group is probably plesiomorphic since most species of the SEGAB group also share this trait (except for *H. gracilis* which has short spines throughout).

Given their short genetic and geographic distance from *H. earlei*, it is likely that the Cuban group is most closely related to the progenitors of the Florida and SEGAB groups. Both *H. eriophora* and *H. taetra* from west Cuba have fairly large seeds and reddish flowers, characters also found in the Florida group. Both *H. fernowii* and *H. taylorii* from east Cuba are very similar to the SEGAB group, all having relatively small seeds and greenish flowers.

The SEGAB group contains *H. brookii* (Bahamas), *H. caymanensis* (Cayman Islands), *H. divaricata* (Hispaniola), *H. gracilis* (Jamaica), and *H. portoricensis* (Puerto Rico). This group is not morphologically cohesive unless *H. fernowii* and *H. taylorii* (SE Cuba) are also included, which is supported somewhat by the haplotype network, sequence data of *isi1*, and geography. With their inclusion, this group is characterized by the greenish flowers and relatively small seeds (largest in *H. brookii*).

In the neighbor-net (Fig 3.3), Bayesian (Fig. 3.2), and parsimony trees (Fig. 3.2), *H. portoricensis* was nested within *H. divaricata*. The spines of *H. portoricensis* densely overlap on the stem whereas the spines of *H. divaricata* are more sparse on the stem. Though regarded as endemic to Puerto Rico, herbarium specimens clearly indicate the

presence of *H. portoricensis* on Hispaniola (e.g. *Judd et al. 3002* (FLAS, JBSD), *Liogier 11241* (GH, NY, US) and *Zanoni et al. 33542* (JBSD); Chapter 4). However, the *ycf1* sequence of *Liogier 11241* (NY) was the same as two samples of *H. divaricata (1,3).*

A unique *xdh1* allele characterized by three substitutions was shared by *H. brookii* and *H. caymanensis* and appears to strongly favor their relationship. In phylogenetic analyses without the *xdh1* marker (not shown), the relationship between these species is more ambiguous leaving them somewhat unaffiliated within the SEGAB group. The spines on the lower stem of *H. brookii* and *H. caymanensis* are longer but do not appear to become as thick as those of *H. divaricata*, *H. fernowii*, and *H. portoricensis*. All the analyses supported a monophyletic *H. gracilis* (Figs. 3.2–3.5), which has relatively short spines on the lower and upper stem. *Harrisia gracilis* was supported as sister to *H. divaricata* and *H. portoricensis* (Figs. 3.2–3.3 and 3.5)

The Florida group consisted of three species, *H. aboriginum*, *H. fragrans*, and *H. simpsonii*. This group was monophyletic in the seven-marker neighbor-net (Fig. 3.3) and haplotype networks (Fig. 3.4). The Florida was fairly distinct in the AFLP analysis (Fig. 3.5) but paraphyletic in the Bayesian tree (Fig. 3.2). The Florida species are morphologically characterized by their relatively large seeds, reddish flowers, and relatively short spines which are not noticeably longer on the basal parts of the stems.

The samples of *H. fragrans* were monophyletic in the AFLP analysis (Fig. 3.5) and the samples of *H. aboriginum* were monophyletic in the Bayesian tree (Figs 3.2). The plastid data strongly support a relationship between *H. aboriginum* and the central peninsular population of *H. fragrans* in St. Lucie Co. This population of *H. fragrans*, which is morphologically similar to the other populations of *H. fragrans*, has apparently

experienced plastid introgression from *H. aboriginum*. The plastid haplotype shared by *H. aboriginum* and the St. Lucie Co. population of *H. fragrans* is characterized by one substitution and one indel in the *rpl16* intron and one substitution and one indel in the *ycf1* region.

In the Bayesian tree (Fig. 3.2), *H. aboriginum* grouped with the SEGAB group. *Harrisia aboriginum* does share the green pericarpel with the SEGAB group. The Bayesian tree (Fig. 3.2) and seven-marker neighbor-net network (Fig. 3.3) recovered a monophyletic clade of *H. fragrans* and *H. simpsonii*, excluding the sample of *H. fragrans* exhibiting plastid introgression from *H. aboriginum*. Both *H. fragrans* and *H. simpsonii* have reddish pericarpels and red mature fruits. These two species also tend to have longer spines than *H. aboriginum*. Examination of herbarium specimens and live plants does not reveal a reliable character to distinguish *H. fragrans* and *H. simpsonii*. These two names may be better treated as one species, though the AFLP data contradicts this.

Because the bi-furcating trees employed by the Bayesian and parsimony methods could potentially obscure complex patterns in the data, phylogenetic networks (i. e. neighbor-net) were used to enable a better summary of the information in the data especially in the presence of conflicting signals (Huson & Bryant 2006). Phylogenetic networks are particularly useful for groups that may have be experiencing reticulate evolution (e. g. hybridization) or deep coalescence (Linder & Rieseberg 2004). In addition to the plastid regions, four low-copy nuclear regions were sequenced to maximize the number of potentially independent regions available to reconstruct reticulate evolution (Linder & Rieseberg 2004). Although more DNA regions would likely improve understanding of the relationships, an increase in sampling might be more

valuable given the paucity of samples of many species used in the phylogenetic analyses. Since the origin of *Harrisia* and its highest diversity is in Cuba, denser sampling is needed in Cuba.

Since the AFLP data presumably reflects patterns throughout the genome, the AFLP data should be more sensitive than the sequence data and produce a more robust phylogeny. The AFLP neighbor-net network (Fig. 3.5) produces the same overall groupings as the sequence data (Figs. 3.2–3.5) such as the Florida and SEGAB groups. However, the error rate was on the high end of the range reported in the literature (Bonin et al. 2004; Meudt & Clarke 2007; Holland et al. 2008) and the AFLP data may therefore not be as accurate as the sequence data. Additionally, several samples were not used in the AFLP analysis because their DNA extractions were of low quality.

Origin & Dispersal of Harrisia in the Caribbean. *Harrisia* likely first colonized west Cuba, where *H. earlei* is endemic, based on several lines of evidence. *Harrisia* is most diverse in Cuba and is absent from the Lesser Antilles. *Harrisia earlei* also was recovered as basal in the phylogeny (Fig. 3.2) and is very unique morphologically. *Harrisia* may have reached the Caribbean from northern South America or nuclear Central America in the last 500 Ka (Chapter 2). As the speciation in the Caribbean occurred in the late Pleistocene when all the islands were in their current positions (Graham 2003b; Iturralde-Vinent 2006), over-water dispersal by animal vectors is the most plausible explanation for the current distribution of *Harrisia* in the Caribbean. The distribution of the Caribbean genus *Leptocereus* was interpreted as vicariant (Areces-Mallea 2003). If overwater dispersal by animal vectors established *Harrisia* in the

Caribbean, then the patterns of bird distribution shared by Central America and the Greater Antilles (Vázquez-Miranda et al. 2007) support colonization of Cuba from an ancestral *Harrisia* in Central America. The colonization of the Caribbean by frugivorous bats (Dávalos 2007) may also have assisted in dispersal.

The Florida group may have originated from a dispersal from west Cuba (Fig. 3.6), as the species of Florida and west Cuba (*H. eriophora* and *H. taetra*) are morphologically similar in seed size and flower color. The SEGAB group then would seem to be derived from east Cuba (Fig. 3.6), as evidenced by their similar seed and flower morphology. However, the resolution provided by the phylogenetic analyses (Figs. 3.2–3.5) is not adequate to support dispersal routes for the Florida or SEGAB group.

Harrisia gracilis may share a recent common ancestor with *H. divaricata* and *H. portoricensis* (Figs. 3.2–3.3 and 3.5), although the seven-marker neighbor-net network (Fig. 3.3) also alludes to a rapid radiation of three lineages within the SEGAB group. *Harrisia portoricensis* must have speciated from *H. divaricata* on Hispaniola and subsequently dispersed to Puerto Rico, given its nested position in the phylogeny (Figs. 3.2–3.4). The similarity between *H. brookii* and *H. caymanensis* in the phylogeny (Figs. 3.2–3.4) is difficult to explain. Perhaps these two species experienced a founder effect from an east Cuba ancestor which had the unique *xdh1* allele that was later lost or remains unsampled in other species. Alternatively, there may have been a long-distance dispersal event between the Bahamas and the Cayman Islands.

The plastid introgression of the *H. aboriginum* haplotype into the St Lucie Co. population of *H. fragrans* (*Franck 472*) suggests these species came in contact historically (Figs. 3.2–3.4 & 3.6). Seeds of *H. aboriginum* probably dispersed across the

peninsula into the population of *H. fragrans*, perhaps when sea levels were 6 m higher 120 Ka ago (Muhs et al. 2003). During this time, the east and west coasts of Florida would have been closer. Plastid introgression in Florida has also been observed in a species of *Chrysopsis* (Nutt.) Elliott (Asteraceae) along the east coast of Florida, which exhibited a haplotype from a morphologically distinct and disjunct species to the west (Clark 2006).

During the last sea level high stand, ca. 120 Ka ago, sea level was likely 6 m above present sea levels (Muhs et al. 2003). The Florida species of *Harrisia* are often found less than 5 m above sea level and nearly all of their present habitat would have been submerged. Under this scenario, these species would have been much further inland. *Harrisia simpsonii* may simply represent the dispersal of *H. fragrans* into the Florida keys after the sea level high stand.

After the sea level high stand 120 Ka ago, sea levels declined to reach 120 m below present levels during the last glacial maximum, ca. 18 Ka ago (Muhs et al. 2003). Coastal habitat would have been much more extensive, and perhaps *Harrisia* had a much greater distribution 18 Ka ago. Arid habitat was probably more widespread during the last glacial maximum as indicated by the fossil distributions of xerophilic vertebrate taxa in the Caribbean region (Pregill and Olson 1981).

Conclusions. The phylogenetic analyses coupled with the morphology have added considerable clarification to the species relationships and dispersal routes of *Harrisia* in the Caribbean region. The species of Cuba and Hispaniola still require additional study to understand relationships and taxonomic limits, as these two geographic regions were

poorly sampled overall. *Harrisia* clearly represents a recent species radiation owing to the similarity in DNA sequences and morphology. The data reveal three species groups: the Cuba, Florida, and SEGAB groups. There is good evidence that *Harrisia* first colonized west Cuba and then dispersed northward and eastward. Further study of other taxonomic groups with recent species radiations in the Caribbean are warranted as there are few phylogenetic and biogeographic studies available to reconstruct the patterns of plant diversity within the Caribbean region.

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TABLE 3.1. New primers used in this study.

REGION	PRIMER NAME	PRIMER SEQUENCE (5′ to 3′)
isi1	isi-HarrF	TTCTATTTCCATCAAGTCAGTTGG
	isi-HarrR	ACTGATCTGATTTGTGAAGATACG
ycf1	ycf-HarrF	TATCAATGAAGATATAGGTCGAAT
	ycf-HarrR	GATATTGATGATGAAATTATTGATGAG
xdh1	xdhF	GGCCCATYATTGATGCTTTCCG
	xdhR	CACCWAGCAACTCCTTGTTCCC
	xdh722R	GCTTTGCAAGATGATACTTCATG

TABLE 3.2. Statistics of the seven-marker combined alignment for 26 samples. Tree length, consistency index (CI), and retention index (RI) were obtained from the parsimony analysis.

	Alignment length (bp)	Parsimony informative nucleotides	Parsimony uninformative nucleotides	Parsimony informative gaps	Parsimony uninformative gaps	Tree length	CI/RI
at103	386	10	6	1	0	-	-
isi1	325	4	11	0	2	-	-
nhx1	466	18	13	2	1	-	-
xdh1	466	4	6	0	0	-	-
atpB-rbcL IGS	867	28	31	11	7	-	-
rpl16 intron	321	4	3	6	0	-	-
ycf1	792	62	62	7	5	-	-
3-plastid	1980	94	96	24	12	253	0.94/0.93
4-nuclear	1643	36	36	3	3	87	0.92/0.91
7-combined	3623	130	132	27	15	346	0.92/0.90

TABLE 3.3. Statistics of the seven markers for sect. *Harrisia*. Parsimony informative is abbreviated to Pars. inf.

	Pars. inf. / variable sites	Pars. inf. / variable indels
at103	1/1	1/1
isi1	5/8	0/1
nhx1	3/3	0/0
xdh1	4/4	0/0
atpB-rbcL IGS	1/1	0/0
rpl16 intron	1/2	2/2
ycf1	5/8	2/2

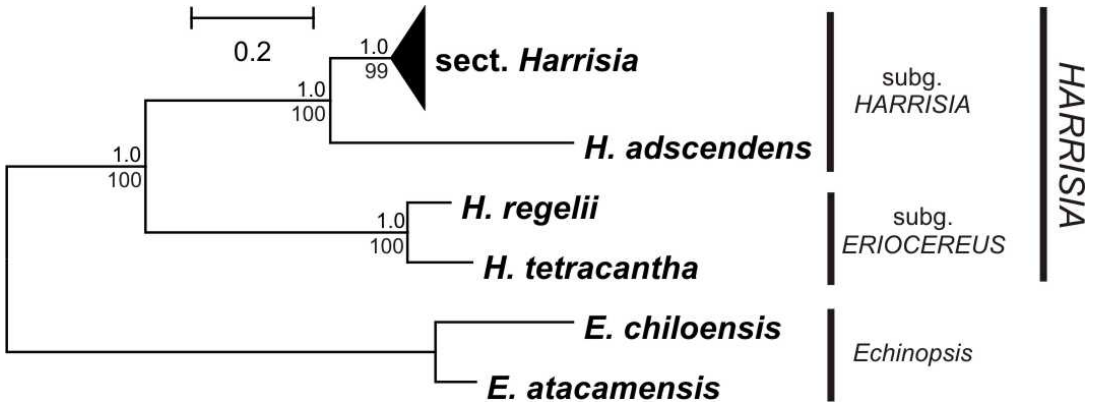

Figure 3.1. Bayesian tree of seven-marker phylogeny. The 21 samples of sect. *Harrisia* are condensed with their topology revealed in Fig . Posterior probabilities and bootstrap percentages 50 and above are above and below the branches, respectively.

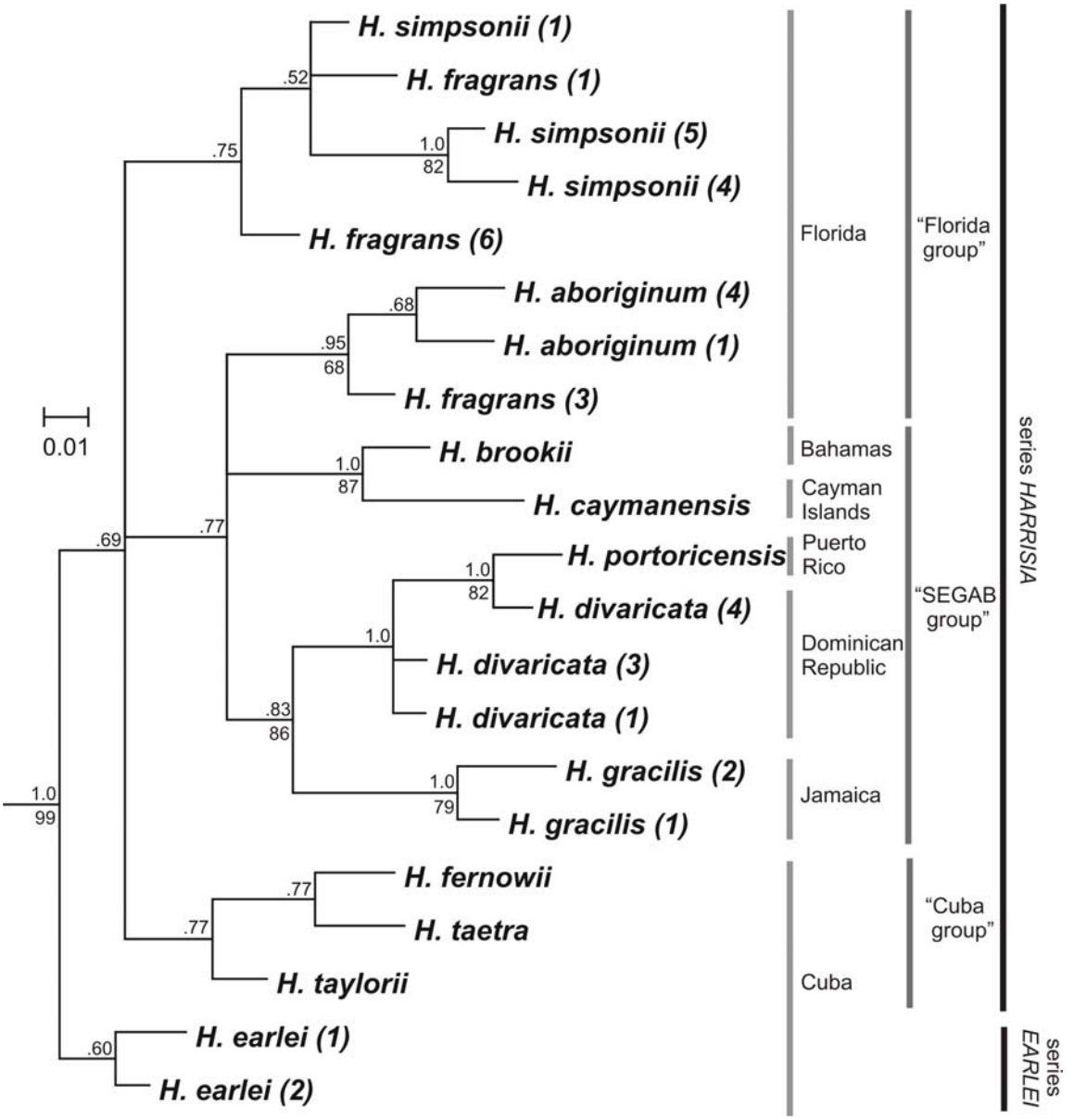

Figure 3.2. Branch of the 21 samples of sect. *Harrisia* condensed in the seven-marker Bayesian tree (Fig. 3.1). Posterior probabilities and bootstrap percentages 50 and above are above and below the branches, respectively.

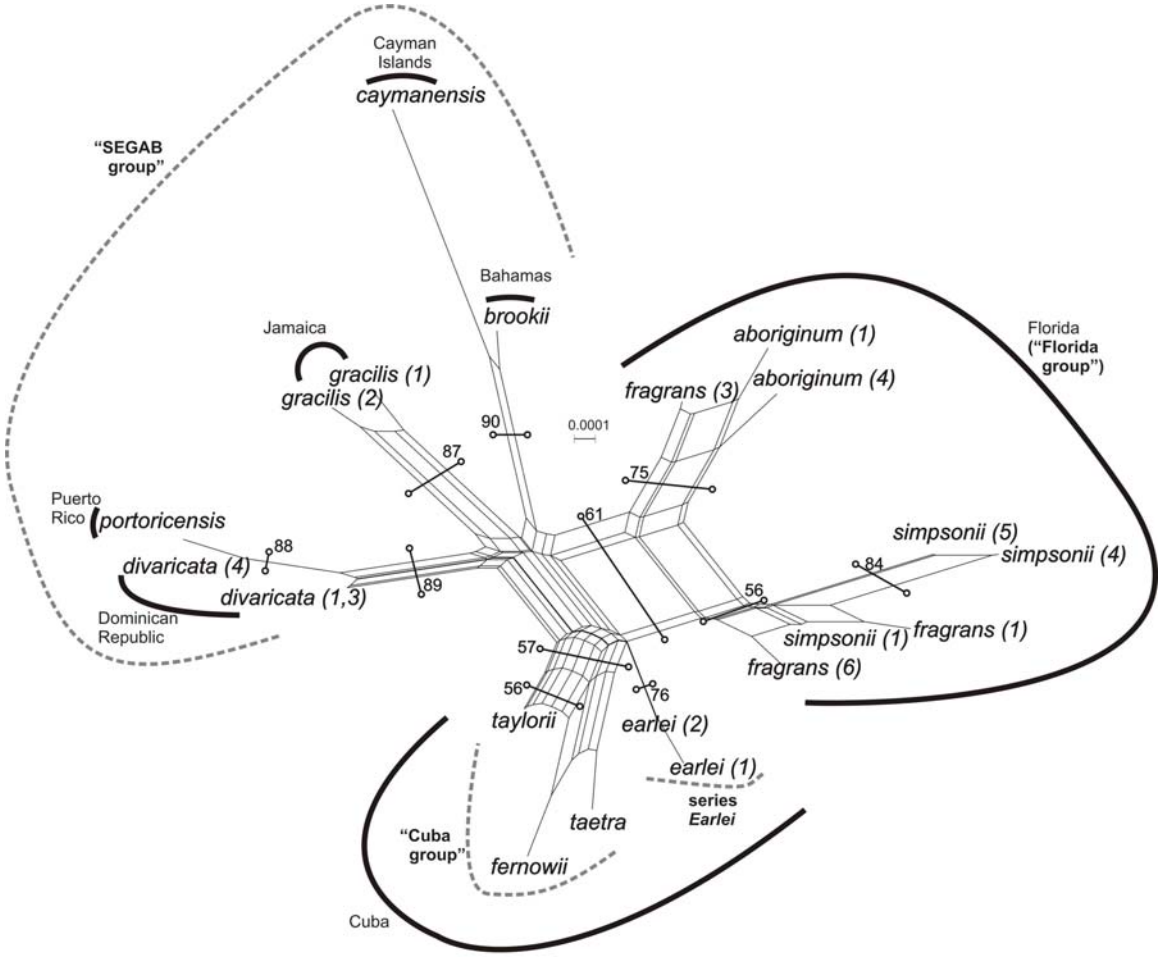

Figure 3.3. Neighbor-net network from the seven-marker alignment of the 21 samples of sect. *Harrisia*. Bootstrap percentages 50 and above are indicated by the lines with circle ends crossing the branches of the tree.

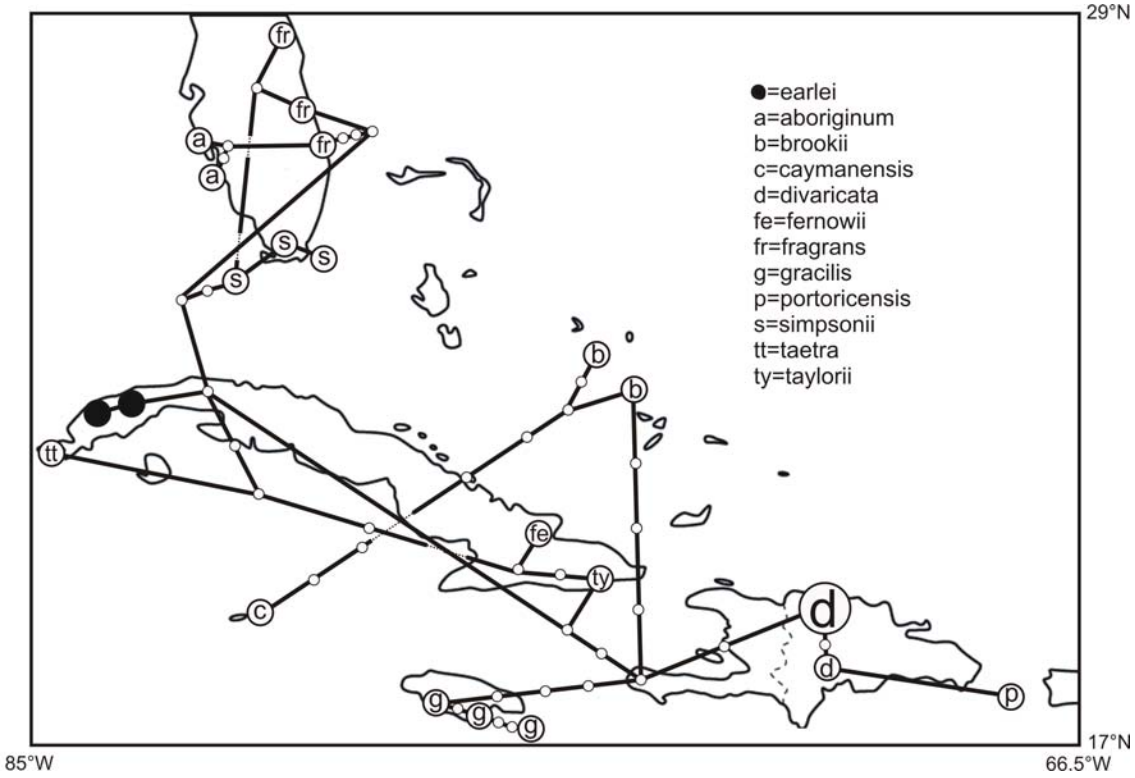

Figure 3.4. Haplotype network from the seven-marker alignment imposed on a map of the Caribbean region. Non-intersecting lines that cross on the map are indicated by dotted lines. Each line segment represents one substitution and the small circles on the lines represent intermediate haplotypes. All samples on the map represent one sample except the sample from the Dominican Republic with a larger circle.

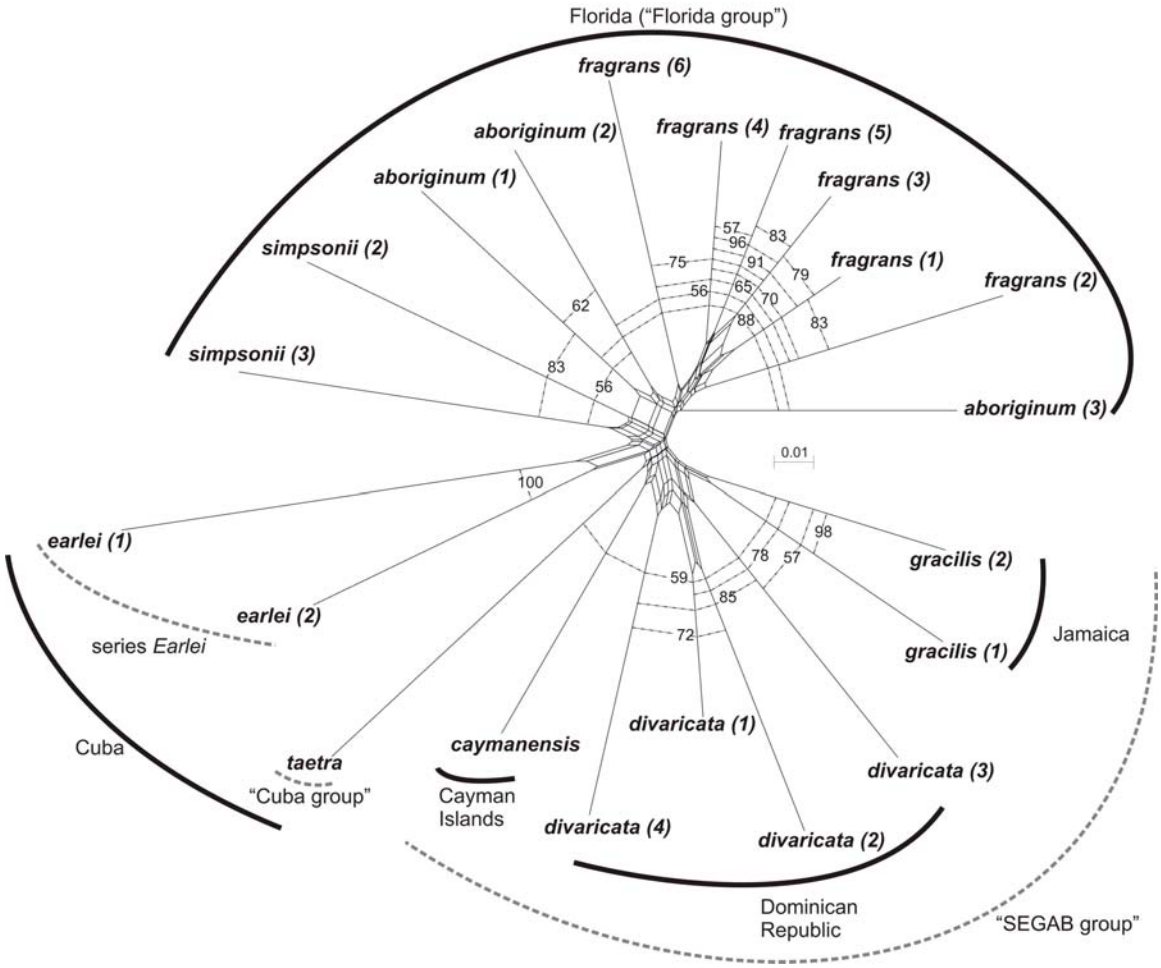

Figure 3.5. Neighbor-net network of the 10 primer sets from the AFLP analysis. Bootstrap percentages 50 and above are indicated by the dashed lines that connect the branches.

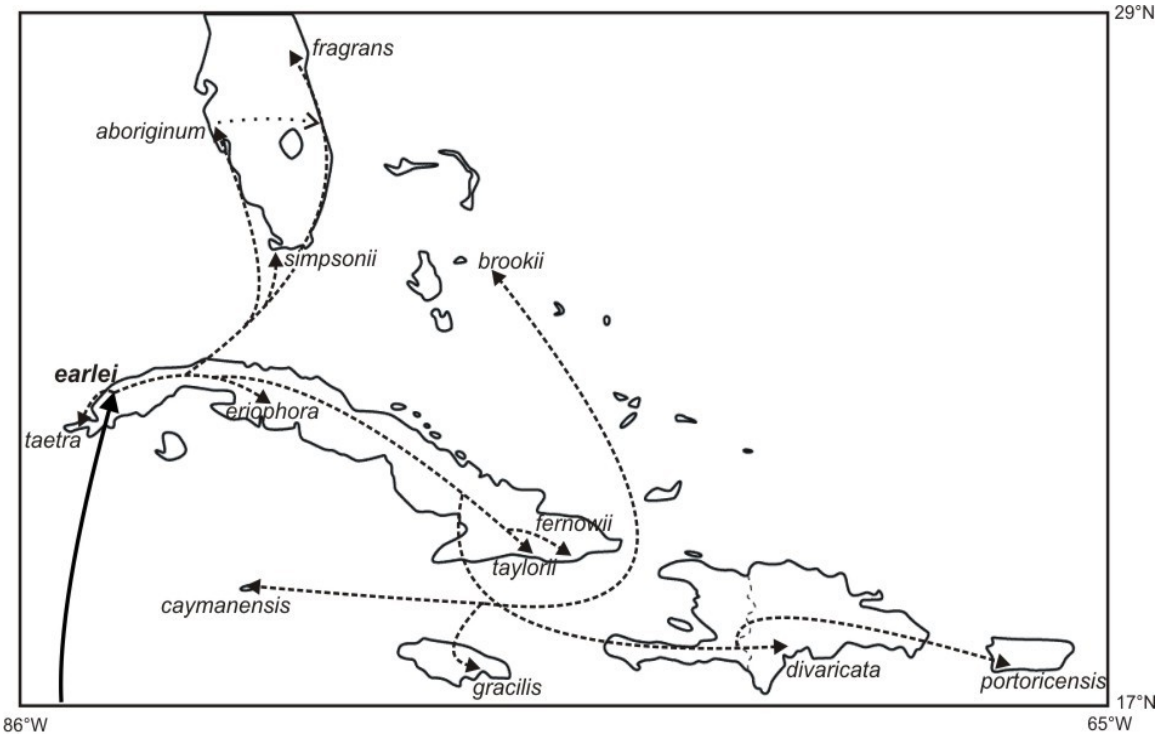

Figure 3.6. Biogeographic diversification of sect. *Harrisia* in the Caribbean. The solid black line represents the initial colonization of the Caribbean where *H. earlei* occurs. The dashed lines represent possible dispersal and diversification of series *Harrisia*. The dotted line represents the introgression of the *H. aboriginum* plastid haplotype into the central peninsular population of *H. fragrans*.

CHAPTER 4: MONOGRAPH OF *HARRISIA* (CACTACEAE)

INTRODUCTION

Harrisia Britton comprises 18 species occurring in west-central and eastern South America, the Greater Antilles, the Bahamas, and Florida, U.S.A. They are spiny, columnar cacti with elongate stems and large, nocturnal, ephemeral flowers. The genus is most easily recognized by seed morphology with enlarged apical testa cells and a cavernous hilum-micropylar region (Fig. 4.1). Within the Cactaceae, the genus is placed within the subtribe Trichocereinae of tribe Cereeae of subfamily Cactoideae. Of the ~250 species in subtribe Trichocereinae (Nyffeler & Eggli 2010) only *Harrisia* has a native distribution outside of South America. The genus likely originated in the Andes in the late Miocene and later dispersed and speciated in the Plio- and Pleistocene (Chapter 2).

Two subgenera, four sections, and two series were recognized within *Harrisia* in Chapter 2 of this dissertation (Table 4.1). The northern range of *Harrisia* is represented by subg. *Harrisia* and is divided into two sections. Section *Adscendens* is endemic to northeast Brazil (Fig. 4.2) and contains one species, *H. adscendens*. Section *Harrisia* occurs in the Caribbean (Fig. 4.3) and is further divided into two series. Series *Earlei* is monotypic with *H. earlei*, confined to western Cuba. Series *Harrisia* consists of 10 closely related species distributed throughout the Caribbean region except the Lesser Antilles. The southern range of *Harrisia* is represented by subg. *Eriocereus* (Fig. 4.4) and

contains two sections. Section *Roseocereus* is endemic to Bolivia and contains one species, *H. tetracantha*. Section *Eriocereus* contains five closely related species in the Gran Chaco region.

Section *Adscendens* and sect. *Eriocereus* have both been the subject of modern taxonomic treatments (Kiesling 1996; Leuenberger 1995, 2000a, 2000b, 2001; Taylor & Zappi 2004). Species of sect. *Harrisia* and sect. *Roseocereus* generally lack recent descriptions and many are in need of typification. Though 10 species occur in the Caribbean sect. *Harrisia*, few have received significant taxonomic effort since the Cactaceae monograph of Britton & Rose (1920). Their taxonomy is perhaps most influenced by the necessary access to many geo-politically isolated islands. Additionally, under the Convention on International Trade in Endangered Species of Wild Fauna and Flora, *Harrisia* requires a permit for export.

This effort provides the most complete analysis of the morphology and nomenclature of *Harrisia* while designating and correcting several typification issues.

TAXONOMIC HISTORY

The genus *Harrisia* was erected by Britton (1908) to accommodate the Caribbean representatives (sect. *Harrisia*). Britton (1908) described five new species and made three new combinations. The earliest published species in the genus, *Cereus gracilis* (=*H. gracilis*), was elected the type of the genus (Britton 1908). In the same year, *Cereus adscendens* (=*H. adscendens*) from the caatinga of Brazil was described (Gürke 1908). In their monograph of the Cactaceae, Britton & Rose (1920) proceeded to unite the South

American species previously placed under *Eriocereus* (Riccobono 1909) with the Caribbean species of *Harrisia*. Britton & Rose (1920) also described three new species from Florida and one species from Cuba. The arborescent *H. tetracantha* from Bolivia was combined in the genus much later (Hunt & Taylor 1987).

Berger (1905) noted the value of the seed characters (Fig. 4.1) in *Harrisia* and was the first to provide the modern sense of the genus by uniting several species from the Caribbean (series *Harrisia*) and South America (sect. *Eriocereus*, sect. *Roseocereus*) under *Cereus* subg. *Eriocereus*, with the South American species later promoted to the genus level (Riccobono 1909). Before Berger (1905), Labouret (1853) and Schumann (1899) had delimited the Caribbean and South American species in unrelated infrageneric groups under *Cereus* s. l.

After the description of the genus *Harrisia*, several authors subsumed the species placed in *Harrisia* by Britton & Rose (1920) under the genus *Cereus* s. l. (e.g. Urban 1910; Vaupel 1913; Fawcett & Rendle 1926; Werdemann 1931; Moscoso 1943; Little 1945; Benson 1969; Long & Lakela 1971; Wunderlin 1982). Backeberg (1938; 1977) chose to recognize three genera, *Eriocereus* (=sect. *Eriocereus*), *Harrisia* (=subg. *Harrisia*), and *Roseocereus* (=sect. *Roseocereus*) while Berger (1929) and Marshall (1941) recognized only *Eriocereus* (=subg. *Eriocereus*) and *Harrisia* (=sect. *Harrisia*). Some authors retained use of *Harrisia* s. l. (e.g. Small 1933; Kreuzinger 1935; Castellanos and Lelong 1938; Moscoso 1941; Liogier 1953; Correll & Correll 1982; Proctor 1984; Liogier 1994; Kiesling 1996; Wunderlin 1998), which is widely accepted by modern treatments (e.g. Anderson 2001; Hunt et al. 2006).

The classification of *Harrisia* within taxonomic divisions of Cactaceae has changed frequently. Britton & Rose (1920) classified *Harrisia* into a broad subtribe Cereinae (as Cereanae). This classification was modified and further split, whence *Harrisia* was allied with groups containing *Nyctocereus* (A. Berger) Britton & Rose (=*Peniocereus* (A. Berger) Britton & Rose) due to the vegetative similarities (Berger 1929; Backeberg 1934; Backeberg 1938; Buxbaum 1958; Backeberg 1977). Barthlott (1988) placed *Harrisia* in the tribe Echinocereeae (=Echinocereinae), apparently based on seed morphology. The genus was also once regarded as primitive in the Cactoideae subfamily (Mauseth et al. 1998; Terrazas & Arias 2003). Molecular work put *Harrisia* in tribe Trichocereeae (Wallace 1995; Applequist & Wallace 2002), later refined to subtribe Trichocereinae (Nyffeler & Eggli 2010).

The systematics of sect. *Eriocereus* of the Gran Chaco region of South America were revised by Kiesling (1996) and Leuenberger (1995, 2000a, 2000b, 2001). A detailed synopsis of the one species in eastern Brazil, *H. adscendens*, was provided by Taylor & Zappi (2004). After the study by Britton & Rose (1920), a few more taxa were described from the Caribbean (Marshall 1941; Marshall 1943; Areces 1980; Hooten 1991). Areces (1980) described a new species from west Cuba and provided a key to the five described species found in Cuba. Benson (1969) and Austin (1984) made varietal combinations of the Florida taxa. In a self-described provisional assessment, Hunt et al. (2006) recognized only 1–2 species in the Caribbean much like Schumann (1899), whereas Anderson (2001) and regional floras (Areces 1980; Correll & Correll 1982; Liogier 1994; Wunderlin & Hansen 2011) have used a narrower species circumscription.

SPECIES CONCEPT

A utilitarian approach (Levin 1979; Gilmour 1989) has been employed to lend taxonomic recognition to groups of individuals which share unique morphological characters, with the supposition that morphology reflects recent common ancestry. Although the sampling in the molecular analyses (Chapters 2–3) was not adequate to ascertain monophyly of many species, the relationships recovered (Chapters 2–3) were used to guide the taxonomic treatment. Additionally, since the possibility of paraphyly and polyphyly in plants is considerable in traditional species concepts (Riesberg & Brouillet 1994), molecular monophyly should not be the rule for taxonomic recognition (Nixon & Wheeler 1990; Riesberg & Brouillet 1994), especially since phylogenetic context often relies on relatively few characters taken from relatively few individuals. Hybridization, which is quite common in Cactaceae (Friedrich 1974; Machado 2008), may easily obscure real relationships. The polyphasic taxonomy approach (Colwell 1970) promoted in microorganisms (Vandamme et al. 1996; Samson & Varga 2009) is probably ideal for all organisms, including large eukaryotes, by analyzing several types of evidence to produce a classification with a minimum number of contradictions.

MATERIALS & METHODS

Approximately 400 herbarium specimens (A, B, BRI, FLAS, FTG, GH, HAC, HAJB, HNT, IJ, JBSD, K, LPB, MAPR, MO, NCU, NY, PRE, PTBG, RSA, SI, US, USC, USF, UWI, ZSS) were examined to prepare the taxonomic treatment. Live plant

photos (K.A. Bradley, pers. comm.; E. Guerrero, pers. comm.; M. Nee, pers. comm.; Z. Rigerski, pers. comm.; N. Taylor, pers. comm.) in situ and ex situ with known provenance were also used. Field studies in the Cayman Islands, Dominican Republic, Jamaica, and Florida, U.S.A. were also conducted.

MORPHOLOGY

Habit. Only one species forms large trees, the Bolivian *H. tetracantha* (sect. *Roseocereus*). Series *Harrisia* generally has erect shrubs though species can range from reclining shrubs to single-trunked small trees. The Brazilian *H. adscendens* (sect. *Adscendens*) and sect. *Eriocereus* grow as reclining shrubs or scrambling thickets. A semi-epiphytic habit in *H. bonplandii* has been reported from the Pantanal of Brazil (Braun & Hofacker 2006). The Cuban endemic, *H. earlei* (series *Earlei*), grows as a prostrate to pendent shrub.

Stems. The green photosynthetic stems of *Harrisia* are indeterminate long-shoots which contain numerous short-shoots. A cactus short-shoot is referred to as an areole, where the cluster of spines and trichomes arise (Figs. 4.5 & 4.6). The photosynthetic region below each areole is the tubercle, an undifferentiated combination of a modified leaf base and stem (Gibson & Nobel 1986). The term stem in cactus nomenclature, thus technically also refers to leaf bases. Tubercles may be somewhat distinct on young stems but become amorphous on mature stems. The tubercles are vertically connected to form ribs. The number of ribs on a stem ranges from 3 to 14. The young stems of all species

are generally erect to ascending but may begin to turn downwards due to the tenuous support of the stem. Stems of *H. adscendens* and sect. *Eriocereus* are often flexible and may become curvaceous. Stems of *H. tetracantha* and sect. *Harrisia* are generally less flexible and more rigid.

The surface of the photosynthetic stems are densely and evenly covered with stomata. The cuticle is fairly thin, 1–6 μm (Gibson & Horak 1978; Mauseth et al. 1998) and may be slightly rough (Gibson & Horak 1978). Under the cuticle, is a thin epidermis (Mauseth et al. 1998; Arruda et al. 2005) with an outer surface that may be roughened (Mauseth et al. 1998). The epidermal cells of *H. adscendens* have straight anticlinal walls and parallelocytic stomata (Arruda et al. 2005). The collenchymatous hypodermis may be one-cell thick in *H. martinii* (Gibson & Horak 1978) or three- to five cells thick in other species (Mauseth et al. 1998; Arruda et al. 2005). Neither the epidermis nor the hypodermis contain crystals (Mauseth et al. 1998; Arruda et al. 2005). The palisade cortex (Fig. 4.7) is at least 1.5 mm thick (Mauseth et al. 1998). Both the cortex and pith may be mucilaginous (Mauseth et al. 1998). The cortex of *H. pomanensis* is reported to be non-mucilaginous with calcium oxalate dihydrate (Hartl et al. 2007) crystal aggregates concentrated in the ribs (Gibson & Horak 1978). Mucilaginous cells and an absence of amyloplasts were observed in *H. adscendens* (Arruda et al. 2005).

In addition to the central ring of vascular bundles, the pith is vascularized with medullary bundles and the cortex vascularized with cortical bundles (Mauseth et al. 1998). Medullary and cortical bundles are unique to all of subfamily Cactoideae except for the basal lineage *Blossfeldia* Werderm. (Mauseth 2006).

Harrisia produces a hard fibrous wood (Mauseth & Plemons-Rodriguez 1998; Mauseth et al. 1998) that is interwoven within the stem (e.g. *Franck 2370*, *2896*, *2897*, *2898*, *2899*). The wood fibers are septate (Metcalfe & Chalk 1950; Mauseth et al. 1998) and libriform (Gibson 1973; Mauseth et al. 1998). Wide-band tracheids have been observed in seedlings (Mauseth 2004) but are absent in adults (Mauseth 2004; Arruda et al. 2004). Relatively long vessel elements of the xylem have been observed (Gibson 1973) with irregularly divided, multiperforate plates (Metcalfe and Chalk 1950). The wood of *H. divaricata* has been characterized as a monomorphic fibrous wood (Stevenson & Mauseth 2004). At the stem base, plants are woodiest and produce bark from the epidermis (Mauseth et al. 1998). Heartwood is not produced and cactus wood does not undergo apoptosis (Mauseth 2006).

Roots. The base of the stem grades into one to several taproots from which arise several branches to form a fibrous root system. A ±parenchymatous mono- to dimorphic wood has been observed in the roots of *H. divaricata* (Stevenson & Mauseth 2004)

Stem Areoles. The areoles of the stem are tomentose with pellucid, septate, uniseriate trichomes (Fig. 4.8) and contain a cluster of spines. The spine clusters and trichomes arise near the adaxial base of a greatly reduced leaf. The tiny, inconspicuous leaves can be seen at the abaxial base of areoles of new stem growth near the shoot apical meristem (Britton & Rose 1920; Mauseth 2007). New growth, such as spines, trichomes, or long-shoots, may continually appear from the adaxial portion of the areoles over the life of the stem. Thus older spines and trichomes are at the base of the areoles and newer ones near

the top. Long-shoots that arise from areoles may be both new stems or flowers (Fig. 4.9). Trichomes of the stem are usually white but newly-emergent trichomes can be reddish.

Spines. The spines may be modified bud scales or leaves of the axillary bud (Mauseth 2006). Mature spines are generally gray, smooth (striate under magnification), round to somewhat flattened basally, and dilated at the base. Newly-emergent spines are more brightly colored, varying from white to yellow-green to red, with darker or blackish tips. Young spines may become straw-colored, yellowish, or blackish before turning gray. Spine clusters at each areole consist of spines of varying lengths. Longer and thicker spines are usually produced later and the older spines at the base of the areole are often thinner and shorter. The longest and thickest spines are usually found at the base of the plant.

Flowers. Flowers are long-shoots, essentially a stem containing floral parts within. As on the stem, the flowers consist of tubercles each with an areole containing a modified leaf as a scale. In addition to uniseriate trichomes, the areoles of the flower usually contain longer, scaly trichomes (Figs. 4.6 & 4.10) that are sometimes deciduous. The scaly trichomes may sometimes grade into spine-like forms, especially near the base of the flower. Both types of trichomes are usually white on the flower but are sometimes reddish. Flower areoles of the pericarpel and lower hypanthium may resemble stem areoles (having spines but no scaly trichomes) on some species of sect. *Eriocereus*.

The pericarpel is the tissue surrounding the ovary and has areoles with scales (Fig. 4.11 & 4.12). The hypanthium is the flower tube which contains visible areoles above the

pericarpel (Fig. 4.11 & 4.12). Subtending the hairy areoles of the pericarpel and hypanthium are scales which are smallest on the pericarpel and gradually increase in size along the hypanthium, eventually grading into the larger sepals (Fig. 4.11). The sepals are loosely defined as the non-white blades occurring after the hypanthium. The scales and sepals are succulent. The petals are white, sometimes pinkish, very thin, and membranaceous.

Flower buds (Fig. 4.9) are produced on the stem areoles of the distal portions of the stem. The buds first appear as scaly protrusions which then slowly engorge to become acutely globose with densely imbricate scales. The buds then elongate, forming an incipient pericarpel and hypanthium. At maturity, the pericarpel is globose and narrows into a slender hypanthium which leads to an expanding tight cluster of 20–30 sepals and petals. The petals are only initially visible the night of anthesis. The sepals reflex backwards and the petals expand outwards (Fig. 4.13).

The stigma is at the center of the flower, level with the petals and anthers or exserted beyond them in sect. *Harrisia*. The rim stamens are adnate to the tip of the hypanthium and held in a ring around the inner circumference of the flower. The stamens which comprise the dorsal stamen cluster (Fig. 4.13) arise from the middle region of the hypanthium (Fig. 4.12) and the filaments are not sheathed together (*Trichocereus*-type, cf. Schick 2011). The arrangement of the stamens is bilaterally symmetrical with a dense array of stamens in the lower portion of the flower, the dorsal stamen cluster and lower rim stamens, and very few in the upper portion, the upper rim stamens. The filaments of both stamen types may curve upwards distally. At the basal portion of the hypanthium is the beige-colored nectar chamber (Fig. 4.12).

Pollen. The pollen is tri- to hexacolpate, ~60–70 μm (Kurtz 1963; Leuenberger 1976) in diameter with numerous spinulae (Fig. 4.14) and shallow sulci (Leuenberger 1976).

Chromosomes. The karyotype of *H. portoricensis* from Guayanilla, Puerto Rico has been recorded as diploid, 2n=22 (Spencer 1955). The base haploid number of Cactaceae is 11 with polyploidy being quite frequent (Ross 1981). Deviations from 11 chromosomes are extremely rare in Cactaceae (Ross 1981).

Fruits. The pericarpel matures into a yellow, orange, or red fruit containing a sweet, edible, white pulp and hundreds to thousands of tiny seeds (Rojas-Sandoval & Meléndez-Ackerman 2009c; McFadyen 2012). The tubercles of the fruit are often well-defined by the sulci, especially when immature (Fig. 4.6). A withered flower often persists on the immature fruit (Fig. 4.6). The withered flower, scales, and trichomes are usually deciduous on the ripened fruit. The fruit scales are retained in *H. bonplandii*. In the Brazilian *H. adscendens* and subg. *Eriocereus*, the fruit splits open along lateral or apical tears, exposing the mesh of pulp and seeds (Fig. 4.5).

Seeds. The testa cells of the seedcoat are black, sometimes becoming reddish around the hilum-micropylar region (HMR). The testa cells are hollow and at the junctures on the seed coat form pits (Gibson & Nobel 1986; Barthlott & Hunt 2000; Doweld 2001). The surface of the cells are vermiculate with striate cell junctures (Gibson & Nobel 1986; Barthlott & Hunt 2000; Doweld 2001). The top of the seed (opposite the HMR) has a

curved crest of a few rows of testa cells noticeably larger (to 0.4 mm long; Fig. 4.1) than the rest of the seed coat (Zuccarini 1838; Barthlott & Voit 1979; Bregman 1988; Barthlott & Hunt 2000; Doweld 2001). The HMR is cavernous (Fig. 1) and is surrounded by the seed coat with only the bottom exposed (Bregman 1988; Barthlott & Hunt 2000; Doweld 2001) to contain a medial sclerified band (Barthlott & Hunt 2000). The interior of the seed is composed of endosperm (Bregman 1988). Perisperm is not evident (Bregman 1988). Seeds weigh ~1.5–2.0 mg (Serrano & Guzmán 1994; Alzugaray et al. 2007).

Seedlings. Seedlings have a green, succulent hypocotyl to 1 cm tall, above which are two cotyledons (Zuccarini 1838). The cotyledons are deltoid or sometimes cleft (Ganong 1898; pers. obs.) and indistinct from the hypocotyl (Zuccarini 1838). The apical meristem of the epicotyl soon produces areolate tubercles (Zuccarini 1838). Ribs of seedlings are often fewer and spines more setaceous than in mature individuals. The epicotyl of *H. bonplandii* reportedly first develops four ribs, then increases to eight, and finally reduces to 4–5 ribs (Ganong 1898).

ECOLOGY

Distribution & Habitat. The genus has a wide range from the eastern Andes of Bolivia to the Gran Chaco region of south-central Argentina to the Caatinga of northeast Brazil to western Puerto Rico to the Swan Islands of Honduras and up to northeast peninsular Florida (Figs. 4.2–4.4). A few species have also been naturalized in Australia (McFadyen 1986), South Africa (Henderson 2007), and Hawaii (Lorence et al. 1995).

The genus is found in seasonally dry forest or shrubland. The rainy season in South America is roughly Nov–Mar and May–Oct in the Caribbean where the species occur. Annual precipitation is generally around 500–1200 mm. At its northern range in Florida and its southwestern range in Argentina and Bolivia, occasional freezing temperatures are experienced. The highest elevation is experienced by *H. tetracantha* at ca. 2600 m (*Cárdenas 5021*), while some populations in the Caribbean are near sea level and only a few meters inland from the high tide line (*Franck 473*, *Franck 1236*).

Phenology. From the initial appearance of the flower buds, several weeks pass until anthesis occurs. Flower bud production and efflorescence seem to be dependent on temperature and rainfall. Flower bud formation in *H. portoricensis* was strongly correlated with monthly mean temperature and monthly minimum temperature while efflorescence was strongly correlated with rainfall (Rojas-Sandoval & Meléndez-Ackerman 2011a). Efflorescence of *H. fragrans* was observed to have two peaks, in May and October (Rae 1995) coinciding with the beginning and end of the rainy season. Plants of *H. fragrans* in the shade rarely flowered but invested more energy in stem growth (Rae 1995), perhaps to gain access to sunnier locations. Efflorescence seems to peak after heavy rain in *H. adscendens* (Lima 2007) and in *H. tetracantha* (M. Mendoza & M. Nee, pers. comm.).

Pollination. The flowers are nocturnal and open only once, opening after sunset and remaining open until near sunrise. Kaiser and Tollsten (1995) characterize *H. adscendens* as moth-pollinated due to its nocturnal anthesis and distinctly pleasant odors, composed

mainly of, in descending order, geraniol, geranial, neral, and methyl benzoate. Scogin (1985) identifies *H. pomanensis* as having a hawkmoth-pollinated syndrome as its nectar is low in energy. The flowers of *Harrisia* may also be chiropteriphilous due to the presence of clustered stamens and hexose sugars, characters amenable to pollination by the New World glossophagine bats (Fleming et al. 2009). Scogin (1985) found the flowers of *H. pomanensis* to have hexose as the dominant nectar sugar. Pollen from *Harrisia* has been reported in the stomach contents of two species of bats in Cuba (Silva Taboada 1979). Several different insects have been observed visiting flowers, including hawk moths (Sphingidae) (*H. portoricensis*, Rojas-Sandoval & Meléndez-Ackerman 2011a), sap-feeding beetles (Nitidulidae) (*H. simpsonii*, Dobson 1972; pers. obs.), long-horned beetles (Cerambycidae) (*H. fragrans*, Hutchinson & Pazara 2004), scarab beetles (Scarabaeidae) (*Nee 51239* (NY)), and stingless bees (*H. pomanensis*, Meliponidae) (Allier et al. 2010). Flowers appear to be self-compatible (Rojas-Sandoval & Meléndez-Ackerman 2009c; pers. obs.) and self-pollination may be assisted by the wind (Rojas-Sandoval & Meléndez-Ackerman 2011b).

Fruit Set & Consumption. Fruits take several weeks to mature, ~60 days after efflorescence (Rojas-Sandoval & Meléndez-Ackerman 2009c; pers. obs.) and another ~30 days to split open in South American species (pers. obs.). Mature fruits can remain on the plant for months (Rae 1995; pers. obs.). The internal pulp of the fruits of *Harrisia* are sweet, fleshy, and likely palatable to a variety of animals such as birds (Rae 1995; Rojas-Sandoval & Meléndez-Ackerman 2009a), mice (Rojas-Sandoval & Meléndez-Ackerman 2009a), lizards (Rojas-Sandoval & Meléndez-Ackerman 2009a), foxes

(Bianchi 2009), coatis (Bianchi 2009), crabs (Areces-Mallea 2003), goats (Meléndez-Ackerman et al. 2008), and social wasps (Santos et al. 2007). The fruit rind is usually not consumed. Seed dispersal of *Harrisia* has likely been assisted by birds (ornithochorous) and/or bats (chiropterochorous) as many populations are limited to isolated islands. The bat subtribe Stenodermatina (Chiroptera) are frugivores with distributions in South America, Central America, and the Caribbean (Dávalos 2007) that may consume *Harrisia* fruits. Seed dispersal by water may also be possible (Bregman 1988).

Germination & Growth. Seeds of *Harrisia* do not appear to experience much dormancy (~ 8 weeks, Rojas-Sandoval & Meléndez-Ackerman 2009c), as they sometimes germinate in the fruit, e.g. in *H. martinii* (Cota-Sánchez 2004) and in *H. simpsonii* (pers. obs.). Gibberellic acid and scarification by sulfuric acid has been used to increase germination (Dehgan & Pérez 2004) as well as soaking the seeds in water (L. Boehner, pers. comm. 2011). Seeds of *H. martinii* experienced maximum germination three days after sowing washed seeds (Alzugaray et al. 2007). Seeds of *H. simpsonii* have germinated up to at least one year after sowing (pers. obs.) while seeds of *H. fragrans* are viable for at least 19 months (Bradley & Hines 2007). Seeds from cross-pollinated flowers were larger and germinated better than those from self-pollination in *H. portoricensis* (Rojas-Sandoval & Meléndez-Ackerman 2009c). Seeds germinate by a fissure between the HMR and upper seed coat like an operculum (Bregman and Bouman 1983).

Mycorrhizal arbuscles and vesicles were reported to be absent from the roots of *H. fragrans* (Fisher & Jayachandran 2005), and is apparently a common observation among

cacti (Muller et al. 1994; Bashan et al. 2000). Seedlings of many cactus species benefit from nurse plants (Godínez-Álvarez et al. 2003) and nurse plant association has been implicated in species of series *Harrisia* (Rae 1995; Rojas-Sandoval & Meléndez-Ackerman 2009b; Moore 2011), with most seedlings of *H. fragrans* appearing in shade (Bradley & Hines 2007). Invasive plants have been correlated with negatively impacting the demography of *H. portoricensis* (Rojas-Sandoval 2007; Guerrero et al. 2011; Rojas-Sandoval & Meléndez-Ackerman 2012).

Vegetative reproduction may occur when stems break and detach from the parent plant. The stems can proceed to produce roots along tissue nearest to the ground and produce a new plant. The ribs of the stem may contract during water stress. The stems function as the photosynthetic region of the plant utilizing crassulacean acid metabolism, or CAM photosynthesis as documented in all cacti so far (Nobel 2002; Hernández-González and Villarreal 2007).

Herbivory. Various insects predate on the stems such as armored scale insects (Diaspididae) (Claps & de Haro 2001; pers. obs.), weevils (O'Brien 1976; McFadyen 1979a), long-horned beetles (McFadyen & Fidalgo 1976), moth larvae (McFadyen 1980), cochineal insects (Claps de Haro 2001), midges (McFadyen 2012), fly larvae (McFadyen 2012), and mealybugs (McFadyen 1979b; Claps de Haro 2001). The mealybug *Hypogeococcus pungens* Granara de Willink has been successfully used to control invasive species of sect. *Eriocereus* in Australia (McFadyen 1986) and South Africa (Klein 1999), especially *H. martinii*. The invasive *Cactoblastis cactorum* avoids *Harrisia* (Tate et al. 2009). Myxomycetes have been observed on decaying *Harrisia* (Eliasson

2004). Moth larvae (Pyralidae) predate on developing fruits (*Franck 1278*, pers. obs.; McFadyen 2012). One of the principal dry season foods of the Chacoan peccary are the stems of *Harrisia* (Mayer and Brandt 1982). Exotic rhesus macaques have been observed feeding on stems of *H. portoricensis* (Brecken 2000).

ETHNOBOTANY

The internal flesh of the fruits of *Harrisia* are edible and used locally by humans (Fawcett & Rendle 1926; Morton 1962; Barton et al. 1990; Schmeda-Hirschmann 1994; Andrade 2002; Arenas and Scarpa 2006), although they should be avoided if already visited by other animals because of the possibility of disease transmission (Martínez 2010). The flowers of *H. bonplandii* are sometimes boiled by the Ayoreo of Paraguay (*Schmeda 1185 & 1489* (US), Schmeda-Hirschmann 1994) and the Chorote Indians of Argentina (*Scarpa 562* (SI), Arenas and Scarpa 2006). The boiled or roasted roots of sect. *Eriocereus* are sometimes eaten (Scarpa & Arenas 2002). Decoctions of the roots of *H. adscendens* are used medicinally (Andrade et al. 2006; Agra et al. 2007; Rocha and Agra 2011). *Harrisia pomanensis* has been used for forage (Palacio et al. 2011). The use of the flowers and stems of *Harrisia* in homeopathy has been reported (Waizel-Bucay 2009). The dried woody stem of *H. gracilis* was said to be used as torch, which when held out from the canoe baited fish for harpooning (Edwards & Lindley 1818; Fawcett & Rendle 1926). Descourtilz (1821) described the reserved use of the juice of the stem of *H. divaricata* in vinegar or castor oil topically as a depilatory and against toothache, warts, and worms in the stomach.

CHEMISTRY

The *n*-alkane content of the cuticular wax was relatively high in *H. tetracantha*, composed of a fairly even distribution of C_{18}–C_{34} and C_{36} *n*-alkanes (Maffei et al. 1997). The *n*-alkane content of *H. martinii* has been reported as dominant in C_{37} with significant fractions of C_{33}, C_{35}, and C_{39} (Herbin & Robins 1968). Presumably, the wax is partly responsible for the low epidermal absorbance of water (Barthlott & Capesius 1974). Alkaloids of the stem tissue remain unknown although tyramine and hordenine are common in the related genus *Echinopsis* s. l. as well as other species of subfamily Cactoideae (Agurell 1969; Agurell et al. 1971). The alkaloid toxicity of *Harrisia* has been characterized as low with mescaline type alkaloids (Levin & York 1978). The sap of *H. gracilis* is very dilute, in accord with other succulents (Harris & Lawrence 1917).

Betacyanin pigments have been characterized in the fruit of *H. guelichii* (=*H. bonplandii*), with the total betacyanin content being 41% phyllocactin, 23% betanin, 18% isobetanin, and 18% isophyllocactin (Piattelli & Imperato 1969). Seeds of *H. pomanensis* are composed of ~11% protein, ~8% water ~2% ash, and a relatively low oil content (~15%) (Serrano & Guzmán 1994). The majority of the oil is 18:2 (62%) with significant amounts of 18:1 (21%) and 16:0 (13%) (Serrano & Guzmán 1994). The highest mineral content was, in descending order, potassium, magnesium, calcium, and sodium (Serrano & Guzmán 1994). Polyacrylamide gel electrophoresis of seed protein patterns of *H. pomanensis* have been compared to other cacti (Carreras et al. 1997). Caffeine has been reported in the seeds of *H. adscendens* (Friese 1935).

CLASSIFICATION

The Cactaceae are part of the order Caryophyllales as demonstrated by the presence of betalains (Wohlpart & Mabry 1968) and sieve-element plastids with peripheral protein filaments (Behnke 1976; Behnke 1993). The axillary areoles consisting of a cluster of spines, bristles, and/or hairs are apomorphic for the Cactaceae. Four subfamilies are recognized within Cactaceae. The largest one is Cactoideae, distinguished by having only microscopic leaves evident in shoot apical meristems (Mauseth 2007). The core Cactoideae (Nyffeler 2002; Hernández-Hernández et al. 2011) are further distinguished by the long-shoot flowers arising from the areoles.

Core Cactoideae is divided into at least five tribes (Nyffeler & Eggli 2010). Tribe Cereeae, with about 600 species in 20 genera (Nyffeler & Eggli 2010), contains ribbed globose to columnar cacti and is predominantly South American with only three genera native to the Caribbean, Central America, and/or Mexico. Tribe Cereeae is further divided into three subtribes (Nyffeler & Eggli 2010). Subtribe Trichocereinae (=tribe Trichocereeae) is characterized by flowers with hairy, acuminate bracts, and an areolate pericarpel (tissue surrounding the ovary). Molecular studies using plastid markers have helped define the subtribe (Wallace 1995; Applequist & Wallace 2002; Nyffeler 2002; Crozier 2005; Ritz et al. 2007; Hernández-Hernández et al. 2011) which contains approximately 250 species in 20 genera (Nyffeler & Eggli 2010). With the exception of *Harrisia* in the Caribbean, Trichocereinae is confined to South America from Ecuador, Peru, and Brazil southward (Hunt et al. 2006).

PHYLOGENY

The monophyly of *Harrisia* and its infrageneric classifications are supported by the molecular phylogenies in this dissertation (Chapter 2). The generic relationships of *Harrisia* within subtribe Trichocereinae are not clear from molecular phylogenies (Chapter 2) although the dorsal stamen clusters (Schick 2011) found in some species of *Echinopsis* s. l. suggest a close relationship. Species within sect. *Eriocereus* are very closely related (Chapter 2). The same is true for the species of sect. *Harrisia* (Chapters 2 & 3). Interspecies relationships in these two sections are unclear and probably represent recent radiations (Chapter 2).

The monotypic series *Earlei* is supported as basal in sect. *Harrisia* (Chapters 2 & 3). Within series *Harrisia*, three main groups emerge from molecular analyses (Chapter 3). The Cuba group comprises all the Cuban species of series *Harrisia* (*H. eriophora*, *H. fernowii*, *H. taetra*, *H. taylorii*). The Florida group consists of all the Florida species (*H. aboriginum*, *H. fragrans*, *H. simpsonii*). The SEGAB group contains all non-Cuban and non-Florida species (*H. brookii*, *H. caymanensis*, *H. divaricata*, *H. gracilis*, *H. portoricensis*). The SEGAB group may also contain or be most related to *H. fernowii* and *H. taylorii* from southeast Cuba (Chapter 3). Morphology suggests the Florida group would be most closely related to *H. eriophora* and *H. taetra* from west Cuba (Chapter 3).

BIOGEOGRAPHY

Diversification. Because of the high diversity and endemism of Cactaceae in the central Andes, sympatric with *H. tetracantha*, the genus is presumed to originate in the central Andes (Wallace 1997; Chapter 2). Its dispersal into Brazil from the central Andes would then match the distribution patterns of many other cacti (Chapter 2). Its diversification in the Gran Chaco (sect. *Eriocereus*) and in the Caribbean is presumed a recent event in the Pleistocene (Chapter 2). The Caribbean sect. *Harrisia* is disjunct from the Brazilian sect. *Adscendens*. The colonization of the Caribbean occurred by dispersal from Brazil to west Cuba (Chapter 2).

Range. Many species of *Harrisia*, including the three monotypic lineages (sect. *Roseocereus*, sect. *Adscendens*, and series *Earlei*), are narrow endemics. Given the narrow range of many species, it is likely that the distribution of *Harrisia* has repeatedly expanded and shrunk in response to climatic alterations of arid habitat. The climate was cooler and more arid 0.7–1.2 Ma ago (Clark et al. 2006), near the estimated dispersal of *Harrisia* into the Gran Chaco and Caribbean (Chapter 2). Numerous cycles of increasing and decreasing aridity throughout the Pleistocene (Johnson 1982) are indicated by various regional studies from South America (van der Hammen 1974; Leyden 1985; Oliveira et al. 1999; Strecker et al. 2007; Werneck et al. 2011), nuclear Central America (Leyden 1984; Leyden et al. 1994), Jamaica (Street-Perrott et al. 1993), Haiti (Hodell et al. 1991), and Florida, U.S.A. (Watts 1975; Grimm et al. 1993).

CHARACTER EVOLUTION

The best-defined apomorphy for *Harrisia* is the cavernous HMR of the seed (Fig. 4.1). The scaly trichomes of the flower may also be apomorphic in *Harrisia*, though it is speculative without having adequate knowledge of flower trichomes in the rest of Trichocereinae. Characters of the Bolivian *H. tetracantha* such as its many-ribbed, thick, erect stems may be plesiomorphic as these stems are common in subtribe Trichocereinae. Slender stems, few ribs, and flexible stems may then be apomorphic or homoplasious, being quite rare in subtribe Trichocereinae. If *Harrisia* is sister to the arborescent *Echinopsis* as suggested by the plastid data (Chapter 2), then arborescence may be plesiomorphic in *H. tetracantha*.

Reduction in rib number may have occurred twice, with sect. *Eriocereus* and series *Earlei*. Rib number then increased with series *Harrisia*. Splitting or dehiscing fruits are likely plesiomorphic as they are present in three sections of *Harrisia* and absent in sect. *Harrisia*. Stigma lobes are relatively long and plesiomorphic in three sections of *Harrisia* and reduced in sect. *Harrisia*. Yellow fruits are apomorphic, rare in sect. *Adscendens* and common in sect. *Harrisia*. The reappearance of red fruits in two Florida species in series *Harrisia* is either a reversion to the ancestral red fruits or homoplasious. Seeds are relatively large in sect. *Adscendens*, series *Earlei*, the west Cuban species, and the Florida species. The smaller seeds in some species of series *Harrisia* appear to be derived. The flower scales in subg. *Eriocereus*, sect. *Adscendens*, and series *Earlei* are usually reddish and the green to yellow-green scales in some species of series *Harrisia* are probably

derived. All species except *H. bonplandii* and *H. regelii* have ribs distinctly separated by a line at the sulcus. The indistinct ribs of these two species may be synapomorphic.

TAXONOMY

HARRISIA Britton, Bull.Torrey Bot. Club 35: 561. 1908. *Cereus* Mill. [unranked] *Attenuati* Labour., Monogr. Cact.: 335. 1858. *Cereus* Mill. series *Attenuati* (Labour.) K. Schum., Gesamtbeschr. Kakt. 54, 95. 1899.—TYPE: *Harrisia gracilis* (Mill.) Britton.

Columnar cacti. Shrubs or trees. Stems pendent, prostrate, decumbent, arching, reclining, clambering, ascending, or erect; green to blue-green; with 3–14 ribs; elongate, indeterminate, basally becoming woody. Stem sulci defined by a sinuous line, rarely absent. Bark smooth to slightly flaking. Spines 3–20 on stem areoles, smooth, straw-colored to gray, microscopically striate, subulate, round to somewhat flattened basally, to 8 cm long and 1.8 mm thick, fewest on distal areoles and becoming more numerous on basal areoles of stems. Young spines white to yellow to red to nearly black with darker tips. Stem areoles tomentose with short septate, uniseriate trichomes to 1.5 mm long. Tiny leaves evident on tips of vigorous shoot apical meristems, deltoid, 0.5–1.1 mm long. Flower buds globose, ovoid, or obovoid, usually hairy. Flowers lateral on distal stems, 14–26 cm long and 8.5–16.5 cm wide, nocturnal, open only once. Pericarpel and hypanthium areoles villous with wavering, scaly trichomes, 2.5–12 mm long, sparse to copious, persistent to deciduous; trichomes sometimes grading into spines. Pericarpel

green to purplish red, with deltoid to lanceolate scales, green to brown to red. Hypanthium green to brown to red, scales green to brown to red, rounded-truncate, acuminate, deltoid to lanceolate, somewhat erect basally. Sepals green to brownish to reddish, linear, acute. Petals white to pinkish, ovate with slender limb, apiculate, margins roughly entire to irregularly denticulate. Stamens adnate to inner hypanthium, filaments straight or often upcurved, arrangement bilaterally symmetrical, ca. 100 stamens concentrated in the lower region of the flower, ca. 50 sparsely arranged in upper ring. Filaments basally light green to white distally. Anthers beige. Pollen spherical, tricolpate to hexacolpate, covered with numerous spinulae, sulci very shallow. Pistil exserted beyond anthers, style light green to white, stigma 10–15 lobed, fimbriate. Fruits yellow to red, 3.5–8 x 3.5–8 cm, spherical to oblong, with hundreds to thousands of seeds, pulp white and sweet. Seeds black, oblong rectangular to squarish to clam-shaped, 1.3–2.4 x 1.8–3.35 mm, hilum-micropylar region cavernous, apical margin testa cells enlarged.

Distribution. Five species occur in the Gran Chaco region of South America (Argentina, Bolivia, Brazil, Paraguay, and Uruguay), one species is restricted to the inter-Andean valleys of southeast Bolivia, one species is found in the caatinga of northeast Brazil, and 13 species occur in the Caribbean region (Greater Antilles, Bahamas, southern coastal Florida) (Figs. 4.2–4.4), also naturalized in Australia, South Africa, and Hawaii.

Etymology. William Harris (1860–1920), Jamaican botanist.

SUBGENERIC KEY FOR HARRISIA

1. Seeds oblong, >0.5 mm longer than wide, glossy ..... subg. *Harrisia*

1. Seed length and width subequal, differing by <0.5 mm, seeds semi-matte.....

..... subg. *Eriocereus*

I. *Harrisia* subg. *Harrisia*

Shrubs or small trees. Stems pendent, prostrate, arching, reclining, clambering, ascending, or erect, dark green to glaucous green, 5–14 ribs. Spines to 12 cm long, 1.5 mm thick. Newly emergent spines white to yellow to red with darker tips. Seeds oblong rectangular, 1.3–2.4 × 1.9–3.35 mm.

Distribution. Eastern Brazil and Caribbean region (Greater Antilles, Bahamas, southern coastal Florida) (Figs. 4.2 and 4.3), naturalized in Hawaii; seasonally dry forest and scrubland; 0–2600 m.

One species is found in the caatinga of northeast Brazil and 11 species occur in the Caribbean region.

This subgenus contains species which have oblong seeds. Compared to subg. *Eriocereus*, this subgenus also tends to have thinner spines and ribs, and as shrubs are usually taller and more upright. Labouret (1853) may have recognized the similarity between the Caribbean species and the Brazilian *H. adscendens* if the name he used, *Cereus platygonus*, is the same plant as *H. adscendens*. These species were placed together in the unranked *Attenuati* group under the genus *Cereus* (Labouret 1853).

KEY TO SECTIONS OF SUBGENUS *HARRISIA*

1. Stigma lobes 0.7–1.0 mm long, exserted; fruits indehiscent; Caribbean ....sect. *Harrisia*

1. Stigma lobes 1.2–2 mm long, not exserted; fruits dehiscent; Brazil ..... sect. *Adscendens*

Ia. *Harrisia* sect. *Harrisia*

Shrubs or small trees. Stems pendent, prostrate, arching, reclining, clambering, ascending, or erect, dark to light green, 5–14 ribs. Spines to 12 cm long, spines of basal and distal areoles sometimes dissimilar in size. Newly emergent spines white to yellow to red. Pericarpel and hypanthium scales green to brownish to reddish, deltoid to lanceolate, sepals lanceolate. Petals white to pinkish. Fruits yellow to red, indehiscent. Seeds 1.25–2.0 × 2.0–3.35 mm.

Distribution. Caribbean region (Greater Antilles, Bahamas, southern coastal Florida) (Fig. 4.3), naturalized in Hawaii; scrubland and scrub forest; 0–400 mm.

Four species occur in Cuba, two in Florida, two on Hispaniola, one in Puerto Rico, one in the Bahamas, one in Jamaica, and one in the Cayman Islands and the Swan Islands.

KEY TO SERIES OF SECTION *HARRISIA*

1. Ribs 8–14; newly emergent spines white to yellow to brown; erect to reclining shrub .....series *Harrisia*

1. Ribs 5–7; newly emergent spines red; prostrate to pendent shrub..... series *Earlei*

Ia-1. *Harrisia* series *Harrisia*

Shrubs or small trees. Stems arching, reclining, clambering, ascending, or erect, 8–14 ribs. Spines to 8 cm long. Young spines white to straw-colored basally, becoming brown to red-black with a black tip distally, eventually becoming gray. Pericarpel green to red. Hypanthium yellow-green to green to red. Scales yellow-green to green to red. Sepals yellow-green to pale red. Fruits yellow to red. Seeds 1.25–2.0 × 2.0–3.35 mm.

Distribution. Three species occur in Cuba, two in Florida, two on Hispaniola, one in Puerto Rico, one in the Bahamas, one in Jamaica, and one in the Cayman Islands and the Swan Islands (Fig. 4.3), and an unknown species naturalized in Hawaii (*Dunn & Wood 298* (PTBG) and *Lorence 7623a* (PTBG); scrubland and scrub forest; 0–400 m.

This study favors recognition of 10 species in the Caribbean, though many species could benefit from additional observations. Four names that are sometimes recognized are here put into synonymy (*H. hurstii*, *H. nashii*, *H. simpsonii*, *H. taylorii*). Should new evidence suggest their reinstatement, infraspecific status is recommended.

Most of the Caribbean species are allopatric and morphologically uniform. The species of Cuba and Hispaniola are in need of further morphological (and molecular) characterization to verify taxonomic delimitation across multiple populations. *Harrisia divaricata* and *H. portoricensis* appear to be sympatric on Hispaniola.

Flower buds of some species were distinguished as being depressed-truncate, subglobose, obovoid, or ovoid (Britton 1908; Britton & Rose 1920). The shape of the flower bud varies greatly as it ages and matures. This character is very difficult to assess

or apply without having some indication of the age of the flower bud and measurements on its dimensions.

Two species, *H. aboriginum* and *H. fernowii*, were described as having tawny trichomes although all observations of living material show white trichomes (Hooten 1991; pers. obs.). Indeed, all species have been observed to have white trichomes on the flower areoles in living material. Furthermore, all old herbarium specimens have tawny trichomes. Additionally, the short, uniseriate trichomes of the stem areoles of many species can be tawny when first emerging.

Flower petals margins were described as denticulate or entire (Britton 1908; Britton & Rose 1920). The margins of the flower petals of all species appear irregularly denticulate to varying degrees. None appear strictly entire. The petals are delicate and thin, and as such, their margins are not well preserved on herbarium specimens. Stem thickness has also been used (Areces 1980) based upon the original descriptions (Britton 1908). This character varies with age and the degree of hydration of the stem.

In the following key, characterization of the flower and overall spine morphology makes identification easiest. Seeds are measured near the middle of their dimensions.

KEY TO SPECIES OF SERIES *HARRISIA*

1. Spines similar on distal and basal areoles of stem; longest spines <3.5 cm long
    2. Pericarpel, hypanthium, and/or scales with pink or red
        3. Pericarpel and immature fruit reddish; scales of the pericarpel crowded and overlapping; mature fruit orange-red, rarely yellow; spines to 3.5 cm long

.....*H. fragrans*

3. Pericarpel and immature fruit green; scales of the pericarpel short, not overlapping; mature fruit yellow; spines to 1.5 cm long.....*H. aboriginum*

2. Pericarpel, hypanthium, and/or scales without any pink or red..... *H. gracilis*

1. Spines longer and thick on basal areoles of stem; longest spines to 3–12 cm long

5. Longest spines <3.5 cm on plant; mature fruit oblong, ellipsoid .....*H. brookii*

5. Longest spines >5 cm on plant; mature fruit globose (rarely ellipsoidal)

6. Most seeds 2.6–3.35 × 1.7–2.2 mm; sepals and upper scales purplish to reddish

7. Hypanthium and lower scales reddish; spines densely crowded and overlapping, stem nearly inaccessible.....*H. taetra*

7. Hypanthium and lower scales green; spines fairly sparse or moderately overlapping, stem accessible.....*H. eriophora*

6. Most seeds 2.0–2.8 × 1.4–1.7 mm; sepals and upper scales green to yellow-green to brown-green

8. Flowers to 15 cm long; longest spines 0.7 mm thick .....*H. caymanensis*

8. Flowers 16.5–21 cm long; longest spines 1.0–1.2 mm thick

9. Hypanthium scales ovate-lanceolate, acuminate, faintly arched at base; immature fruit without well-defined sulci, faintly tuberculate .....*H. fernowii*

9. Hypanthium scales deltoid-subulate, strongly arched at base; immature fruit strongly tuberculate with well-defined sulci

10. Spines fairly sparse or moderately overlapping, stem accessible

spines..... *H. divaricata*

10. Spines densely crowded and overlapping, stem nearly spines inaccessible ..... *H. portoricensis*

1. *Harrisia aboriginum* Small ex Britton & Rose, Cact. 2: 154. 1920. *Cereus aboriginum* (Small ex Britton & Rose) Little, Amer. Midl. Naturalist 33: 495. 1945. *Cereus gracilis* Mill. var. *aboriginum* (Small ex Britton & Rose) L. D. Benson, Cact. Succ. J. (Los Angeles) 41: 126. 1969. *Harrisia gracilis* (Mill.) Britton var. *aboriginum* (Small ex Britton & Rose) D. B. Ward, Novon 14: 366. 2004. — TYPE: U.S.A., Florida, Manatee Co., Terra Ceia Island, collected by John K. Small on April 1919. —LECTOTYPE (designated by Benson 1969): U.S.A., Florida, [Manatee Co.], western shore Terra Ceia Island, hammock, 29 Apr 1919, *Small et al*. *s.n*. (lectotype: NY!; isolectotype: US!).

Shrubs, trunk not evident or poorly developed. Spines not crowded, not overlapping spines of adjacent areoles, shorter than width of the stem, to 1.5 cm, 0.5 mm thick, spines of similar size on distal and basal areoles of stems. Flowers 18 cm long. Pericarpel green, scales not crowded and overlapping. Hypanthium pinkish brown, scales pinkish brown to red-brown, deltoid becoming deltoid-ovate distally. Sepals pale pink. Immature fruit green and lightly tuberculate, turning orange and smooth near maturity. Mature fruit yellow, smooth. Seeds 1.7–2.0 × 2.7–3.0 mm.

Distribution. Gulf coast, central peninsula of Florida, U.S.A (Fig. 4.3); coastal strands and maritime hammock, on sandy or shell fragment soils; 0–5 m.

ADDITIONAL SPECIMENS EXAMINED. **U.S.A.** FLORIDA: Lee Co.: Buck Key, *Hooten s.n.* (US); Buck Key, 29 Oct 2007, *Franck s.n.* (USF); Buck Key, *Possley & Fellows 29* (FTG); Bokeelia Island, Jun 2007, *Franck s.n.* (USF); Buck Island, *Austin & Austin 6863* (FTG). Manatee Co.: Terra Ceia Island, 1917, *Swingle s.n.* (US); Terra Ceia, Jan 1920, *Robinson s.n.* (K). Sarasota Co.: Osprey, 1906, *Webb s.n.* (NY, US); Longboat Key, *Lakela & Long 28145* (USF); Manasota Key, *Hansen & Richardson 6819* (USF); Longboat Key, *Lakela & Long 27609* (FLAS, USF); Little Sarasota Key, *Coville 24* (US); Manasota Key, *Beckner 1715* (FLAS); Manasota Key, *Franck 1237* (USF); Longboat Key, Jun 2007, *Franck s.n.* (USF).

Etymology. For the "shell heaps formed by the aborigines" (Britton & Rose 1920).

Harrisia aboriginum can be recognized by its consistently short spines, brownish hypanthium, green pericarpel, green immature fruit, and yellow mature fruit. Vegetatively it is most like two other short-spined species, *H. gracilis* and *H. fragrans*, although longer spines are also observed in these two (<3.5 cm). Hooten (1991) used a strict interpretation of the protologue to attempt to name a new species (*H. donae-antoinae*) because of the white hairs observed. This is undoubtedly synonymous with *H. aboriginum*. Additionally, all flower hairs seem to age to a tawny color on herbarium specimens, including the (now designated) type specimen of *H. donae-antoinae*.

2. *Harrisia brookii* Britton, Bull.Torrey Bot. Club 35: 564. 1908. *Cereus brookii* (Britton) Vaupel, Monatsschr. Kakteenk. 23: 24. 1913.—TYPE: Bahamas, Long Island, Clarence Town and vicinity, scrublands, 16–19 Mar 1907, *Britton &*

Millspaugh 6337 (holotype: NY!; isotype: US!); —EPITYPE (designated here): near Mortimers, dry open coppice on high rocky ridge, 24 Apr 1980, *Correll 51278* (epitype: FTG!, isoepitype: NY!).

Shrubs, trunk not evident or poorly developed. Spines of distal areoles sometimes overlapping spines of adjacent areoles, shorter than width of stem, to 1.5 cm long and 0.3 mm thick, spines of basal areoles somewhat crowded and overlapping, to 3 cm long and 0.6 mm thick. Flowers to 20 cm long. Pericarpel green, scales crowded and overlapping. Hypanthium green to reddish, scales deltoid-subulate, yellow-green to green distally. Sepals pinkish brown-green. Immature fruit green, tuberculate. Mature fruit yellow, oblong, ellipsoid, lightly tuberculate. Seeds 1.55–1.7 × 2.65–2.85 mm.

Distribution. Bahamas (Fig. 4.3); scrublands (Correll & Correll 1982) or coastal coppice (Correll 1979); 0–20 m.

ADDITIONAL SPECIMENS EXAMINED. **BAHAMAS**. ELEUTHERA ISLAND: *Vincent 13393* (MU). LONG ISLAND: Clarence Town, *Britton & Millspaugh 6337* (NY,US); Mortimers, *Correll 44980* (FTG); Clarence Town, *Hill 2291* (FTG, NCU); Clarence Town, 11 May 1974, *O'Kelly s.n.* (A); Deadman's Cay, *Correll 45038* (FTG). NEW PROVIDENCE ISLAND: *Robinson 475* (K).

Etymology. Hon. Herbert A. Brook, Registrar of the Bahamas.

Harrisia brookii can be recognized by its ellipsoid fruit (Vincent 2007), and spines that only become slightly longer and thicker on the lower stem. Although there is a

specimen made in 1880 labeled from New Providence Island (*Robinson 475* (K)), recent observations have only been made from Eleuthera and Long Island. Correll & Correll (1982) mentions that the specimen *G.R. Proctor 8812* (IJ) from Grand Turk is possibly referable to this species, but the specimen was not located in a recent search at IJ.

The holotype and isotype consist only of a flower bud. The designated epitype, illustrated in Correll & Correll (1982, Fig. 426), consists of stem, immature and mature fruit, flower bud, and a withered flower.

3. *Harrisia caymanensis* sp. nov. —TYPE: Cayman Islands, Cayman Brac, cutting grown in Florida, 10 June 2012, *A. R. Franck 3035* (USF!).

Shrubs, trunk not evident or poorly developed. Spines of distal areoles often overlapping those of adjacent areoles, often as long or longer than stem width, to 2.5 cm long and 0.5 mm thick, spines of basal areoles crowded and overlapping, to 6.5 cm long and 0.7 mm thick. Flowers to 14.5 cm long. Pericarpel green, scales crowded and overlapping. Hypanthium light green, scales light green to green, deltoid-subulate. Sepals greenish brown. Immature fruit green, lightly tuberculate. Mature fruit yellow, smooth. Seeds 1.4–1.5 × 1.9–2.1 mm.

Distribution. Caymans Islands and Swan Islands (Honduras) (Fig. 4.3); coastal scrub and scrubby woodland on exposed limestone rock; 5–50 m.

ADDITIONAL SPECIMENS EXAMINED. **CAYMAN ISLANDS**. CAYMAN BRAC: *Franck 2370*, *2371*, *2622*, *2630* (USF). **HONDURAS**. SWAN ISLANDS: Little Swan, 30 Jul 1974, *Clough s.n.* (IJ); Eastern Swan Island, 14 Nov 1973, *Moyne s.n.* (K).

Etymology. For the Cayman Islands.

The shortest flowers in the genus occur in *Harrisia caymanensis*. This species is also characterized by long spines on the lower areoles and small seeds. Its status as a new taxon was intimated previously (Areces-Mallea 1997). The name *H. gracilis* had been applied to these populations (Adams 1972; Proctor 1984). The populations of *Harrisia* on the Swan Islands, identified as *H. eriophora* (Sandoval et al. 2007), are here tentatively referred to this species, although no critical comparison has been made with the populations on the Cayman Islands.

4. *Harrisia divaricata* (Lam.) Backeb., Die Cactaceae 4: 2101. 1960 . *Cactus divaricatus* Lam., Encycl. 1: 540 1783. *Cereus divaricatus* (Lam.) DC., Prodr. 3: 466. 1828. *Pilocereus divaricatus* (Lam.) Lem., Rev. Hort. (Paris) 427. 1862. —TYPE: Haiti, Cul-de-sac, Plumier, plate 193, p. 187 in Burman, Pl. amer. 1758.

Cereus divergens Pfeiff., Enum. diagn. Cact. 95. 1837. —TYPE: Dominican Republic, Santo Domingo.

Harrisia nashii Britton, Bull.Torrey Bot. Club 35: 564. 1908. *Cereus nashii* (Britton) Vaupel, Monatsschr. Kakteenk. 23: 27. 1913. —TYPE: Haiti, between Gonaives and Plaisance, 1905, *Nash & Taylor 1765* (holotype: NY!).

Harrisia nashii Britton var. *straminea* W. T. Marshall, Cact. Succ. J. (Los Angeles) 15: 3. 1943. —TYPE: Dominican Republic, Monte Cristi Prov., Hurst finca near Monte Christi, 25 Nov 1936, *Marshall s.n*. (holotype: GH!).

Harrisia serruliflora (Haw.) Lourteig, Bradea 5: 408. 1991. *Cereus serruliflorus* Haw., Philos. Mag. Ann. Chem. 113. 1830.—TYPE: Haiti, Cul-de-sac, Plumier, plate 195, fig. 1, p. 188 in Burman, Pl. amer. 1758.

Shrubs or small trees, with well-developed erect trunk to 1 m. Spines of distal areoles not much overlapping those of adjacent areoles, shorter than stem width, to 2.0 cm long and 0.5 mm thick, spines of basal areoles crowded and overlapping, to 5.5 cm long and 1.0 mm thick. Flowers 17–23 cm long. Pericarpel green, scales crowded and overlapping. Hypanthium green to brownish green distally, scales light green to green, deltoid-subulate. Sepals greenish brown. Immature fruit green and strongly tuberculate, becoming ellipsoid, yellow, and strongly tuberculate near maturity. Mature fruit yellow, smooth, globose. Seeds 1.4–1.65 × 2.05 –2.6 mm.

Distribution. Lowlands and coastal areas of Hispaniola (Fig. 4.3); scrub forest and scrubland; 5–400 m.

ADDITIONAL SPECIMENS EXAMINED. **DOMINICAN REPUBLIC**. AZUA PROV.: Azua, *Rose et al*. *3831* (GH, NY, US); Azua, *Lavastre 2207* (NY); Puerto Tortuguero y Hatillo, *García et al*. *137* (JBSD); Hatillo, *Zanoni 31122* (JBSD, USF); Palmar de Ocoa, *Clase & García 2859* (JBSD); Hatillo, *Veloz 1779* (JBSD); Hatillo, *Franck et al*. *2305* (USF); Hatillo, *Franck et al*. *2306* (USF); Azua, *Lavastre 1914* (NY). BARAHONA PROV.: Las

Salinas, *Fuertes 974* (NY); Valley of Neiba, *Howard & Howard 8336* (GH, NY, US). INDEPENDENCIA PROV.: Municipio La Descubierta, Las Caritas, *Clase et al. 5637* (JBSD); Duverge, *Camejo & Feliz 60* (JBSD). MONTE CRISTI PROV.: Villa Elisa, *Franck et al. 2309* (USF); Monte Cristi, *Franck et al. 2307* (USF); Monte Cristi, *Franck et al. 2308* (USF); Villa Elisa, *Sauleda et al. 7546* (USF); Guayubín, *Abbott 1016* (US). PERAVIA PROV.: Punta Caballera, *Zanoni et al. 22005* (JBSD); Baní, *Zanoni et al. 14970* (JBSD). PERDENALES PROV.: Perdenales, *Liogier & Liogier 25260* (JBSD); Cabo Rojo, *Veloz et al. 942* (JBSD); Cabo Rojo, *Zanoni & Mejía 16753* (JBSD, NY); El Guano, *Zanoni & Pimentel 25903* (JBSD); Cabo Rojo, *Fisher-Meerow 793b* (FLAS). SAN JUAN PROV.: San Juan de la Maguana, *Liogier & Liogier 26641* (JBSD); Guanito, *Sauleda et al. 7579* (USF). SANTIAGO PROV.: Santiago to Jacagua, *Liogier 11041* (GH, NY, US); Navarrete, May 1985, *Kimnach s.n.* (HNT); Santiago de los Caballeros, *Read 1076* (FTG). VALVERDE PROV.: Mao, *Abbott 1043* (US). **HAITI**. ARTIBONITE DEPT.: Gonaïves, *Clover 19396* (US); Gonaïves, *Clover 11015* (US). NORD-OUEST DEPT.: Port de Paix, *Ekman 3994* (K, NY, US); Port de Paix, *Leonard & Leonard 15642* (GH, NY, US); Baie des Moustiques, *Leonard & Leonard 12003* (K, US). OUEST DEPT.: Étang Saumâtre, *Leonard 3513* (NY, US); Étang Saumâtre, *Leonard 4249* (NY, US); Plaine du Cul de Sac, 1916, *Buch s.n.* (IJ, illustration); Anse-à-Galets, *Leonard 3256* (US).

Etymology. Divaricate branching.

Harrisia divaricata forms a well-defined trunk with spreading branches in the sub-canopy and also has a strongly tuberculate immature fruit, small seeds, and long spines on the basal parts of the stem. This species may be sympatric and interbreed with *H.*

portoricensis as several specimens from Hispaniola are difficult to ascribe to either species. The description of *H. hurstii* appears to be based on a specimen of *H. portoricensis*. If *H. portoricensis* and *H. divaricata* interbreed, then they might be better classified as subspecies. However, *H. portoricensis* is traditionally defined as endemic to Puerto Rico. It has been suggested that *H. divaricata* is the only species in DR (Corman 2005). *Harrisia fernowii* requires careful study to verify its distinction from *H. divaricata*, given the subtle differences separating the species in the identification key. In addition to the specimens examined, its distribution is also mapped from Hilaire (2008).

Britton & Rose (1920) doubted that Plumier's plate, one of the earliest known illustrations of a species of *Harrisia* (Burman 1758), could be referred to any cactus species and as such introduced the name *H. nashii*. The plate is undoubtedly a *Harrisia* (Hunt 1984). Moscoso first recognized *H. divaricata* and *H. nashii* as two distinct species (1941) and later *H. hurstii* and *H. nashii* (1943).

5. *Harrisia eriophora* (Pfeiff.) Britton, Bull.Torrey Bot. Club 35: 562. 1908. *Cereus eriophorus* Pfeiff., Enum. diagn. Cact. 94. 1837. —TYPE: Cuba. —NEOTYPE (designated here): Pfeiffer & Otto, Abbild. Beschr. Cact., Tab. 22. 1843.

Shrubs or small trees, with well-developed erect trunk to 1 m. Spines of distal areoles not overlapping those of adjacent areoles, shorter than stem width, to 2.0 cm long and 0.5 mm thick, spines of basal areoles crowded and overlapping, to 6.5 cm long and 1.0 mm thick. Pericarpel green, scales not crowded and overlapping. Hypanthium green, scales green to green with red tips. deltoid to deltoid subulate. Sepals greenish pink.

Immature fruit green, tuberculate. Mature fruit yellow, smooth. Seeds 1.7–2.1 × 2.6–3.35 mm.

Distribution. West-central and western Cuba (Fig. 4.3); scrub forest and scrubland; 5–50 m.

ADDITIONAL SPECIMENS EXAMINED. **CUBA**. ARTEMISA PROV.: Mariel, *León 13415* (GH, HAC); Bay of Mariel, *Britton & Gager 7600* (K, NY, US). CIENFUEGOS PROV.: Cienfuegos, *Jack 5406*, *5527*, *& 5556* (GH); Paso Caballos, *Wood*, *Jr. & Atchison 7446* (A); Jagua, *Carabia 18058* (HAC). HAVANA PROV.: Cojimar, *León 7179* (GH, HAC, NY); Cojimar, *Baker 2828* (US). ISLA DE LA JUVENTUD PROV.: Sierra de los Caballos, *Britton & Wilson 15122* (NY, US); Isle of Pines, s.d., *Jennings s.n.* (US, acc. no. 3045199). MATANZAS PROV.: Espada, *León 16111* (HAC). MAYABEQUE PROV.: Cruz del Norte, *León & Daniel 7802* (HAC, NY); Jibacoa, *León 16833* (HAC). PINAR DEL RÍO PROV.: Sierra Mendoza, *Shafer 11135* (NY); Pan de Azúcar, *E. del Riseo et al. 27580* (HAC); Remates de Guane, *Roig 3938*, *4604* (HAC). SANCTI SPÍRITUS PROV.: Las Villas, *Shafer 12091* (A); Sancti Spíritus, *León 4091 & 4093* (HAC, NY); Sancti Spíritus, *Shafer 12090* (NY, US). VILLA CLARA PROV.: Coralillo, *Bermúdez 14270* (HAC).

Etymology. Woolly, for the flower buds.

Harrisia eriophora has flowers with relatively short, reddish scales, relatively large seeds, and long spines on the basal parts of the stem The designated neotype may be from the type specimen, although it is in not absolutely certain.

Areces (1980) explained that red-tipped scales are found in southern Havana Prov. (now Mayabeque Prov.) and that plants along the northern coast have green scales without red tips (Britton & Rose 1920, plate 28). However, plate 28 clearly shows that the upper scales-lower sepals do have the red tips.

6. *Harrisia fernowii* Britton, Bull.Torrey Bot. Club 35: 562. 1908. —TYPE: Cuba. —LECTOTYPE (designated here): Cuba, Santiago de Cuba Prov., Río Grande to Río Ubero, 1906, *Taylor 254* (lectotype: NY!).

Harrisia taylorii Britton, Bull.Torrey Bot. Club 35: 565. 1908. *Cereus taylorii* (Britton) Vaupel, Monatsschr. Kakteenk. 23: 37. 1913 —TYPE: Cuba, Santiago de Cuba Prov., sea-beach between Rio Grande and Rio Ubero, 1906, NYBG 25767 [living collection number], *Taylor 253* [not found]. —LECTOTYPE (designated here): Britton & Rose, The Cactaceae 2: 153, Fig. 224. 1920. —EPITYPE (designated here): Cuba, Guantánamo Prov., Novaliches, *Hioram 1806* (epitype: NY!, isoepitype: HAC!).

Shrubs or small trees with well-developed erect trunk to 1 m. Spines of distal areoles sometimes overlapping those of adjacent areoles, sometimes longer than stem width, to 3.0 cm long and 0.5 mm thick, spines of basal areoles crowded and overlapping, to 7.5 cm long and 1.2 mm thick. Pericarpel green, scales crowded and overlapping. Hypanthium dirty green to slightly reddish distally, scales green, ovate-lanceolate, acute. Sepals yellow green. Immature fruit green, moderately tuberculate. Mature fruit yellow, smooth, globose. Seeds 1.4–1.65 × 2.05–2.6 mm.

Distribution. East Cuba (Fig. 4.3); scrub forest and scrubland; 0–50 m.

ADDITIONAL SPECIMENS EXAMINED. **CUBA**. *Wright 2623* (BM, GH, K, NY, US), *2624* (BM, GH, K). CAMAGÜEY PROV.: entre Nuevitas y Minas, *León & Dahlgren 23393* (HAC). GRANMA PROV.: Sierra Maestra, *Meszaros 6* (ZSS), Cabo Cruz, *León 16358* (HAC). GUANTÁNAMO PROV.: Novaliches, *Maxon 4517* (NY, US); Guantánamo, *León 3931* (HAC, NY); Novaliches, *Britton 2003* (NY, US); Guantánamo Bay, *Britton 1969* (NY, US); Imías, *León & Pérez 12557* (HAC, NY, US); Imías, *León & Pérez 12484* (HAC, NY); Imías, *León 16854* (HAC); Novaliches, *Ekman 2962* (K); Pueblo de Maisí, *León 18495* (HAC); Maisí, *León 20097* (HAC). HOLGUÍN PROV.: Holguín, Jan 1984, *Dice s.n.* (HNT). SANTIAGO DE CUBA PROV.: Reuter, *León 3744* (HAC, NY); Daiquirí, *Britton & Cowell 12656* (NY); Santiago de Cuba, *León 3745* (HAC, NY); Bacanao, *Rauh 70007* (ZSS); Renté, *Chrysogone 2620* (HAC); Cabañas Bay, *Britton & Cowell 12814* (NY). U.S. NAVAL STATION GUANTANAMO BAY: Cuzco Beach, *Areces-Mallea et al. 6619* (MAPR).

Etymology. Professor B. E. Fernow.

The hypanthium and ovate-lanceolate scales of *Harrisia fernowii* are yellow-green to green. Its spines are much longer on the lower areoles of the stem. Here it is only marginally separated from *H. divaricata* by the flower scales.

Harrisia taylorii is here synonymized under *H. fernowii*, as intimated by Britton & Rose (1920) who stated that "specimens of the two [*H. fernowii* and *H. taylorii*] appeared to be different when first studied, but subsequent observations indicate that they may not

be distinct; additional evidence is needed to determine this question." The labels of several specimens at NY have had the name *H. taylorii* noticeably removed and replaced with *H. fernowii*.

The two were originally separated by the length and copiousness of hairs on the flower buds which appears quite untenable, especially since the hairs are variably deciduous. The protologue of *H. fernowii* had much shorter spines (Britton 1908) than in the redefined description (Britton & Rose 1920) where *H. taylorii* was tentatively synonymized, first characterized as having longer spines. Spine lengths appears to be quite variable among the specimens. The notion that stem thickness is a useful character seems quite dubious as this characters varies with the age, vigor, health, and degree of hydration of the stems.

The lectotype selected for *H. fernowii* is from the type collection, though the specimen from the type collection was made in 1912, years after the original description in 1908. The lectotype here designated for *H. taylorii* is a photograph, presumably of the type specimen, in its natural habitat, though there is little detail to discern its morphology. An epitype is designated to characterize the longer, ascending spines described in the protologue of *H. taylorii*.

The names *Cereus pitajaya* (Jacq.) DC and *C. pellucidus* Pfeiff were misapplied to this species (Grisebach 1866). Its distribution is further mapped from two literature sources that are presumably of this species (González Gutiérrez et al. 2006; Reyes & Acosta Cantillo 2007).

7. *Harrisia fragrans* Small ex Britton & Rose, Cact. 2: 149. 1920. *Cereus fragrans* (Small ex Britton & Rose) Little, American Midland Naturalist 33: 496. 1945. *Cereus eriophorus* Pfeiff. var. *fragrans* (Small ex Britton & Rose) L. D. Benson, Cact. Succ. J. (Los Angeles) 41: 126. 1969. *Harrisia eriophora* (Pfeiff.) Britton var. *fragrans* (Small ex Britton & Rose) D. B. Ward, Novon 14: 366. 2004. —TYPE: U.S.A., Florida, St. Lucie Co., 6 mi. S of Ft. Pierce, collected by John K. Small on Dec 1917. —LECTOTYPE (designated here): U.S.A., Florida, St. Lucie Co., 6 mi. S of Ft. Pierce, hammock on sand dune, 20 Dec 1917, *Small 8457* (lectotype: NY!, isotypes: FLAS!, GH!, NY!, US!).

Harrisia simpsonii Small ex Britton & Rose, Cact. 2: 152. 1920. *Cereus gracilis* Mill. var. *simpsonii* (Small ex Britton & Rose) L. D. Benson, Cact. Succ. J. (Los Angeles) 41: 126. 1969. *Harrisia gracilis* (Mill.) Britton var. *simpsonii* (Small ex Britton & Rose) D. B. Ward, Novon 14: 367. 2004.—TYPE: U.S.A., Florida, Monroe Co., between Cape Sable and Flamingo, collected by John K. Small on 29 Nov 1916 [not found]. —LECTOTYPE (designated here): U.S.A., Florida, Monroe Co., Pumpkin Key, 18 Jun 1917, *Small s.n.* (lectotype: NY!, isolectotype: NY!, US!).

Shrubs, trunk not evident or poorly developed. Spines of distal areoles sometimes overlapping those of adjacent areoles, sometimes as thick as stem width, to 3.5 cm long and 0.8 mm thick, spines of distal and basal areoles similar. Flowers 15–20 cm long. Pericarpel red-green, scales crowded and overlapping. Hypanthium red basally to brownish yellow-green distally, scales deltoid subulate, long acuminate, brownish

yellow-green with red tips. Sepals brownish yellow-green to pale pink-brown. Immature fruit reddish green to dark red-purple, tuberculate. Mature fruit red to pale red to yellow-orange, smooth to lightly tuberculate. Seeds 1.7–2.0 × 2.7–3.0 mm.

Distribution. Atlantic Coast of upper peninsula, lower Everglades and Keys, Florida, U.S.A (Fig. 4.3); coastal berm, scrub, shell mound, rockland hammock, and maritime hammock; 0–10 m.

ADDITIONAL SPECIMENS EXAMINED. **U.S.A.** FLORIDA: Between Indian River and ocean, *Curtiss 963* (NY, US). Brevard Co.: Malabar, 30 Jul 1912, *Small s.n.* (NY); Malabar, *Rolfs 78* (NY); Melbourne Beach, 7 Jan 1983, *Hames s.n.* (FLAS). Indian River Co.: Vero Beach, *Popenoe 2411* (FTG). Miami-Dade Co.: Madeira Hammock, 10 May 1919, *Small et al. s.n.* (NY); Cuthbert Lake, Apr 1916, *collector not given* (NY); Biscayne Bay, *Franck 1236*, *1465*, *2845*, *2899* (USF). Monroe Co.: Pumpkin Key, 1919, *Small s.n.* (NY, US); Flamingo, May 1919, *Small s.n.* (NY); Key Largo, 22 May 1919, *Small & DeWinkeler s.n.* (NY); Key Largo, *Small 29964*. (NY); Cape Sable, *Moldenke 5861* (NY); Upper Matecumbe Key, *Moldenke 5832* (NY); Everglades National Park, *Hill 2948* (FTG, UNC); Key Largo, *Long et al. 1863* (USF); Key Largo, 23 Feb 1968, *Skinner & Weymouth s.n.* (FTG); Flamingo, 16 May 1965, *Craighead s.n.* (FTG); Everglades, 16 Apr 1965, *Craighead s.n.* (USF); Tavernier, *Franck 1278* (USF); Everglades National Park, *Franck 1277* (USF); Everglades National Park, *Benson 16578* (RSA); Plantation Key, *Franck 1279* (USF); Harry Harris Park, *Franck 2203*, *2722* (USF); Key Largo, *Franck 2204* (USF); Key Largo, *Austin et al. 6880* (FTG); Key Largo, *Austin & Austin 6868* (FTG); Turkey Gobbler Key, *Austin et al. 6896* (FTG); Big Pine Key: 8–9 May

1919, *Small et al. s.n.* (NY, US); *Miller, Jr. 1713* (US); *Killip 41330* (US); *Killip 32033* (FLAS, K, US); *Killip 44348* (US); *Benson et al. 16575* (RSA); *Austin et al. 6942* (FTG), 1986, *Tabb s.n.* (FTG); *Franck 2627* (USF). Palm Beach Co.: *Avery 1920* (FLAS), *Avery 1893* (FTG). St. Lucie Co.: Ft. Pierce, Aug 1916, *Vosburg s.n.* (US); Savannas Preserve State Park, *Franck 472* (USF); Ft. Pierce, *Benson & Benson 15375* (RSA); Stuart, *McCart 11289* (FLAS), Savannas Preserve State Park, *Bradley et al. 2426* (FTG). Volusia Co.: Cape Canaveral National Seashore, *Franck 473* (FTG, USF); Turtle Mound, *Norman 101* (RSA).

Etymology. Odorous flower.

Harrisia fragrans is the only species with red fruit (Small 1932) in the Caribbean. Yellow fruits are occasionally observed but may later change to red (Austin 1984). The name *H. simpsonii* is here treated as a synonym of *H. fragrans*. The DNA sequence data (with the exception of the St. Lucie Co. population) and morphology support synonymization of *H. simpsonii* with *H. fragrans* (Chapter 3). The St. Lucie Co. population experienced an introgression of the plastid haplotype from *H. aboriginum* (Chapter 3). The AFLP data does not support the synonymy proposed, though the error rate of the AFLP analyses was rather high (Chapter 3).

It had been asserted that *H. fragrans* occurs only near Malabar and Ft. Pierce (Austin 1984) and all other populations from Volusia Co. southward were *H. gracilis* (presumably as =*H. gracilis* var. *simpsonii*). Various authors have attempted to separate populations from south Florida (*H. simpsonii*) and east coast Florida (*H. fragrans* s. s.) (Britton & Rose 1920; Small 1933; Austin 1984; Chafin 2000) but the characters cited

are not useful. Flower petal margins (Britton & Rose 1920), hypanthium shape (Small 1933), and fruit shape (Small 1933) of plants from south Florida and east coast Florida are similar. Additionally these characters are best observed on live plants and are not preserved well on herbarium specimens. It is not clear how the two were distinguished by Austin (1984), except for an allusion to spine and fruit qualities. Long central spines thought to be unique to *H. fragrans* s. s. (Chafin 2000) have been observed from Big Pine Key (*Benson et al. 16575*), Biscayne Bay (*Franck 2845*), and Savannas Preserve State Park (*Franck 472*).

There may not have been a significant disjunction between *Harrisia* from south Florida (*H. simpsonii*) and east coast Florida (*H. fragrans*) as noted by Small (1933). Two collections by *Avery* (*1893*, *1920*) were labeled "originally from Palm Beach Co." without further details. The overwhelming amount of development along coastal Palm Beach, Broward, and Miami-Dade counties may have easily extirpated any populations of *Harrisia*, which are quite rare overall in Florida.

Benson (1982) actually mapped the distributions of *H. fragrans* s. s. and *H. simpsonii* as overlapping, possibly sympatric. The length of the flower areole trichomes was used to separate the two, though this character is also not useful.

Of the two Florida species, *H. fragrans* has received the most attention (Rae 1995; Rae & Ebert 2002; Hutchinson & Pazara 2004; Bradley & Hines 2007) and is also the only federally endangered *Harrisia* in the U.S.A.

A lectotype is designated for *H. fragrans* because the two potential holotype sheets at NY have different labels and cannot be assumed to intentionally represent a single specimen.

The cited holotype of *H. simpsonii* has never been located, however two other specimens were cited in the protologue (Britton & Rose 1920), a photograph (Fig. 223) and a Pumpkin Key specimen cultivated by the New York Botanical Garden and Charles Deering. Two Pumpkin Key specimens collected by *Small* exist at NY. The herbarium specimen, which consists of a stem and flower, was chosen as the lectotype. Benson (1969) designated a lectotype not cited (thus a neotype) by the authors, which consisted only of a flower, and is here superseded.

The name *H. brookii* has been misapplied to this species (Small 1913a, 1913b). Backeberg (1977) listed an undescribed species as *H. deeringii* that is 2 m tall from Pumpkin Key but it is presumably synonymous with *H. simpsonii* and thus *H. fragrans*.

8. *Harrisia gracilis* (Mill.) Britton, Bull.Torrey Bot. Club 35: 563. 1908. *Cereus gracilis* Mill., Gard. dict., ed. 8. 1768. *Cactus gracilis* (Mill.) Weston, Bot. univ. 1: 33. 1770. —TYPE: British Islands of America, received in 1728. —NEOTYPE (designated here): Jamaica, St. Andrew Par., Palisadoes, *Franck et al. 2265* (neotype: NY!, isoneotype: USF!). —EPITYPE (designated here): St. Andrew Par., Port Henderson, *Higgins s.n.* (photo, NY!).

Shrubs, or small trees, with well developed trunk to 1m. Spines not overlapping those of adjacent areoles, shorter than stem width, to 2.5 cm long and 0.5 mm thick, distal and basal spines similar. Flowers 21 cm long. Pericarpel green. Hypanthium greenish brown, scales yellow-green to green deltoid subulate. Sepals pale greenish brown.

Immature fruit green, moderately tuberculate. Mature fruit yellow, smooth. Seeds 1.3–1.6 × 2.1–2.3 mm.

Distribution. Coastal southern Jamaica (Fig. 4.3); scrubland forest; 5-100 m.

ADDITIONAL SPECIMENS EXAMINED. **JAMAICA**. CLARENDON PAR.: Salt Island, *Britton 3070* (NY); Harris Savannah, Dec 1998, *Douglas s.n*. (UWI); Harris Savannah, *Franck 2661* (USF). ST. ANDREW PAR.: Palisadoes, *Franck et al*. *2265* (IJ, USF); Palisadoes, *Asprey et al*. *2422* (UWI); Palisadoes, *West & Arnold 468* (FLAS); Sandy Gully, *Robertson 2333* (UWI); Hope, *Harris 6946* (UWI); Kingston and Gregory Park, *Maxon & Killip 335* (GH, NY, US); Kingston, *Britton 413* (NY). ST. CATHERINE PAR.: Hellshire Hills, *Scott 288* (UWI); Hellshire Hills, *duQuesnay 453* (UWI); Hellshire Hills, s.d., *Fleming s.n*. (ZSS, acc. no. AX 12819); Spanish Town, *Proctor 36874* (FTG). ST. ELIZABETH PAR.: Great Pedro Bay, *Britton 1255* (NY).

Etymology. Gracile, slender stems.

Harrisia gracilis is the only short-spined species outside of Florida. The epitype clearly shows the short spines on the trunk. It differs from the Florida species by having a greenish brown hypanthium (Britton 1917) and smaller seeds. The name *C. eriophorus* has been misapplied to this species (Grisebach 1860). Some of the earliest known descriptions of *Harrisia* are of *H. gracilis* from Jamaica (Sloane 1696; Trew 1751).

9. *Harrisia portoricensis* Britton, Bull.Torrey Bot. Club 35: 563. 1908. *Cereus portoricensis* (Britton) Urb., Symb. antill. 4: 430. 1910. —TYPE: Puerto Rico,

mainland, near Ponce, 1906, *Britton & Cowell 1324* (holotype: NY!). —EPITYPE (designated here): Puerto Rico, Mona Island, 6 Mar 1994, *Areces-Mallea s.n.* (2 sheets, NY!).

Harrisia hurstii W.T. Marshall, Cactaceae, with illustrated keys of all tribes, subtribes and genera, 96. 1941. —TYPE: Dominican Republic, Monte Cristi Prov., Hurst finca near Monte Cristi, 25 Nov 1936, *Marshall s.n.* (holotype: GH!).

Shrubs, trunk not evident or poorly developed. Spines of distal areoles usually overlapping those of adjacent areoles, often as long or longer than stem width, to 3.0 cm long and 0.7 mm thick, spines of basal areoles to 7.5 cm long, 1.0 mm thick. Flowers 17–22 cm long. Pericarpel green, scales crowded and overlapping. Hypanthium green to greenish brown, scales deltoid subulate, yellow green to green. Sepals pale greenish brown. Immature fruit green. Mature fruit yellow, smooth. Seeds 1.4–1.7 × 2–3 mm.

Distribution. Southwest mainland and western and southern islands of Puerto Rico, northern coast of Haiti, and west Dominican Republic (Fig. 4.3); scrubland on exposed limestone rock; 0-150 m.

ADDITIONAL SPECIMENS EXAMINED. **DOMINICAN REPUBLIC**. INDEPENDENCIA PROV.: Isla Cabritos, *Mejía & Pimentel 17228* (JBSD). MONTE CRISTI PROV.: El Morro, *Judd et al. 3002* (FLAS, JBSD); Monte Cristi, 25 Nov 1936, *Marshall s.n.* (GH). SANTIAGO PROV.: Jaiqui Picado, *Liogier 11241* (GH, NY, US). **HAITI**. NORD-OUEST DEPT.: Mole St. Nicolas, *Zanoni et al. 33542* (JBSD). **PUERTO RICO**. *Britton 1957* (BM). PONCE MUNICIP.: Ponce to Playa Las Cucharas, *Britton et al. 1959* (NY, US); Caja

de Muertos Island, *Breckon & López 7095* (MAPR). DESECHEO ISLAND: *Britton et al. 1599* (NY, US); *Woodbury et al. D-152* (MO, NY); *Breckon & Carraro 4780* (MAPR). MONA ISLAND: *Britton et al. 1737* (NY, US); *Chardon & Otero 1001* (NY); *Little, Jr. & Kuns 16540* (NY, US); s.d., *Fumero-Cabán & Meléndez-Ackerman s.n.* (USF, acc. no. 252611); *Breckon & Kolterman 6747* (MAPR). MONITO ISLAND: *Breckon et al. 5185* (MAPR). **CULTIVATED** (origin unknown): *Peláez 1474* (JBSD).

Etymology. Puerto Rico.

Harrisia portoricensis is recognized by its densely overlapping and numerous spines in combination with its green scales and hypanthium. Because the holotype consists solely of a flower specimen (made in 1912, after the original publication in 1908), an epitype is designated to characterize the distinctive stem morphology and also includes an immature fruit with withered flowers. It is apparently extirpated from the mainland Puerto Rico (Liogier 1994; Medina & Cuevas 2009) where it was found near Ponce (Britton 1908) and Guayanilla (Spencer 1955). Traditionally *H. portoricensis* is defined as an endemic of Puerto Rico. The several specimens here cited from Hispaniola match this species.

10. *Harrisia taetra* Areces, Revista Jard. Bot. Nac. Univ. Habana 1: 17. 1980.—TYPE: Cuba, Pinar del Río Province, península Guanahacabibes ad latus merid. gradum septr. 22° 53’ ext. secus viam ad extremun San Antonio in Terraza la Iguana locis calcareis corallinis apertis valde sterilibus, 5–8 m alt. s. m., men. Mart. 1973 fl. et fruct., *Areces 37990* (HAJB, not found). —NEOTYPE (designated here): Cuba,

Pinar del Río Province, Peninsula de Guanahacabibes, Terraza, costera la Iguana, en la carretera al cabo de San Antonio, 27 Mar 1973, *Areces 37991* (neotype: HAJB!).

Shrubs, trunk not evident or poorly developed. Spines of distal areoles usually overlapping those of adjacent areoles, often as long or longer than stem width, to 10.3 cm long and 0.9 mm thick, spines of basal areoles to 12 cm long, 1.5 mm thick. Flowers 16.5–20 cm long. Pericarpel pale green. Hypanthium vinaceous to rose-purple to rose-pink, sometimes reddish green distally, scales deltoid-ovate, pale purple to yellowish with red tips. Sepals purple, slightly pinkish. Immature fruit greenish red to dark red-purple, moderately tuberculate. Mature fruit yellow, smooth to lightly tuberculate. Seeds 1.8–2.0 × 2.9–3.2 mm.

Distribution. Southwest Pinar del Río Prov., Cuba (Fig. 4.3); scrubland on exposed limestone rock; 5–20 m.

ADDITIONAL SPECIMENS EXAMINED. **CUBA**. PINAR DEL RÍO PROV.: Guanahacabibes, 17 Sep 2008, *ZSS staff s.n.* (ZSS); Corrientes Bay, *Britton & Cowell 9880* (NY); Península Corrientes, *Alain 6917* (HAC); Las Martinas, *Shafer 11096* (A, NY).

Etymology. From *taeter*, meaning horrid, repulsive, etc. referring to the thick, spiny stems.

Harrisia taetra has densely overlapping spines and flowers with red-purplish scales and hypanthium. Though apparently unrelated, this species has a similar vegetative morphology to *H. portoricensis*, also occurring on exposed limestone rock.

Ia-1. *Harrisia* series *Earlei* A. R. Franck —TYPE: *Harrisia earlei* Britton & Rose.

Shrubs. Stems pendent, prostrate, clambering, only young stems ascending, or erect, 5–7 ribs. Young spines bright red with darker tips, becoming black to gray. Flower buds very hairy. Pericarpel green. Hypanthium light green. Scales brownish, reddish tips. Sepals pale yellow-greenish with brown-red tips. Immature fruit green with scales at least half as long as the tubercles. Fruits yellow. Seeds 1.65–1.8 x 2.55–2.95 mm.

Distribution. One species in Pinar del Río Province, Cuba (Fig. 4.3); scrub forest over limestone boulders; 100–400 m.

It seems quite probable that this series was once more diverse or widespread historically, perhaps sympatric with other species of *Hylocereus* (A. Berger) Britton & Rose and *Strophocactus* Britton & Rose that occupy similar habitats, found prostrate on boulders in Central America and Mexico (Chapter 2).

1. *Harrisia earlei* Britton & Rose, Cact. 2: 154. 1920. —TYPE: Cuba, Pinar del Río Prov., San Diego de los Baños, limestone rocks, 31 Aug–3 Sep 1910, *Britton et al. 6667* (holotype: NY!).

Shrubs. Stems pendent, prostrate, clambering, only young stems ascending, or erect, 5–7 ribs. Young spines bright red with darker tips, becoming black to gray. Flower buds very hairy. Pericarpel green. Hypanthium light green. Scales brownish, reddish tips. Sepals pale yellow-greenish with brown-red tips. Immature fruit green with scales at least half as long as the tubercles. Fruits yellow. Seeds 1.65–1.8 × 2.55–2.95 mm.

Distribution. Pinar del Rio Provínce, Cuba (Fig. 4.3); scrub forest over limestone boulders; 100–400 m.

ADDITIONAL SPECIMENS EXAMINED. **CUBA**. PINAR DEL RÍO PROV.: Pan de Azucar, *Morton s.n*. (HNT, UC, US); Sumidero, *Shafer* [& *León*] *13397* (GH, HAC, NY, US); Río Guao, *Britton et al*. *9653* (NY, US); Sierra Guayaba, *Shafer 13862* (NY, US); Sierra Guane, *Shafer 10524* (NY, US); Viñales, *Ardisson 99.15* (USF); San Diego de Los Baños, *Agathange 5045* (HAC, NY); San Diego de los Baños, *Earle s.n*. (NY); Guane, *Britton et al*. *9747* (NY); San Diego de los Baños, *León 4231* (NY); 2 May 2006, *ZSS staff s.n*. (ZSS).

Etymology. Franklin S. Earle (1856–1929), mycologist.

Harrisia earlei is the most distinctive species in the Caribbean with its prostrate habit and 5–7 ribs. A description of the flowers was provided by Delanoy (2004) and a description of its habitat by Ardisson & Delanoy (2002).

Ib. *Harrisia* sect. *Adscendens* A. R. Franck —TYPE: *Harrisia adscendens* (Gürke) Britton & Rose.

Shrubs. Stems arching, reclining, clambering, ascending, or erect, glaucous green, 6–10 ribs. Spines to 3 cm long, spines of basal and distal areoles similar in size. Newly emergent spines red. Pericarpel and hypanthium green, scales deltoid to deltoid-subulate, green with reddish tips. Sepals green to pale reddish green. Petals white. Fruits orange to red, rarely yellow-orange, tearing at sides to expose inner pulp and seed. Seeds 2–2.3 × 2.8–2.9 mm.

Distribution. Northeastern Brazil (Fig. 4.2); Caatinga; 100–600 m.

As in series *Earlei*, this section is monotypic and also might have been historically more widespread given the presence of other columnar cacti in northern South America (Chapter 2).

1. *Harrisia adscendens* (Gürke) Britton & Rose, Cact. 2: 155. 1920. *Cereus adscendens* Gürke, Monatsschr. Kakteenk.18: 66. 1908. *Eriocereus adscendens* (Gürke) A. Berger, Kakteen 129. 1929. —LECTOTYPE (designated by Taylor & Zappi 2004): Brazil, Bahia, Tambury, den Niederungen der catinga, Oct 1906, *Ule 7072* (lectotype: B; isolectotype: HBG , K [photo]).

Shrubs. Stems arching, reclining, clambering, ascending, or erect, glaucous green, 6–10 ribs. Spines to 3 cm long, spines of basal and distal areoles similar in size. Newly emergent spines red. Pericarpel and hypanthium green, scales deltoid to deltoid-subulate, green with reddish tips. Sepals green to pale reddish green. Petals white. Fruits orange to

red, rarely yellow-orange, tearing at sides to expose inner pulp and seed. Seeds 2–2.3 × 2.8–2.9 mm.

Distribution. Northeastern Brazil (Fig. 4.2); Caatinga; 100–600 m.

ADDITIONAL SPECIMENS EXAMINED. **BRAZIL**. BAHIA: Juazeiro, *Rose & Russell 19730* (NY, US); Salgada, *Rose & Russell 19715* (NY, US); Barrinha, *Rose & Russell 19794* (NY); Joazeiro, *Zehntner 729* (US); Teofilandia, *Taylor et al. 1349* (ZSS); Uauá, *Taylor et al. 1362* (ZSS); Juazeiro, *Taylor et al. 1388* (ZSS); Iaçú, *Taylor et al. 1580* (ZSS); Ipirá, *Hofacker 403* (USF, ZSS). **CULTIVATED** (origin unknown): *Schumann 99* (NY); 1901, *Britton s.n.* (NY); *Franck 2641*, *2896* (USF).

Etymology. Climbing, ascendant.

Harrisia adscendens is characterized by its oblong seeds, glaucous stem, dehiscent fruit, and long stigma lobes. This species has been treated in detail by Taylor & Zappi (2004). The unknown *Harrisia* reported from the Cerrado of Unaí, Brazil (Junqueira et al. 2010) may be *H. adscendens*.

II. *Harrisia* subg. *Eriocereus* (A. Berger) Britton & Rose ex R. Kiesling, Darwinia 34: 390. 1996. *Cereus* Mill. series *Tortuosi* K. Schum., Gesamtbeschr. Kakt. 54, 135. 1899. *Cereus* Mill. subg. *Eriocereus* A. Berger, Rep. (Annual) Missouri Bot. Gard. 16: 74. 1905. *Eriocereus* (A. Berger) Riccob., Boll. Reale Orto Bot. Palermo 8: 238. 1909. *Harrisia* Britton [unranked] *Eriocereus* (A. Berger) Britton

& Rose. 1920. Cact. 2:148. —TYPE: *Harrisia tortuosa* (J. Forbes ex Otto & A. Dietr.) Britton & Rose.

Shrubs or large trees. Stems arching, reclining, clambering, ascending, or erect, 3–9 ribs. Spines to 4.6 cm long, 1.7 mm thick. Newly emergent spines yellow-green to red. Seed length and width subequal, 1.7–2.2 × 1.8–2.5 mm.

Distribution. One species in the Eastern Cordillera of Bolivia and five species in the Gran Chaco region of southeast Bolivia, north Argentina, Paraguay, southwest Brazil, and west Uruguay (Fig. 4.4), and naturalized in Australia, South Africa, and Hawaii; scrub forest and scrubland; 50–2600 m.

Berger (1905) unambiguously designated the type of *Cereus* subg. *Eriocereus*, though Hunt et al. (2006) referred to Backeberg (1960) as lectotypifying the name. Britton & Rose (1920) used an unranked group *Eriocereus* within *Harrisia* and did not clearly denote it as a subgenus. Kiesling (1996) appears to be the first to satisfactorily publish *Harrisia* subg. *Eriocereus*, though he gives credit to Britton & Rose (1920).

KEY TO SECTIONS OF SUBGENUS *ERIOCEREUS*

1. Shrubs; stems curvaceous and arching, scrambling, clambering, or prostrate; ribs 3–8; fruits widely & laterally dehiscent; Gran Chaco and adjacent areas .....sect. *Eriocereus*
1. Trees; stems mostly stiffly erect; ribs 7–9; fruits narrowly apically dehiscent; inter-Andean valleys of Bolivia..... sect. *Roseocereus*

IIa. *Harrisia* sect. *Eriocereus*

Shrubs. Stems clambering, ascending, to 4 cm thick, ribs 3–7. Flower buds white to reddish hairy. Pericarpel green. Hypanthium green to pinkish green. Scales deltoid to deltoid-subulate. Sepals brownish green. Fruits, tearing at sides to expose inner pulp and seed.

Distribution. Five species in the Gran Chaco region (Fig. 4.4) and naturalized in Australia, South Africa, and Hawaii; chaco serrano, chaco arido, chaco semi-arido, chaco humedo, espinal, pantanal, pampeana; 50–1200 m.

KEY TO SPECIES OF SECTION *ERIOCEREUS*

1. Most areoles with 1–4 long, central spines to 3.2 cm that are 1.5–2 times as long as most radials spines; ribs mostly 3–5
    2. Pericarpel and lower hypanthium areoles with reddish scaly hairs and usually without spines; fruit without spines; sulcus undefined or only faintly so.... *H. regelii*
    2. Pericarpel and lower hypanthium areoles resembling stem areoles, without scaly hairs and usually with spines; scaly hairs white; fruit usually with spines; sulcus evident as a line between the ribs .....*H. martinii*

1. Most spines of similar length; ribs 3-7
    3. Sulcus undefined, no line between ribs; scales of mature fruit persistent; sulcus undefined or only faintly so ..... *H. bonplandii*
    3. Sulcus evident as line between ribs; scales of mature fruit usually deciduous

4. Stem glaucous green to light green; ribs not tuberculate or only lightly so; ribs 4–6; fruit not spiny.....*H. pomanensis*

4. Stem green; ribs lightly to moderately tuberculate; ribs 6–8; fruit often spiny..... .....*H. tortuosa*

1. *Harrisia bonplandii* (Parm. ex Pfeiff.) Britton & Rose, Cact. 2: 157. 1920. *Cereus bonplandii* Parm. ex Pfeiff., Enum. diagn. Cact. 108. 1837. *Eriocereus bonplandii* (Parm. ex Pfeiff.) Riccob., Boll. Reale Orto Bot. Palermo 8: 238. 1909. *Harrisia pomanensis* (F. A. C. Weber ex K. Schum.) Britton & Rose subsp. *bonplandii* (Parm. ex Pfeiff.) Braun & Esteves, Succulenta (Netherlands) 73: 131. 1995. —TYPE: Brazil. —NEOTYPE (designated by Kiesling 1996): Argentina, Formosa Prov., Pilcomayo Dept., Parque Nacional Pilcomayo, 14 Dec 1988, *Guaglianone & Múlgura 2228* (neotype: SI; isoneotype: B!).

Cereus acutangulus Pfeiff., Enum. diagn. Cact. 107. 1937. —TYPE: Mexico [perhaps dubious, cf. Leuenberger 2001]. —NEOTYPE (designated by Leuenberger 2001): cult. hort. Berol., 9 Feb 1983, *Schwerdtfeger 15056* (neotype: B).

Cereus balansae K. Schum., Fl. bras. 4: 210. 1890. *Harrisia balansae* (K. Schum.) N. P. Taylor & Zappi, Cactaceae Consensus Init. 3: 7. 1997. —TYPE: In saxosis rei publicae Paraguariae prope l'Assumption, floret Decembri, *Balansa 2504*. (holotype: B, destroyed). —LECTOTYPE (designated by Kiesling 1996): Paraguay, Asunción, dans le forest, *Balansa 2504* (lectotype: G!; isolectotype: K, P).

Cereus guelichii Speg., Anales del Mus. Nac. Buenos Aires 11: 482. 1905. *Eriocereus guelichii* (Speg.) A. Berger, Kakteen 130. 1929. *Harrisia guelichii*

(Speg.) Britton & Rose, Cact. 2: 158. 1920. —TYPE: not indicated. —LECTOTYPE (designated by Kiesling 1996): Britton & Rose, Cact. 2: 158, Fig. 228. 1920. (isolectotype: NY!, photo at NY is mounted along with 3 other photos presumably of same plant, "Chaco, Argentina, *Spegazzini*, Rec'd 1915").

Cereus pomanensis F. A. C. Weber ex K. Schum. var. *grossei* Weing. ex A. Berger, Kakteen 128. 1929. —TYPE:?

Stems green. Ribs not tuberculate, 3–5, indistinct with no demarcating line at sulcus. Spines of similar length. Flower buds mostly glabrous. Pericarpel and lower hypanthium areoles not similar to stem areoles. Flowers 17–24 cm long. Pericarpel green. Hypanthium light green, scales deltoid-subulate, red-brown to green basally, with reddish hairs. Sepals brownish green to green. Fruits without spines, retaining scales.

Distribution. Southeast Bolivia, north Argentina, Paraguay, and southeast Bolivia (Fig. 4.4), and naturalized in Hawaii; chaco serrano, chaco árido, chaco semi-árido, chaco húmedo, espinal, pantanal; 80–900 m (*Nee 51221*).

ADDITIONAL SPECIMENS EXAMINED. **ARGENTINA**. BUENOS AIRES PROV.: La Plata, *Rose & Russell 21095* (US). CORRIENTES PROV.: Estancia Santa Teresa, *Pedersen 3083* (A, US). TUCUMÁN PROV.: Trancas Dept., Vipos, *Venturi 3555* (NY, US). Jujuy Prov.: Calilegua, *Shafer 70* (GH, MO, NY, US). Salta Prov.: Salta, *Shafer 39* (GH, MO, NY, US). **BOLIVIA**. SANTA CRUZ DEPT.: Boyuibe, *Cárdenas 5025* (US). Andres Ibáñez Prov.: Puerto Pailas, *Nee 44863* (MO, NY); Cotoca, *Nee 37764* (MO, NY); Río Grande Planta de Gas, *Nee et al. 44630* (MO, NY). Cordillera Prov.: Comunidad Salinas, *Nee*

51221 (MO, NY). **PARAGUAY**. ALTO PARAGUAY DEPT.: Cerro León, *Matilde Zardini & Carlos Rivas 58334* (MO). BOQUÉRON DEPT.: Campo Loro, *Schmeda 1185* (US). DISTRITO CAPITAL: Asunción, *Shafer 138* (NY, US). GUAIRÁ DEPT.: Olimpo, *Schmeda 1460* (US). ITAPÚA DEPT.: Trinidad, *Morong 268* (NY); Isla Alta, *Schmeda 1489* (US). **U.S.A.** HAWAII: Kauai, Poipu Distr.: Waihoai, *Dunn & Wood 288* (PTBG). Koloa Distr.: Poipu, *Lorence & Flynn 6279* (PTBG); Poipu, *Lorence 7698* (PTBG). **CULTIVATED** (origin unknown): *Franck 2642* (USF).

Etymology. Aimé Jacques Alexandre Bonpland (1773–1858), explorer and botanist.

Harrisia bonplandii has a few-ribbed stem with an undefined sulcus, spines of a similar length, and a fruit with persistent scales. This species appears to be the most tolerant to wetter conditions, occurring in the Pantanal (Eggli 2002) where it is said to be common (Junk et al. 2006).

In favor of the use of *H. balansae*, Hunt et al. (2006) stated that the name *H. bonplandii* is "to be rejected" contrary to Art. 51 (McNeill et al. 2006) but no formal proposal has been made. Leuenberger (2001) exhaustively detailed the history of the name and elected to maintain use of the name *H. bonplandii*. Its modern application appears to be consistent and widely accepted. The lectotype designated by Taylor & Zappi (1997) was superseded by Kiesling (1996).

2. *Harrisia martinii* (Labour.) Britton, Addisonia 2: 55. 1917. *Cereus martinii* Labour. Annales Soc. Hort. Haute-Garonne 1: 182. 1854. *Eriocereus martinii* (Labour.) Riccob., Boll. Reale Orto Bot. Palermo 8: 241. 1909.—TYPE: not indicated. —

NEOTYPE (designated by Kiesling 1996): Argentina, Entre Ríos Prov., Colón Dept., Colón, 6 Feb 1985, *Kiesling 5069* (neotype: SI!; isoneotype: CTES).

Cereus martinii Labour. var. *perviridis* Weing., Monatsschr. Kakteenk. 24: 72. 1914. *Harrisia perviridis* (Weing.) Borg, Cacti: a gardener's handbook for their identification and cultivation 145. 1937. —TYPE: Paraguay, [Guairá Dept.], Estancia San Salvador, *Anisits 50*.

Stems green. Ribs tuberculate, 4–5, separated by distinct line at sulcus. Central spines 1–4, 1.5–2 times as long as radial spines. Flower buds white hairy. Flowers 18–26 cm long. Pericarpel green, having areoles similar to stem areoles with spines and without scaly trichomes. Hypanthium green to reddish green, scales red-purple, deltoid, with white hairs, lower areoles often similar to stem areoles with spines and without scaly trichomes. Sepals green to pinkish green. Fruits often spiny, scales deciduous.

Distribution. Southeast Paraguay and northeast Argentina (Fig. 4.4), and naturalized in Australia, South Africa, and Hawaii; chaco húmedo, espinal; 50–100 m.

ADDITIONAL SPECIMENS EXAMINED. **ARGENTINA**. CORRIENTES PROV.: Mburucyá Dept., *Pedersen 5871* (A, US); San Cosme, *Leuenberger & Arroyo 3969* (ZSS). SANTA FE PROV.: San Cristóbal Dept., Ceres a Arrufó, *Kiesling 8365* (NY). **AUSTRALIA**. QUEENSLAND. Brisbane: Leichhardt, 7 Nov 2004, *Batianoff s.n.* (BRI). Darling Downs: Goondiwindi, *Telford 8875* (BRI). Moreton: Mt. Crosby Road, *Booth 5384* (BRI). **PARAGUAY**. CONCEPCIÓN DEPT.: Concepción, *Hassler 7388* (BM). ITAPÚA DEPT.: Trinidad, *Shafer 132* (NY, US). PARAGUARÍ DEPT.: Paraguarí, *Shafer 150* (NY, US).

PRESIDENTE HAYES DEPT.: *Hahn 2152* (MO). **SOUTH AFRICA**. KWAZULU-NATAL PROV.: Ashburton, 18 Mar 1970, *Wells s.n.* (MO); Ashburton, May 1973, *Paterson s.n.* (PRE); near Pietermaritzburg, Jul 1975, *Pickworth s.n.* (PRE); Pietermaritzburg Distr., 13 Sep 1965, *Pickworth s.n.* (PRE); Ashburton area, Jul 1967, *Regional Officer s.n.* (PRE). WESTERN CAPE PROV.: Hout Bay, s.d., *Succulenta Nurseries s.n.* [cultivated?] (PRE). **U.S.A.** HAWAII: Kauai: Koloa Distr.: Poipu, *Lorence 7697* (PTBG); Poi'pu Road, *Lorence 7987* (PTBG). Poipu Distr.: near Waiohai, *Dunn & Wood 303* (PTBG).

Etymology. Raymond Martin (?–?), cactus horticulturalist.

Harrisia martinii has a few-ribbed stem and short radial spines that are often present on the pericarpel and lower hypanthium. It is the most successful species to naturalize, occurring in South Africa, Australia, and Hawaii. The authorship of *H. martinii* was confirmed by Leuenberger (2000a).

3. *Harrisia pomanensis* (F. A. C. Weber ex K. Schum.) Britton & Rose, Cact. 2: 155. 1920. *Cereus pomanensis* F. A. C. Weber ex K. Schum., Gesamtbeschr. Kakt. 136. 1899. —TYPE: Argentina, Catamarca Prov., Poman.—NEOTYPE (designated by Kiesling 1996): Argentina, Santiago del Estero Prov., Ojo de Agua Dept., Quebrada de Pozo Grande, 18 Dec 1981, *Ulibarri 1366* (neotype: SI!; isoneotype: SI!).

Eriocereus polycanthus F. Ritter, Kakteen in Südamerika 2: 436. 1980. —TYPE: Argentina, Catamarca, *F. Ritter 413* (holotype: U, not found).

Eriocereus tarijensis F. Ritter, Kakteen in Südamerika 2: 557. 1980. *Harrisia pomanensis* (F. A. C. Weber ex K. Schum.) Britton & Rose subsp. *tarijensis* Braun & Esteves, Succulenta (Netherlands) 73: 131.1995. —TYPE: Bolivia, [Chuquisaca Dept.], Puente Azero, Grenze der Provinzen Azero und Tomina, *F. Ritter 619* (holotype: U, not found).

Stems glaucous green. Ribs not tuberculate, 4–6, separated by conspicuous line at sulcus. Spines of similar length. Flower buds mostly glabrous. Pericarpel and lower hypanthium areoles not similar to stem areoles. Flowers 14–21 cm long. Pericarpel green. Hypanthium green, scales reddish green, deltoid subulate with small tufts of white hairs. Sepals pale reddish green. Fruits without spines, scales usually deciduous.

Distribution. Southeast Bolivia, west Paraguay, and northwest Argentina (Fig. 4.4); chaco serrano, chaco árido, chaco semi-árido; 200–1200 m.

ADDITIONAL SPECIMENS EXAMINED. **ARGENTINA**. CATAMARCA PROV.: *Leuenberger 4362* (USF). CÓRDOBA PROV.: Villa de Maria, *Leuenberger & Eggli 4639* (ZSS); Cruz del Eje, *Leuenberger & Eggli 4710* (ZSS); Cruz del Eje, *Leuenberger & Eggli 4485* (ZSS). TUCUMÁN PROV.: Trancas, 3 Dec 2003, *Leuenberger & Eggli 4901* (ZSS). **BOLIVIA**. SANTA CRUZ DEPT.: Caballero Prov.: Río Quiñal, *Saldias & Medellín 4409* (NY); Cordillera Prov.: Ipatí, *Nee 51239* (NY). TARIJA DEPT.: Yacuiba, *Pensiero & Marino 4475* (MO). **CULTIVATED** (origin unknown): *Franck 2640* (USF).

Etymology. Poman, place in Argentina.

Harrisia pomanensis has a few-ribbed stem with a defined sulcus between the ribs and a spineless fruit. This species is found in drier habitats than the other species of sect. *Eriocereus*.

4. *Harrisia regelii* (Weing.) Borg, Cacti: a gardener's handbook for their identification and cultivation 145. 1937. *Cereus regelii* Weing., Monatsschr. Kakteenk. 20: 33. 1910. *Eriocereus martinii* (Labour.) Riccob. var. *regelii* (Weing.) W. T. Marshall, Cactaceae, with illustrated keys of all tribes, sub-tribes and genera 98. 1941. *Eriocereus regelii* (Weing.) Backeb., Die Cactaceae 4: 2093. 1960. *Harrisia pomanensis* (F. A. C. Weber ex K. Schum.) Britton & Rose var. *regelii* R. Kiesling, Darwiniana 34: 395. —TYPE: not indicated.—NEOTYPE (designated by Leuenberger 1996): Cultivated, Germany, Berlin-Dahlem Botanical Garden, 10 Aug 1994, *Schwerdtfeger 12552a* (neotype: B!; isoneotype: SI!).

Stems green. Ribs tuberculate, 4–5, not separated by conspicuous line at sulcus. Central spines 1–4, 1.5–2 times as long as radial spines. Flower buds with reddish hairs. Flowers 19–22 cm long. Pericarpel and lower hypanthium areoles not similar to stem areoles. Pericarpel green. Hypanthium green, scales red to reddish green, deltoid to deltoid subulate, with reddish hairs. Sepals pale reddish green. Fruits not spiny, scales deciduous.

Distribution. Entre Ríos Province, Argentina (Fig. 4.4), reportedly naturalized in Queensland, Australia (Leuenberger 2000b; Paskins 2001); espinal; ~50 m.

ADDITIONAL SPECIMENS EXAMINED. **CULTIVATED** (origin unknown): 19 Jun 2008 & 23 Sep 2008, *Franck s.n*. (USF); *Franck 2629*, *2662* (USF).

Etymology. Eduard August von Regel (1815–1892), botanist.

Harrisia regelii has short radial spines like *H. martinii*, of which it was noted as a possible variety of (Borg 1937; Hunt et al. 2006). Unlike *H. martinii*, areoles of the pericarpel and lower hypanthium of *H. regelii* typically have reddish scaly hairs and no spines. Kiesling (1996) regarded *H. regelii* as a variety of *H. pomanensis*, though Leuenberger (1995, 2000b) and Hunt et al. (2006) maintained it as a species. Both Kiesling (1996) and Leuenberger (2000b) identified specimens of this species from Entre Ríos Province, Argentina, which until then this species had only been known from cultivation. *Harrisia regelii* is sympatric with *H. bonplandii* (Kiesling 1996; Leuenberger 2000b) while *H. pomanensis* is widely disjunct. Perhaps, it is a hybrid between *H. bonplandii* and *H. martinii*.

5. *Harrisia tortuosa* (J. Forbes ex Otto & A. Dietr.) Britton & Rose, Cact. 2: 154. 1920.

Cereus tortuosus J. Forbes ex Otto & A. Dietr., Allg. Gartenzeitung 6: 35. 1838. *Eriocereus tortuosus* (J. Forbes ex Otto & A. Dietr.) Riccob., Boll. Reale Orto Bot. Palermo 8: 245. 1909. —TYPE: Argentina, Buenos Aires. —NEOTYPE (designated by Kiesling 1996): Argentina, Corrientes Prov., San L. del Palmar Dept., 18 km SE de San Luis del Palmar, 2 Nov 1979, *Schinini 19451* (neotype: CTES; isoneotype: SI).

Cereus atropurpureus Hocay, Cacteencult 91. 1916. —TYPE:?

Stems green. Ribs tuberculate, 6–8, separated by conspicuous line at sulcus. Spines of similar length. Flower buds with reddish hairs. Flowers 19–22 cm long. Pericarpel brownish green, sometimes having areoles similar to stem areoles with spines and without scaly trichomes. Hypanthium pale pinkish green, scales red, deltoid to deltoid subulate, with white hairs, sometimes the lower hypanthium areoles similar to stem areoles with spines and without scaly trichomes. Sepals pale pinkish green-brown. Fruits with or without spines, scales deciduous.

KEY TO VARIETIES OF *H. TORTUOSA*

1. Fruit spiny ..... var. *tortuosa*

1. Fruit not spiny ..... var. *uruguayensis*

5a. *Harrisia tortuosa* var. *tortuosa*

Fruits with spines.

Distribution. Paraguay and northeast Argentina (Fig. 4.4) and naturalized in Australia; chaco húmedo, espinal; 80-300 m.

ADDITIONAL SPECIMENS EXAMINED. **AUSTRALIA**. QUEENSLAND. Darling Downs: Boondandilla State Forest, *Forster 29482* (BRI). **PARAGUAY**. CORDILLERA DEPT.: Río

Piribebuy, *Zardini & Velazquez 19789* (MO). **CULTIVATED** (origin unknown): *Franck 1281*, *2628*, *2897* (USF).

Etymology. Tortuous, twisting, winding.

Harrisia tortuosa var. *tortuosa* and *H. martinii* are the only taxa of *Harrisia* with spiny fruits. However, with its 6–8 ribs and longer radial spines, var. *tortuosa* is readily differentiated from *H. martinii*.

5b. *Harrisia tortuosa* var. *uruguayensis* Osten, Notas sobre Cactáceas 66. 1941. *Eriocereus pomanensis* var. *uruguayensis* (Osten) Backeb., Die Cactaceae 4: 2095. 1960. — TYPE: Uruguay, Río Negro [Dept.], Estancia Nueva Mehlem, en limo pampeano, Apr 1922, J. Schroeder, photo Apr 1923, Herb. C. Osten 16.720. LECTOTYPE (designated here): Osten, Notas sobre Cactáceas, lámina 57.1941.

Fruits without spines.

Distribution. West Uruguay (Fig. 4.4); pampeana; 70–90 m.

Etymology. Uruguay.

The stem of *Harrisia tortuosa* var. *uruguayensis*, with 6–8 ribs, agrees with its placement under *H. tortuosus*. However, Backeberg (1960) and Kiesling (1996) allied this variety with *H. pomanensis*, which is widely disjunct. The range of *H. tortuosa* var. *uruguayensis* is more agreeable with *H. tortuosa*. This is the only taxon of *Harrisia* known from Uruguay and is known only from the type.

IIb. *Harrisia* sect. *Roseocereus* (Backeb.) A. R. Franck. *Eriocereus* (A. Berger) Riccobono subg. *Roseocereus* Backeb., Blätt. Kakteenf., 1936–6. 1936. *Roseocereus* (Backeb.) Backeb., Blätt. Kakteenf.1938–6. 1938.—TYPE: *Harrisia tetracantha* (Labour.) D. R. Hunt.

Tree to 6 m. Stems erect, ribs 7–9. Upper stems 3–5 cm wide, lower woody stems to 15 cm wide, basal trunk to 30 cm wide. Flower bud white hairy. Flowers 17–21 cm long. Pericarpel green. Hypanthium green. Scales deltoid, red to reddish green apically, green basally. Sepals brownish green. Fruit tearing apically.

Distribution. One species in Bolivia (Fig. 4.4); scrub forest; 1200–2600 m.

1. *Harrisia tetracantha* (Labour.) D.R. Hunt, Bradleya 5: 92. 1987. *Cereus tetracanthus* Labour., Rev. Hort. (Paris) 4: 25. 1855. *Eriocereus tetracanthus* (Labour.) Riccob., Boll. Reale Orto Bot. Palermo 8: 244. 1909. *Trichocereus tetracanthus* (Labour.) Borg, Cacti: a gardener's handbook for their identification and cultivation 137. 1937. *Roseocereus tetracanthus* (Labour.) Backeb. Jahrbücher der Deutschen Kakteen-Gesellschaft 30. 1942. —TYPE: Bolivia, Chuquisaca Dept., Chuquisaca [Sucre], from seeds supplied by Jacques Philippe Martin Cels [no specimens found]. —NEOTYPE (designated here): Bolivia, Santa Cruz Dept., [Manuel María] Caballero Prov., NE of Abra de Quiñe, shrubland, upper limit of arid woodlands, 31 Dec 1995, *Nee 46674* (neotype: NY!; isoneotype: LPB!, MO!, USZ!).

Cereus tetracanthus var. *boliviana* F. A. C. Weber ex K. Schum., Gesamtbeschr. Kakt. 81. 1899. *Cereus bolivianus* (F. A. C. Weber ex K. Schum.) F. A. C. Weber ex K. Schum., Monatsschr. Kakteenk. 12: 21. 1902. *Eriocereus tetracanthus* (Labour.) Riccob. var. *bolivianus* (F. A. C. Weber ex K. Schum.) Backeb., Kaktus-ABC 179. 1936. *Trichocereus tetracanthus* (Lab.) Borg var. *boliviensis* (F. A. C. Weber ex K. Schum.) Borg, Cacti: a gardener's handbook for their identification and cultivation 137. 1937. —TYPE: Bolivia, [Cochabamba Dept.], Cochabamba.

Tree to 6 m. Stems erect, ribs 7–9. Upper stems 3–5 cm wide, lower woody stems to 15 cm wide, basal trunk to 30 cm wide. Flower bud white hairy. Flowers 17–21 cm long. Pericarpel green. Hypanthium green. Scales deltoid, red to reddish green apically, green basally. Sepals brownish green. Fruit tearing apically.

Distribution. Eastern Cordillera of the Andes, Bolivia (Fig. 4.4); seasonally dry scrub forest of inter-Andean valleys; 1200–2600 m.

ADDITIONAL SPECIMENS EXAMINED. **BOLIVIA**: COCHABAMBA DEPT.: San Pedro [Tarata?], *Cárdenas 5021* (US); Esteban Arce Prov., Tiataco, 8 May 2010, *Kamm s.n.* (USF). SANTA CRUZ DEPT.: Florida Prov.: Pampa Grande, *Nee & Vargas C. 44709* (MO, NY); Pampa Grande, *Rente 12* (ZSS). Manuel María Caballero Prov.: Comarapa, *Nee 46559* (MO, NY); Saipina, *Nee et al. 53697* (NY). **CULTIVATED** (origin unknown): *Schumann 80* (NY); 27 Jun 1904, *Schumann s.n.* (NY); 30 Jul 1904, *Schumann s.n.* (NY); 17 Aug 1904, *Schumann s.n.* (NY); *Franck 2262*, *2898* (USF).

Etymology. The description states that the areoles from the top to the bottom increase in the number of spines by four. The epithet has been suggested to mean spines in four series or that it is a misspelling of 'tephracanthus' intended as ashy-spined (Hunt & Taylor 1987).

Harrisia tetracantha is the only arborescent species in *Harrisia* and also has the thickest trunk. The species may be well adapted to disturbance, apparently common in cleared forests with *Cleistocactus parviflorus* (K. Schum.) Gosselin (Barra Ricáldez 1998).

NOTHOTAXA

×*Guillauminara* P. V. Heath, Calyx 1: 111. 1992. =*Echinopsis* Zucc. × *Harrisia* × *Trichocereus* (A. Berger) Riccob.

This is a hybrid allegedly between ×*Harrisnopsis jusbertii* and *Trichocereus lamprochlorus* (Lem.) Britton & Rose.

×*Harricereus* G. D. Rowley, Natl. Cact. Succ. J. 37: 76. 1982. =*Cereus* Mill. × *Harrisia*.

Cereus and *Harrisia*, though both in tribe Cereeae, are in different subtribes and the reported hybrid seems dubious. Ritter (1980) reported a natural hybrid between *H. bonplandii* and *C. stenogonus* K. Schum. in Puerto Casado, Paraguay.

×*Harrisnopsis* G. D. Rowley, Natl. Cact. Succ. J. 37: 77. 1982. ×*Eriocereopsis* Doweld, Tsukkulenty 4: 34. 2001. =*Echinopsis* × *Harrisia*. —TYPE: ×*Harrisnopsis jusbertii* (Rebut ex K. Schum.) P. V. Heath.

Harrisia ×*jusbertii* (Rebut. ex K. Schum.) Frič, Möllers Deutsche Gärtner-Zeitung 36: 421. 1932. ×*Harrisnopsis jusbertii* (Rebut ex K. Schum.) P. V. Heath, Calyx 1: 111. 1992. *Cereus jusbertii* Rebut ex K. Schum., Gesamtbeschr. Kakt. 137. 1899. *Eriocereus jusbertii* (Rebut ex K. Schum.) Riccob., Boll. Reale Orto Bot. Palermo 8: 240. 1909. ×*Eriocereopsis jusbertii* (Rebut ex K. Schum.) Doweld, Tsukkulenty 4: 34. 2001.—TYPE: Argentina oder Paraguay und wird in der Sammlung des verstorbenen Gruson in Magdeburg in schönen Exemplaren kultivert. —NEOTYPE (designated here): U.S.A., California, cultivated at the Huntington Botanical Gardens, 18 Jul 1963, *Kimnach s.n*. (neotype: HNT!).

Harrisia ×*jusbertii* is a putative horticultural hybrid between an *Echinopsis* and a *Harrisia* (Berger 1905) with distinctively short (to 5 mm long), thick (~1–2 mm wide), conical blackish spines. Some characters are agreeable with *H. bonplandii* such as the red, dehiscent fruit with persistent scales, the green stem often lacking a well-defined sulcus, the acicular juvenile spines, and often having 4–5 ribs. The short mature spines of *Harrisia* ×*jusbertii* are also found in *H. martinii* and *H. regelii*. Young spines of *Harrisia* are often blackish at the base as in the spines of *Harrisia* ×*jusbertii*. It has been postulated that it is a chimeric mutant of *H. bonplandii* (Rowley 1980), though the

morphology is quite consistent on a plant.. Henke (1981) claimed to have produced this hybrid by crossing *Echinopsis eyriesii* Pfeiff. & Otto and *Echinocereus pentalophus* (DC.) Haage var. *procumbens* (Engelm.) P. Fourn., which seems extremely doubtful (Drawert 1983). This hybrid is unknown in the wild but it is widespread in cultivation, often used for a grafting stock. The name *Harrisia* ×*jusbertii* is more common in usage, though if it is indeed a hybrid with *Echinopsis* then ×*Harrisnopsis* is the preferred generic name. However, given the poor taxonomy of the likely polyphyletic *Echinopsis* and the uncertainty of its other hybrid parent, it may be ideal to retain use of *Harrisia* ×*jusbertii* until additional information is gathered. As all the vegetative characters are agreeable with *Harrisia*, even the short spines, it may well be an intrageneric hybrid of *Harrisia*. If so then *Harrisia* ×*jusbertii* is the proper name.

×*Selenirisia* G. D. Rowley, Natl. Cact. Succ. J. 37: 79. 1982. =*Harrisia* × *Selenicereus* (A. Berger) Britton & Rose.

This hybrid is allegedly between *H. bonplandii* and *S. pteranthus* (Link ex A. Dietr.) Britton & Rose but as these two genera are distantly related across divergent tribes, it is dubious. Their morphological similarity is probably the cause of misidentification.

EXCLUDED NAMES

Cereus bonplandii Parm. ex Pfeiff. var. *brevispinus* Maass-Zehlendorf, Monatsschr. Kakteenk. 15: 119. 1905, nomen nudum.

Cereus bonplandii Parm. ex Pfeiff. var. *pomanensis* F.A.C. Weber ex K. Schum. Gesamtbeschr. Kakt. 137. 1899, nomen nudum.

Cereus repandus Haw., Syn. pl. succ. 183. 1812, nomen illegitimum (homonym for *Cereus repandus* (L.) Mill., Gard. dict., ed. 8. 1768.).

Cereus undatus Pfeiff., Enum. diagn. Cact. 94. 1837. *Harrisia undata* (Pfeiff.) Britton, Bull.Torrey Bot. Club 35: 564. 1908, nomen illegitimum (homonym for *Cereus undatus* Haw., Philos. Mag. Ann. Chem. 7: 110. 1830. =*Hylocereus undatus* (Haw.) Britton & Rose, Fl. Bermuda 256. 1918.)

Eriocereus (A. Berger) Riccobono subg. *Pseudoharrisia* Backeb., Blätt. Kakteenf. 1934–3, 1936–6. 1934–1936.—TYPE: *Harrisia tortuosa* (J. Forbes ex Otto & A. Dietr.) Britton & Rose, nomen illegitimum.

Eriocereus (A. Berger) Riccob. series *Acanthocarpi* Backeb., Blätt. Kakteenf. 1938–6. 1938, nomen nudum.

Eriocereus (A. Berger) Riccob. series *Eriocarpi* Backeb., Blätt. Kakteenf. 1938–6. 1938, nomen nudum.

Eriocereus cavendishii (Monv.) Riccob., Boll. Reale Orto Bot. Palermo 239. 1909. =*Monvillea cavendishii* (Monv.) Britton & Rose, Cact. 2: 21. 1920.

Eriocereus martianus (Zucc.) Riccob., Boll. Reale Orto Bot. Palermo 240. 1909. =*Disocactus martianus* (Zucc.) Barthlott, Bradleya 9: 88. 1991.

Eriocereus spinosissimus Buining & Brederoo. =*Arthrocereus spinosissimus* (Buining & Brederoo) F. Ritter, Kakteen in Südamerika 1: 244.

Harrisia Britton [unranked] *Euharrisia* Britton & Rose, Cact. 2:148, nomen illegitimum.

Harrisia Lundblad, K. svenska Vetensk. Akad. Handl. 4: 71. 1950, nomen illegitimum.

Harrisia Robineau-Desvoidy, Mem. Pres. Div. Sav. Acad. R. Sci. Inst. Fr. - Paris 2: 323. 1830, [kingdom Animalia, order Diptera].

Harrisia brailovskyi Carvalho, Rev. Bras. Bio. 43: 148. 1983, [kingdom Animalia, order Diptera].

Harrisia brasiliensis Robineau-Desvoidy, Mem. Pres. Div. Sav. Acad. R. Sci. Inst. Fr. - Paris 2: 324. 1830, [kingdom Animalia, order Diptera].

Harrisia deeringii Backeb., Das Kakteenlexicon. 1966, nomen nudum.

Harrisia donae-antoniae M. L. Hooten, Cact. Succ. J. (Los Angeles) 63: 65. 1991, nomen illegitimum.

Harrisia divaricata (Lam.) Lourteig, Bradea 5: 407. 1991, isonym.

Harrisia fimbriata (Lam.) F. M. Knuth, Kaktus-ABC 309. 1936. =*Stenocereus fimbriatus* (Lam.) Lourteig, Bradea 5: 408. 1991.

Harrisia fimbriata (Lam.) F. M. Knuth var. *straminia* W. T. Marshall, Cactaceae, with illustrated keys of all tribes, sub-tribes and genera, 96. 1941, nomen illegitimum.

Harrisia floridana Rose ex J.G. Webb, unpublished (name on his specimen of *H. aboriginum*).

Harrisia floridana Vosburg, unpublished (name on his specimen of *H. fragrans*).

Harrisia hahniana (Backeb.) Kimnach & Hutchison ex Kimnach, Cact. Succ. J. (Los Angeles) 59: 59. =*Echinopsis hahniana* (Backeb.) R. S. Wallace, Cactaceae Consensus Init. 4: 12. 1997.

Harrisia marsilioides Lundblad, K. svenska Vetensk. Akad. Handl. 4: 71. 1950. =*Harrisiothecium marsilioides* (Lundblad) Lundblad, Taxon 10: 23. 1961.

Harrisia rumpalensis Moravec [Trilobita, publication details unknown, cited in Mergl, M., & P. Budil. 2010. Exceptional preservation of trilobite exoskeletons in the Řevnice Quartzite (Libeň Formation, Ordovician) from Ejpovice in the Rokycany area. Paleontologie 2010: 109–112.].

Harrisia scutellaris Robineau-Desvoidy, Mem. Pres. Div. Sav. Acad. R. Sci. Inst. Fr. - Paris 2: 324. 1830, [kingdom Animalia, order Diptera].

INCERTAE SEDIS

Cereus arendtii Hildmann & Mathsson ex K. Schum., Monatsschr. Kakteenk. 4: 173. 1894. *Eriocereus arendtii* (Hildmann & Mathsson ex K. Schum.) F. Ritter, Kakteen in Südamerika 1: 242. 1979. —TYPE: Uruguay [?, probably Argentina], dem Thale von Cordova.

The type of *Cereus arendtii* must be from Argentina if it is indeed from Thale von Cordova [Córdoba Valley] as cited. If so, it is more likely a synonym of *H. pomanensis* which is the only species of *Harrisia* known from there (Kiesling 1996; Zak & Cabido 2002). The protologue only describes the stem and mentions a similarity to *H. tortuosa* but does not synonymize it, although others have (Britton & Rose 1920; Aarsen 1983). The description of the stem could fit both *H. pomanensis* and *H. tortuosus*. If *C. arendtii* were the same as *H. pomanensis*, then *C. arendtii* would take precedence unless Art. 14 were applied (McNeill et al. 2006).

Cereus cubensis Zucc., Allg. Gartenzeitung 2: 244. 1834. —TYPE: Cuba (cultivated at Munich Botanical Garden).

Pfeiffer (1837) listed *Cereus cubensis* as a synonym of *C. eriophorus*. The description of *C. cubensis* describes flowers like *C. grandiflorus* L. (=*Selenicereus grandiflorus* (L.) Britton & Rose), nocturnal, and stems thick like *C. peruvianus* (L.) Mill. As *Harrisia* in Cuba tends to have slender stems that are not distinctly thicker than *S. grandiflorus*, *C. cubensis* may refer to *Dendrocereus* Britton & Rose which has nocturnal flowers and thick stems.

Cereus subrepandus Haw., Suppl. pl. succ. 78. 1819. *Eriocereus subrepandus* (Haw.) Riccob., Boll. Reale Orto Bot. Palermo 8: 243. 1909. —TYPE: Vigebat in hort. Chels. ante A.D. 1817.

This was said to be similar to *H. gracilis* (as *C. repandus*) except with longer spines, possibly representing a taxon from Cuba or Hispaniola. However, Riccobono (1909) mentions a yellow fruit that is completely red at maturity, suggesting it to be *H. fragrans*, the only Caribbean species with red fruits.

Cereus eriophorus var. *laetevirens* Salm-Dyck ex Pfeiff., Enum. diagn. Cact. 94. 1837.

This was described to have a yellow-green stem, and is possibly a synonym of *H. fernowii*, whose stem is light green (Britton 1908).

Harrisia platygona (Otto) Britton & Rose, Cact. 2: 156. 1920. *Cereus platygonus* Otto, Cacteae Horto Dyckensis Cultae, Anno 1849: 199. 1850. *Eriocereus platygonus* (Otto) Riccob. 8: 242. 1909.

The name *Cereus platygonus* Otto was interpreted as *Eriocereus platygonus* by Riccobono (1909) and as *Harrisia platygona* (Otto) by Britton & Rose (1920). Two collections at NY (*Britton s.n.* and *Schumann 99*) annotated as *H. platygona* assuredly match *H. adscendens*. The original description of *C. platygonus* describes erect, slender stems (caule erecto gracili), 8 glaucous ribs (8 gono glaucescent-viridi), 8 spines (aculeis gracilibus parvulis rigidis exterioribus 7 radianter patulis infimo ac centrali solitario longioribus), round ribs distally scarcely incised (costis superne rotundatis sinubus vix incisis), flat ribs basally (inferne omnino applanatis), 8 sulci, nearly absent at the base (sulcis inferne omnino obliteratis), and areoles subtomentose (pulvillis remotis parvulis griseo subtomentosis). This description seems applicable to *H. adscendens*. Labouret (1853) allied species of the Caribbean series *Harrisia* with *C. platygonus* in an unranked group *Attenuati*. Schumann (1899) redescribed *Cereus platygonus* as having 12–15 setaceous spines which Riccobono (1909) interpreted as a description of the juvenile growth. Should it be allied with *H. adscendens*, priority would yield to *H. platygona* unless *H. adscendens* is conserved under Art. 14 (McNeill et al. 2006).

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TABLE 1. Overview of taxonomy of *Harrisia*.

SUBGENUS	SECTION/SERIES		NO. OF SPECIES	INCLUDED SPECIES
Harrisia	sect. *Harrisia*	series *Harrisia*	10	*H. aboriginum*, *H. brookii*, *H. caymanensis*, *H. divaricata*, *H. eriophora*, *H. fernowii*, *H. fragrans*, *H. gracilis*, *H. portoricensis*, *H. taetra*
		series *Earlei*	1	*H. earlei*
	sect. *Adscendens*		1	*H. adscendens*
Eriocereus	sect. *Eriocereus*		5	*H. bonplandii*, *H. martinii*, *H. pomanensis*, *H. regelii*, *H. tortuosa*
	sect. *Roseocereus*		1	*H. tetracantha*

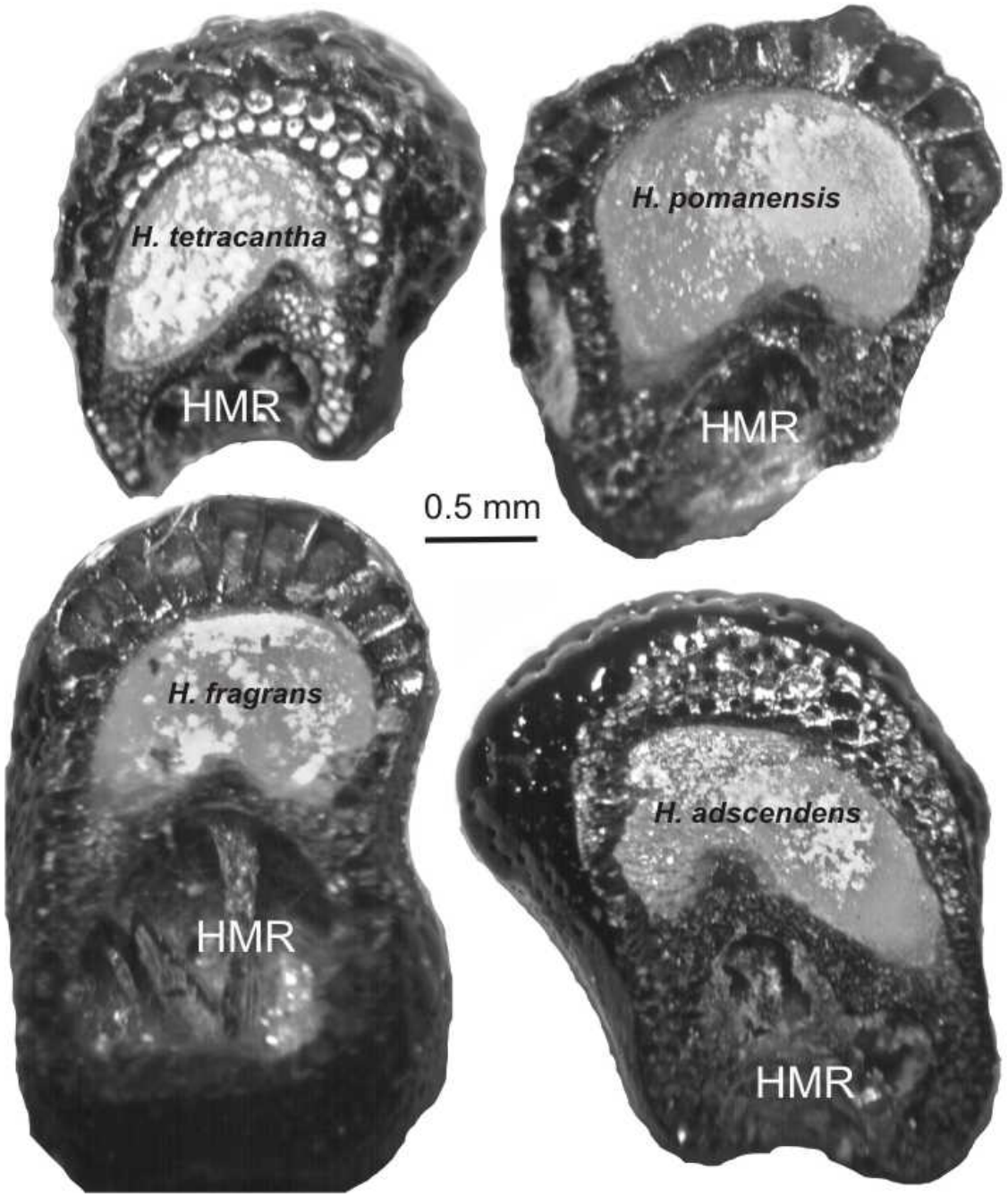

Figure 4.1. Cross-section of seeds of four species of *Harrisia*: *H. tetracantha* (*Kamm s.n.*), *H. pomanensis* (*Leuenberger & Eggli 4362*), *H. fragrans* (*Franck 472*), and *H. adscendens* (*Hofacker 403*). The hilum-micropylar region (HMR) is indicated. Seeds were secured with cyanoacrylate, remnants of which are seen in the HMR of seed of *H. pomanensis*.

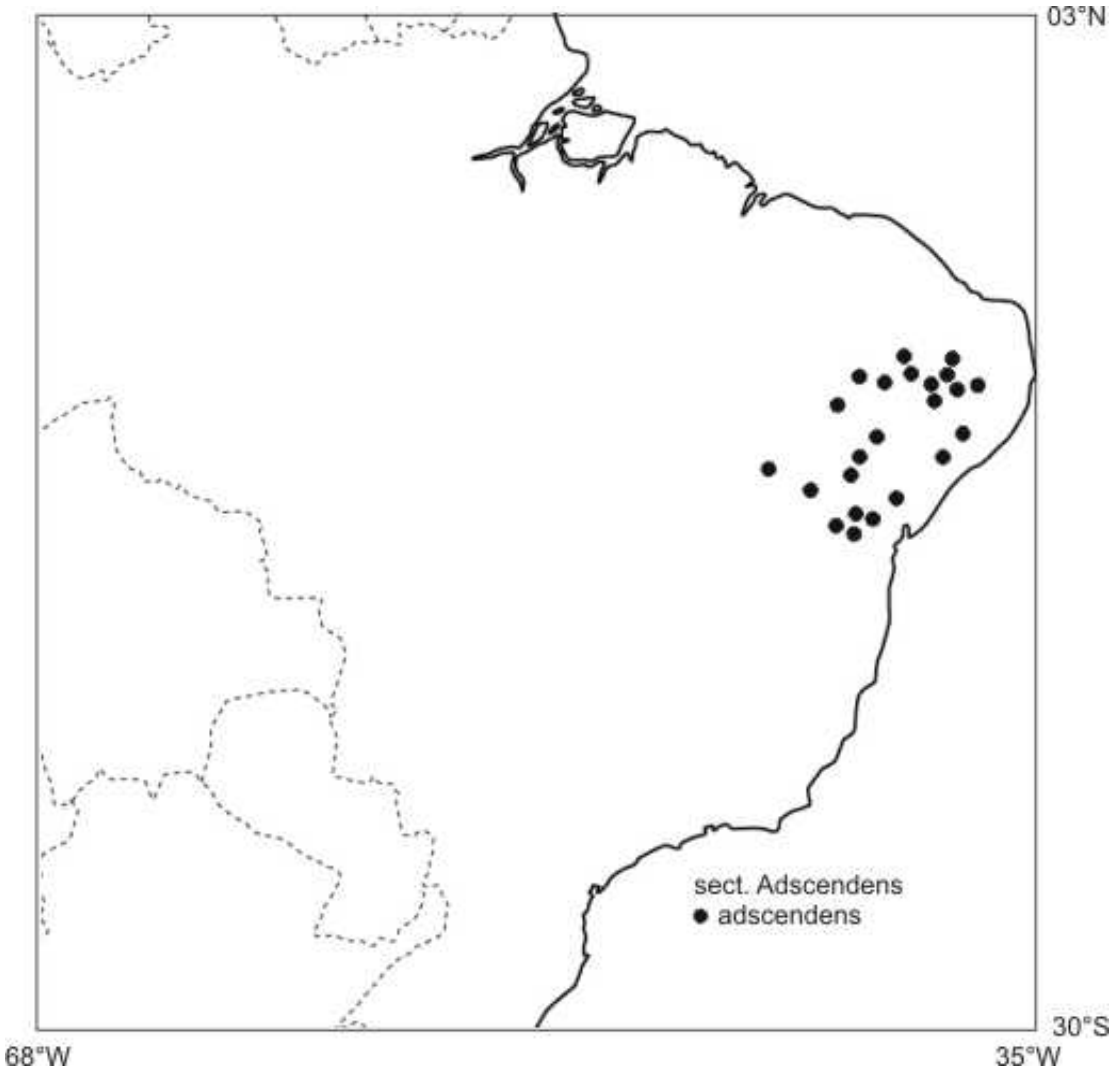

Figure 4.2. Map of distribution of sect. *Adscendens*.

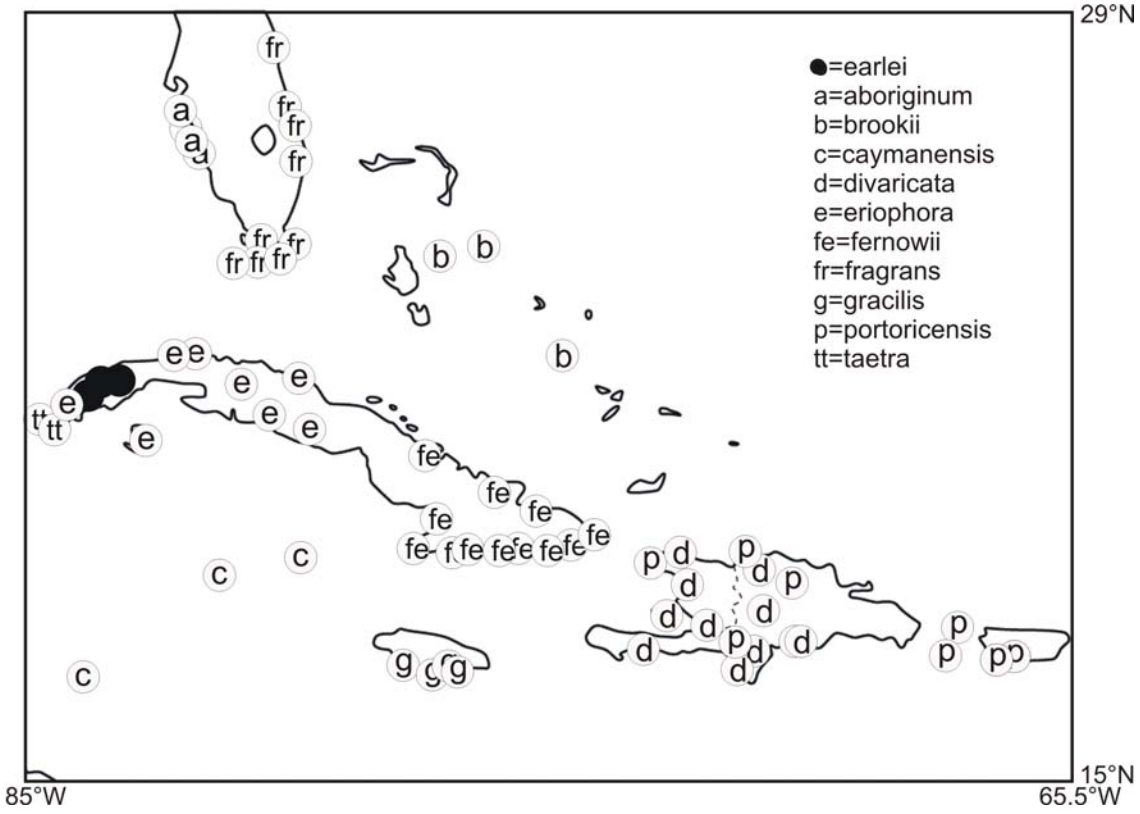

Figure 4.3. Map of distribution of sect. *Harrisia*.

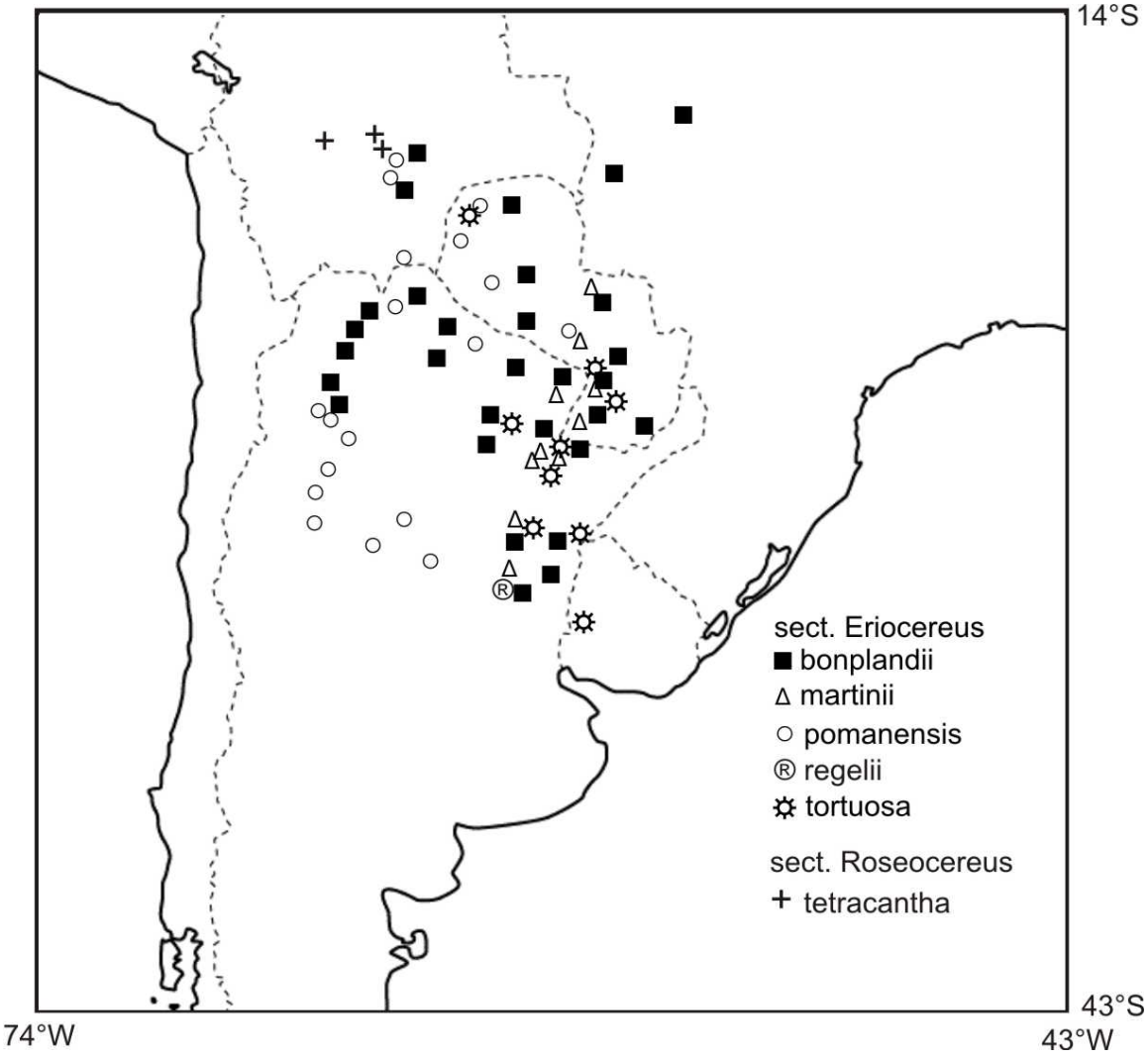

Figure 4.4. Map of distribution of subg. *Eriocereus*. *Harrisia tetracantha* is also known from Chuquisaca, Potosí, and Tarija Departments (Antezana & Navarro 2002) but no specimens were examined or mapped.

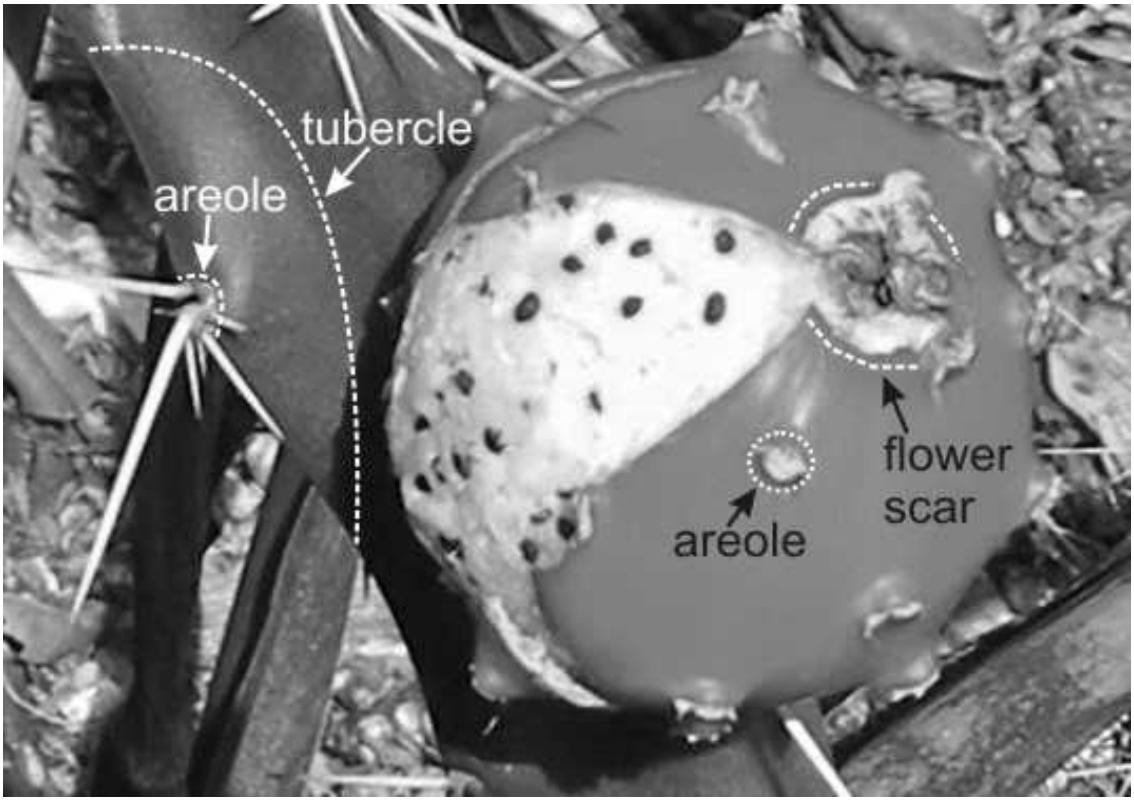

Figure 4.5. Photo of fruit and stem of *H. regelii* (*Franck 2662*), showing the areoles, flower scar, and stem tubercle. The fruit is ca. 4 cm in diameter.

Figure 4.6. Photo of immature fruit and stem of *H. gracilis* (*Franck 2265*), showing the areole, scale, tubercles, scaly trichomes, and uniseriate trichomes. The immature fruit is ca. 4 cm in diameter.

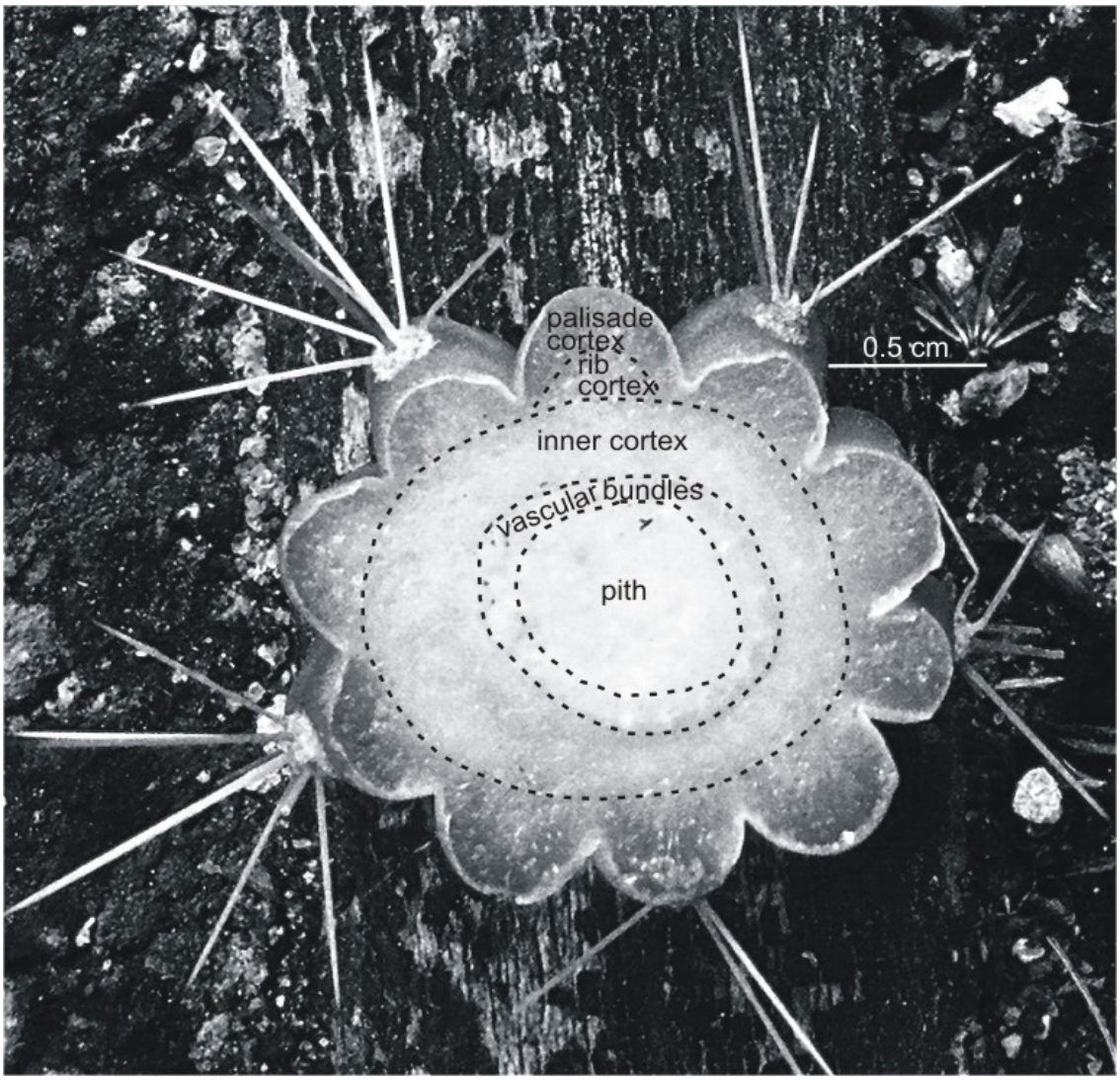

Figure 4.7. Cross-section of a 10-ribbed stem of *H. divaricata* (*Franck 3021*), showing the palisade cortex, rib cortex, inner cortex, vascular bundles, and pith.

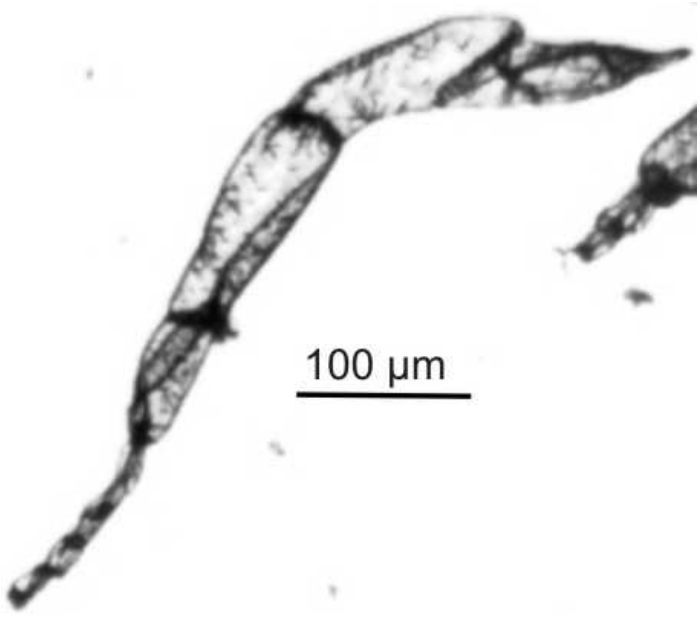

Figure 4.8. Light microscopy photo of uniseriate trichome from stem areole of *H. adscendens* (*Franck 2641*).

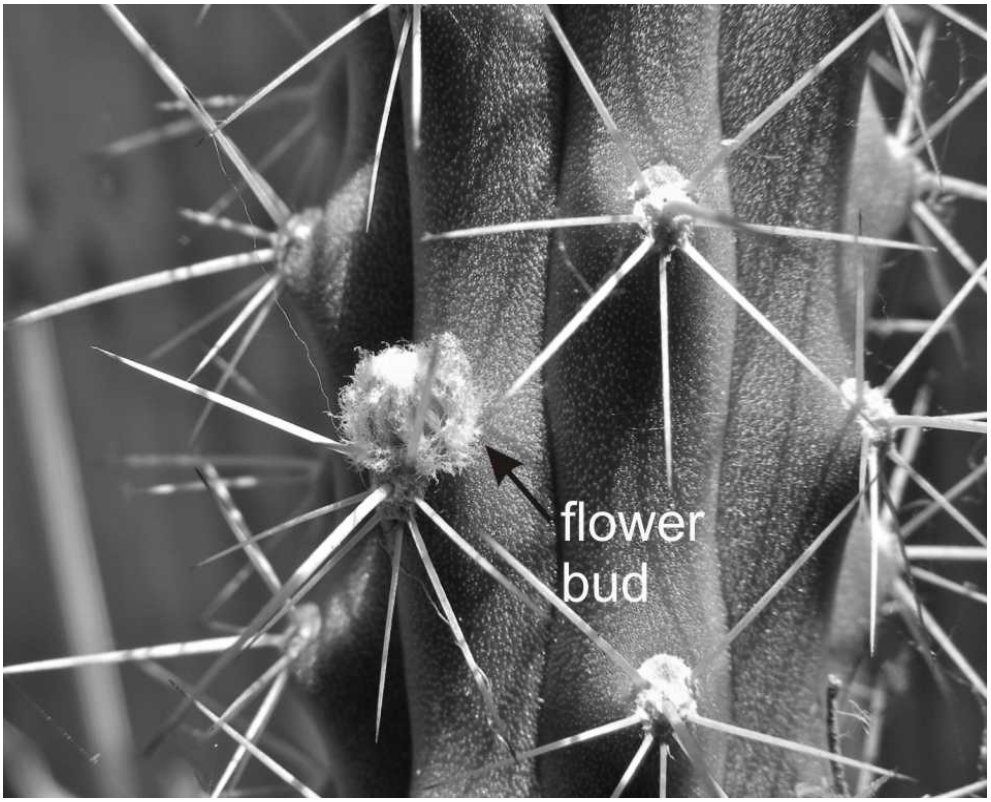

Figure 4.9. Photo of stem of *H. fragrans* (*Franck 472*) showing the flower bud initiating from the adaxial portion of the areole. The diameter of the stem is ca. 3 cm.

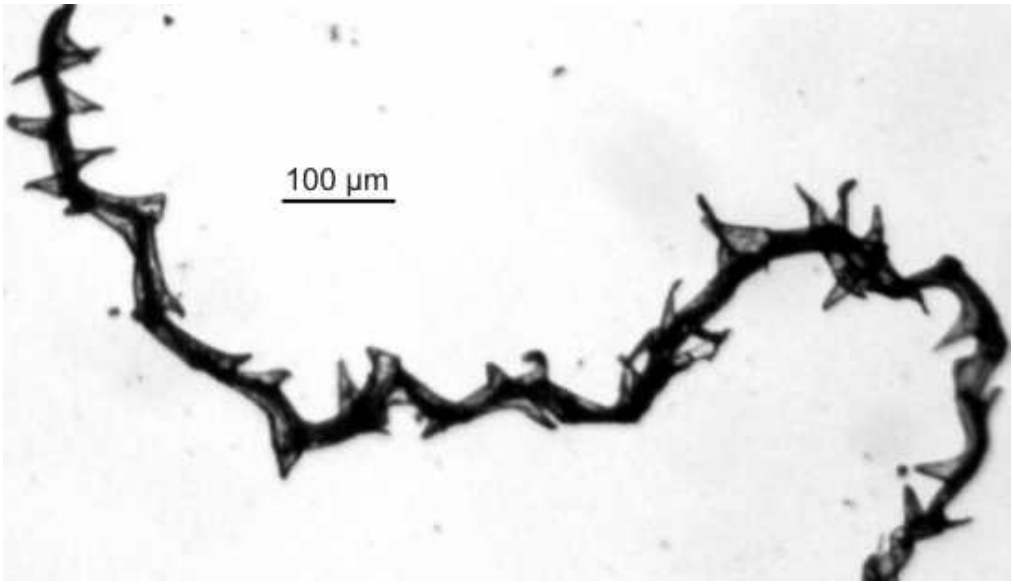

Figure 4.10. Light microscopy photo of scaly trichome from flower areole of *H. regelii* (*Franck s.n.*, 19 Jun 2008).

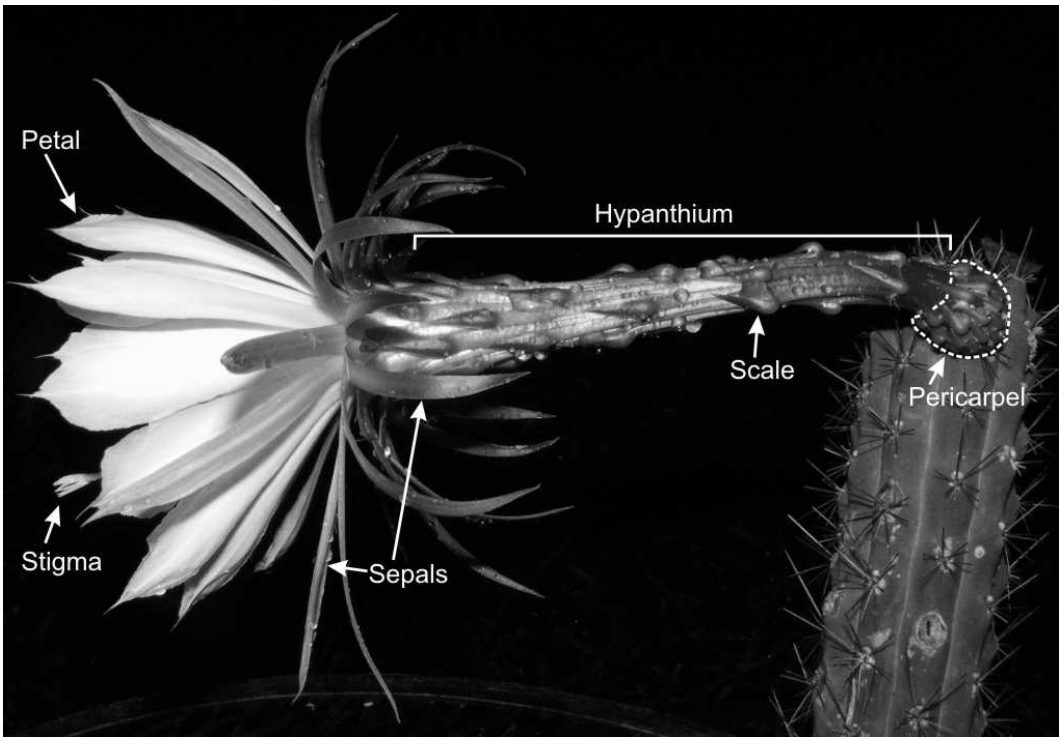

Figure 4.11. Photo of flower and stem of *H. gracilis* (*Franck 2661*) showing the pericarpel, hypanthium, scales, sepals, petals, and exserted stigma. Water droplets are also visible on the hypanthium. The hypanthium is ca. 12 cm long.

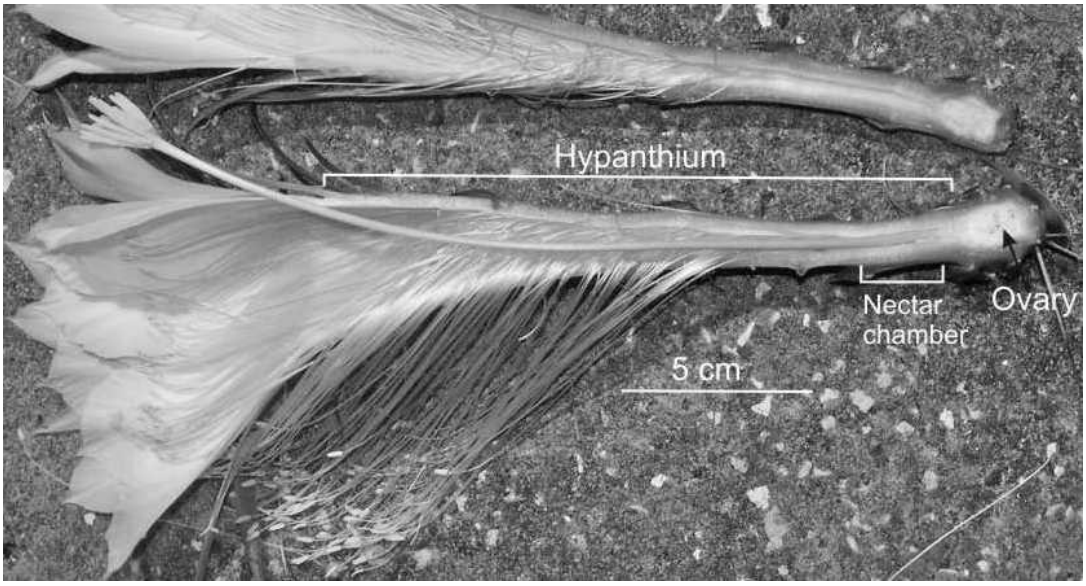

Figure 4.12. Photo of flower of *H. regelii* (*Franck 3022*) showing the hypanthium, nectar chamber, and ovary. Each stamen of the dorsal stamen cluster arises independently from the inside of the hypanthium like the *Trichocereus*-type flower described by Schick (2011).

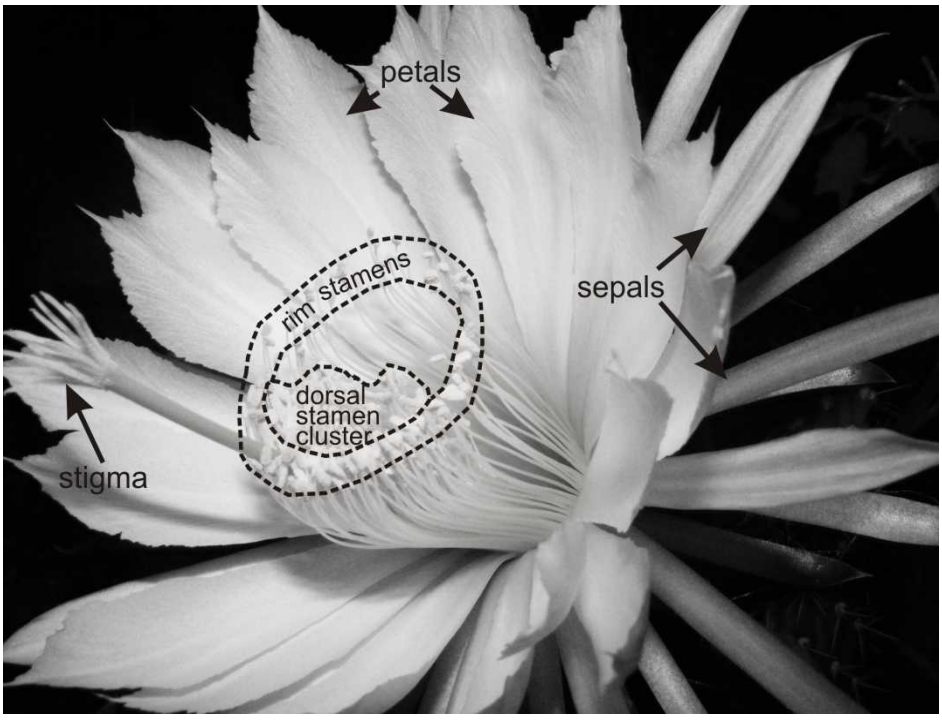

Figure 4.13. Photo of flower of *H. fragrans* (*Franck 1236*) showing the dorsal stamen cluster, rim stamens, sepals, petals, and lobed stigma. The stigma lobes are ca. 8 mm long.

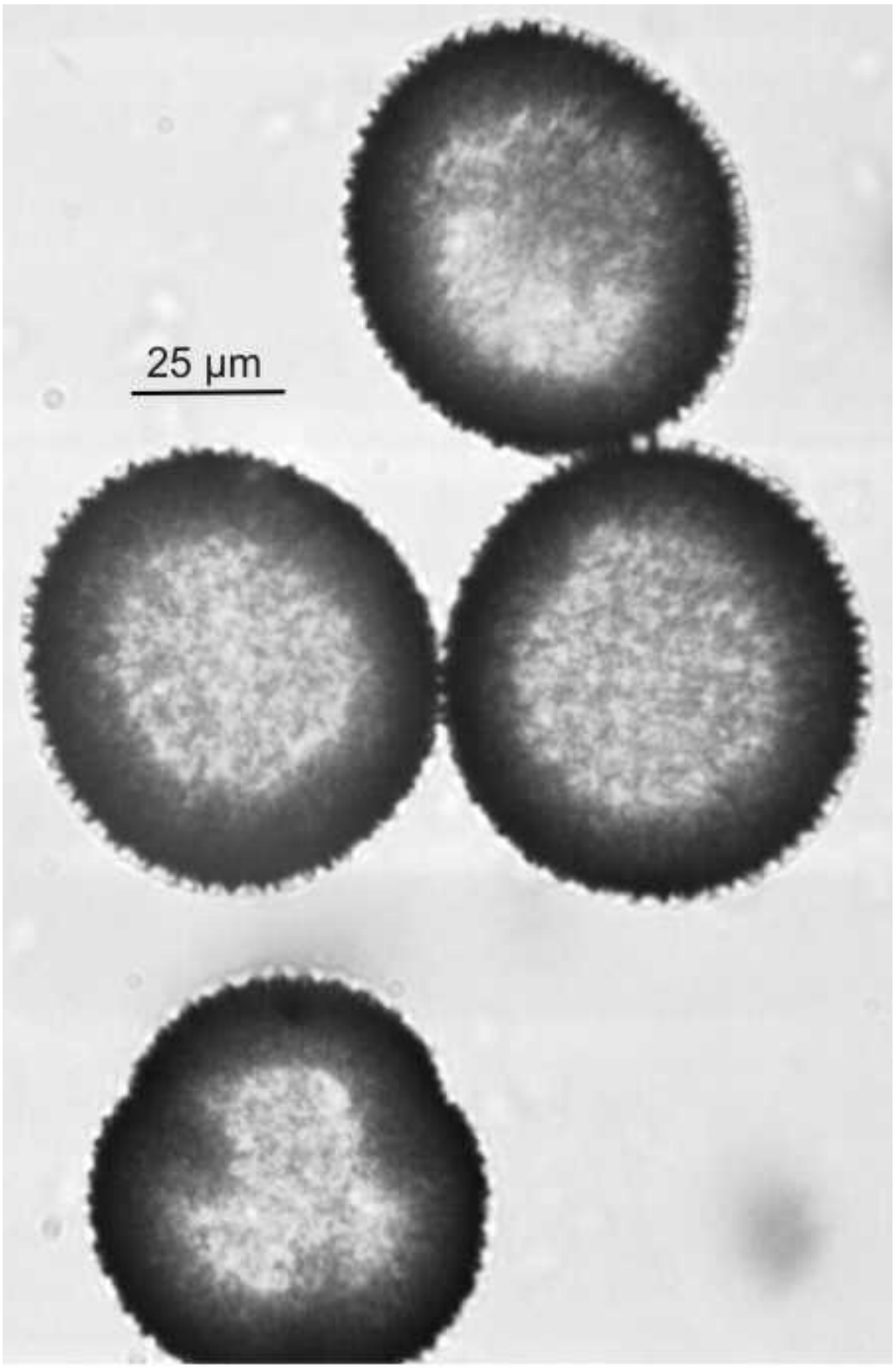

Figure 4.14. Light microscopy photo of pollen of *H. divaricata* (*Franck 3021*). Spinulae are evident at the outer circumference of the pollen.

OVERALL CONCLUSIONS

1. The primers for *isi1* and *nhx1* represent alternative nuclear markers spanning introns and are useful for inferring cactus phylogenies. The *ycf1* primers amplified a region that was much more variable than any other plastid region surveyed in *Harrisia* (Chapter 1).
2. The flower morphology of *Harrisia* suggests a relationship with similar species of *Echinopsis* s. l. However, the molecular phylogeny did not reveal this. The plastid sequences supported a relationship with arborescent species of *Echinopsis* (Chapter 2).
3. Seed morphology and DNA sequences confirm the monophyly of *Harrisia* (Chapter 2).
4. The species of *Harrisia* from the Bolivian Andes and the Gran Chaco region are closely related and here recognized as the monophyletic subg. *Eriocereus*. The species of *Harrisia* from northeast Brazil and the Caribbean are closely related and recognized as the monophyletic subg. *Harrisia*. The Gran Chaco species constitute a monophyletic group recognized as sect. *Eriocereus*. The Caribbean species are monophyletic and recognized as sect. *Harrisia* (Chapter 2).
5. *Harrisia earlei* (series *Earlei*) is very morphologically distinct and likely sister to the rest of the Caribbean species (series *Harrisia*) as evidenced from the molecular phylogenies. The Florida species are closely related and perhaps derived from ancestral populations of west Cuba. The species of the SEGAB group are closely

related and perhaps derived from populations from east Cuba (Chapter 3). *Harrisia portoricensis* is possibly nested within *H. divaricata*, consistent with the molecular data and geography. One population of *H. fragrans* from the east coast of Florida experienced a plastid introgression from *H. aboriginum* on the west coast.

6. *Harrisia* originated in the Bolivian Andes, sympatric with the high endemism of the Cactaceae and subtribe Trichocereinae. An early dispersal event into Brazil must have founded the northeast Brazil/Caribbean clade (subg. *Harrisia*). The species of the Gran Chaco (sect. *Eriocereus*) represent recent radiations in the Pleistocene and the same is true for the Caribbean species (sect. *Harrisia*) (Chapters 2–3). In the Caribbean, *Harrisia* first colonized west Cuba, sympatric with *H. earlei*, before dispersing northward into Florida and eastward to Puerto Rico.
7. There are 18 species in *Harrisia*: one from the Bolivian Andes (sect. *Roseocereus*), five from the Gran Chaco region (sect. *Eriocereus*), one from northeast Brazil (sect. *Adscendens*), and 11 from the Caribbean (sect. *Harrisia*). *Harrisia simpsonii* is synonymized under *H. fragrans*, consistent with morphology and molecular data. *Harrisia taylorii* is regarded as a synonym of *H. fernowii*, also consistent with morphology and molecular data. Several names are typified. One new species is described from the Cayman Islands.

Future directions. This phylogenetic study only sampled a small portion of subtribe Trichocereinae, so perhaps an increase in taxonomic coverage will help to reveal generic level relationships with *Harrisia*. The nuclear regions were adequate to support the monophyly of *Harrisia* yet apparently unsuitable for uncovering generic level

relationships. Though the morphology implicates *Echinopsis* s. l. as a closely related genus, *Echinopsis* s. l. is also likely polyphyletic. *Cleistocactus* s. l. is another diverse genus that may be polyphyletic. Many taxonomic problems in subtribe Trichocereinae require revision to sort out generic level relationships within the subtribe. Since hybridization is common in cacti, reliance on plastid sequences for phylogenies is discouraged. Other variable nuclear regions should also be sought.

The infrageneric classification of *Harrisia* proposed is supported by the molecular data and morphology. Although three infrageneric units are monotypic, their establishment enables the recognition of two groups of closely related species: sect. *Eriocereus* (5 spp.) and series *Harrisia* (12 spp.). The sister group relationship between series *Earlei* and series *Harrisia* is poorly supported in the molecular phylogeny and further molecular analyses are desirable to verify their taxonomic recognition. Further study of other west Cuban species would help to corroborate the position of series *Earlei*.

The biogeographic hypothesis of *Harrisia* in South America and the Caribbean is consistent with the molecular data and morphology. However, there are no fossils available in Cactaceae to confirm this hypothesis. The extremely high endemism in the east Andes of Bolivia supports an Andean origin of *Harrisia*. The distributions of other cacti suggest the dispersal of *Harrisia* from the Andes to northeast Brazil and from Brazil to west Cuba. It would be interesting to see if phylogenetic studies of other genera present in Brazil and the Caribbean such as *Melocactus* and *Pilosocereus* would reveal a similar biogeographic hypothesis since these genera share a similar distribution pattern and habitat preference with *Harrisia*.

The AFLP data and DNA sequences produced an informative network for hypothesizing about the evolution of the Caribbean species of *Harrisia*. Three species groups appear to be present: a Cuba group, Florida group, and southeast Greater Antilles/Bahamas group. With respect to geographic range and species diversity, Cuba and Hispaniola were the least sampled regions in the phylogenetic analyses. Many questions remain concerning the relationships among the species of Cuba and Hispaniola. The distinction between *H. fernowii* from east Cuba, *H. divaricata* from Hispaniola, and *H. portoricensis* from Puerto Rico and Hispaniola is in need of clarification. Corroboration of the distinction between *H. fernowii* from east Cuba, *H. eriophora* from central/west Cuba (and the Isla de la Juventud), and *H. taetra* from west Cuba is also needed. It is possible that some names synonymized here are distinct, and if so should probably be regarded as infraspecific taxa, but this would require additional study of multiple populations.

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APPENDICES

APPENDIX A

GenBank accession numbers of sequenced taxa of *Harrisia* and latitude and longitude of provenance (or "cult." if from cultivation). Vouchers are the same as in Table 1.1.

Taxon	Lat., long.	*isi1*	*nhx1*	*atpB-rbcL*	*rpl16*	*ycf1*
H. adscendens	cult.	JN166835	JN166840	JN166871	JN166899	JQ664554
H. earlei	22.6°, –83.7°	JN166810	JN166839	JN166872	JN166932	JQ664556
H. fragrans	28.9°, –80.8°	JN166811	JN166836	JN166876	JN166930	JQ664555
H. regelii	cult.	JN166807	JN166842	JN166867	JN166902	JQ664557
H. tetracantha	cult.	JN166813	JN166843	JN166866	JN166900	JQ664558

APPENDIX B

Number of species recognized in *Harrisia* by various authors, using the current proposed infrageneric taxonomy. The putative hybrid cultivar *H. jusbertii*, the basionym of uncertain application *Cereus platygonus*, and undescribed taxa (nom. nud.) were not counted.

	Britton & Rose (1920)	Backeberg (1977)	Anderson (2001)	Hunt et al. (2006)	This study
Series *Harrisia*	9	12	13	2	10
Series *Earlei*	1	1	1	0	1
Sect. *Adscendens*	1	1	1	1	1
Sect. *Eriocereus*	5	6	4	5	5
Sect. *Roseocereus*	0	1	1	1	1

APPENDIX C

Voucher information and GenBank accessions numbers for sequenced taxa. Taxon name and authority is followed by provenance (xc=origin unknown, from cultivation), collection no.; GenBank accession numbers of *isi1*, *nhx1*, *atpB-rbcL* IGS, *rpl16* intron. All vouchers are collected by A. R. Franck and deposited at USF unless otherwise noted.

Ingroup: ARTHROCEREUS: *A. spinosissimus* (Buining & Brederoo) F. Ritter, xc, *1331*; JN166832, JN166858, JN166897, JN166920. **CLEISTOCACTUS**: *C. laniceps* (K. Schum.) Gosselin, xc, *2637*; JN166828, -, JN166877, JN166916. **ECHINOPSIS**: *E. atacamensis* (Phil.) Friedrich & G. D. Rowley subsp. *pasacana* (F. A. C. Weber) G. Navarro, xc, *2313*; JN166819, JN166860, JN166881, JN166903. *E. aurea* Britton & Rose, Argentina: Córdoba, *2623*; JN166833, JN166862, JN166878, JN166925. *E. bridgesii* Salm-Dyck subsp. *yungasensis* (F. Ritter) P. J. Braun & Esteves, Bolivia: La Paz Dept., *2639*;

JN166814, JN166857, JN166865, JN166918. *E. camarguensis* (Cárdenas) D. R. Hunt, Bolivia: Chuquisaca Dept., *2311*; JN166820, JN166847, JN166890, JN166912. *E. candicans* F. A. C. Weber, Argentina: Mendoza, *2643*; JN166826, JN166850, JN166885, JN166907. *E. chamaecereus* Friedrich & Glaetzle, xc, *2636*; -, -, JN166883, JN166924. *E. chiloensis* (Colla) Friedrich & G. D. Rowley subsp. *litoralis* (Johow) M. Lowry, xc, *2498*; JN166830, JN166846, JN166882, JN166905. *E. formosa* (Pfeiff.) Jacobi ex Salm-Dyck, Argentina: Mendoza, *2644*; -, -, JN166884, JN166922. *E. haematantha* (Speg.) D. R. Hunt, Argentina: Salta, *2624*; JN166834, JN166863, JN166879, JN166927. *E. hahniana* (Backeb.) R. S. Wallace, Paraguay, *2645*; JN166817, JN166849, JN166888, JN166908. *E. lageniformis* (Foerster) Friedrich & G. D. Rowley, xc, *2635*; JN166823, JN166852, JN166894, JN166915. *E. pachanoi* (Britton & Rose) Friedrich & G. D. Rowley, xc, *2598*; JN166821, JN166853, JN166895, JN166917. *E. schickendantzii* F. A. C. Weber, Argentina: Salta, *2312*; JN166831, JN166854, JN166889, JN166909. *E. strigosa* Friedrich & G. D. Rowley, Argentina: La Rioja, *2634*; JN166822, JN166845, JN166892, JN166911. *E. tacaquirensis* (Vaupel) Friedrich & G. D. Rowley, Bolivia, *1330*; JN166829, JN166851, JN166893, JN166914. *E. terscheckii* (Parm. ex Pfeiff.) Friedrich & G. D. Rowley, Argentina: Salta, *2621*; JN166818, JN166844, JN166880, JN166904. *E. thelegona* (F. A. C. Weber ex K. Schum.) Friedrich & G. D. Rowley, Argentina: Salta, *2638*; JN166816, JN166859, JN166887, JN166910. *E. vasquezii* (Rausch) G. D. Rowley, xc, *2310*; JN166825, JN166861, JN166891, JN166913. **HAAGEOCEREUS**: *H. decumbens* (Vaupel) Backeb., Peru: Arequipa, *Arakaki*

1579 (USM); JQ889315, JQ889321, JQ889304, JQ889310. *H. pseudomelanostele* (Werderm. & Backeb.) Backeb., Peru: Lima, *Arakaki 1575a* (USM); JQ889316, -, JQ889305, JQ889308. **HARRISIA**: *H. adscendens* (Gürke) Britton & Rose, xc, *2641*; JN166835, JN166840, JN166871, JN166899. *H. bonplandii* (Parm. ex Pfeiff.) Britton & Rose, xc, *2642*; -, -, JN166870, JN166926. *H. brookii* Britton, Bahamas: Long Island, *Correll 51278* (NY); JN166812, JN166837, JN166874, JN166928. *H. earlei* Britton & Rose (1), Cuba: Pinar del Río, *Ardisson 99.15*; JN166810, JN166839, JN166872, JN166932. *H. earlei* (2), Cuba: Pinar del Río, *ZSS Staff s.n.* (ZSS); JQ889319, JQ889325, JQ889306, JQ889313. *H. fernowii* Britton, Cuba: Holguin, *Dice s.n.* (HNT); JN166809, JN166864, JN166875., JN166929 *H. fragrans* Small ex Britton & Rose, USA: Florida, *473*; JN166811, JN166836, JN166876, JN166930. *H. gracilis* (Mill.) Britton, Jamaica: Palisadoes, *2265*; JQ889318, JQ889324, JQ889307, JQ889314. *H. pomanensis* (F. A. C. Weber) Britton & Rose, xc, *2640*; -, -, JN166869, JN166923. *H. regelii* (Weing.) Borg, Argentina, *s.n.*; JN166807, JN166842, JN166867, JN166902. *H. simpsonii* Small ex Britton & Rose, USA: Florida, *1236*; JN166808, JN166838, JN166873, JN166931. *H. tetracantha* (Labour.) D. R. Hunt, xc, *2262*; JN166813, JN166843, JN166866, JN166900. *H. tortuosa* (J. Forbes ex Otto & A. Dietr.) Britton & Rose, xc, *2628*; JN166806, JN166841, JN166868, JN166901. **SAMAIPATICEREUS**: *S. corroanus* Cárdenas, xc, *1785*; JN166815, JN166848, JN166886, JN166906. **YUNGASOCEREUS**: *Y. inquisivensis* (Cárdenas) F. Ritter ex Eggli, Bolivia: La Paz Dept., *2633*; JN166827, JN166855, JN166898, JN166919. **Outgroup: MONVILLEA**: *M. cavendishii* (Monv.) Britton & Rose, xc, *2631*; JN166823,

JN166856, JN166896, JN166921. **PILOSOCEREUS**: *P. pachycladus*, xc, *2969*; JQ889317, JQ889320, JQ889303, JQ889309. **RHIPSALIS**: *R. pilocarpa* Loefgr., xc, *1132*; -, JQ889323, -, JQ889312. **SELENICEREUS**: *S. pteranthus* (Link & Otto) Britton & Rose, U.S.A.: Florida, *1314*; -, JQ889322, JQ889302, JQ889311.

APPENDIX D

Voucher information and GenBank accessions numbers for sequenced taxa. Taxon name and authority is followed by provenance (xc=origin unknown, from cultivation), collection no.; GenBank accession no. of *isi1*, *nhx1*, *atpB-rbcL* IGS, *rpl16* intron, *ycf1*, *at103*, *xdh1*. All vouchers are collected by A. R. Franck and deposited at USF unless otherwise noted.

ECHINOPSIS: *E. atacamensis* (Phil.) Friedrich & G. D. Rowley subsp. *pasacana* (F. A. C. Weber) G. Navarro, xc, *2313*; JN166819, JN166860, JN166881, JN166903, JX136751, JX136772, JX162238. *E. chiloensis* (Colla) Friedrich & G. D. Rowley subsp. *litoralis* (Johow) M. Lowry, xc, *2498*; JN166830, JN166846, JN166882, JN166905, JX136750, JX136771, JX162239. **HARRISIA series Harrisia**: *H. aboriginum* Small ex Britton & Rose (1-WC), USA: Florida (Sarasota Co.), Jun 2007 *s.n.*; JX096565, JX112046, JX135007, JX135021, JX136754, JQ898104, JX162215. *H. aboriginum* Small ex Britton & Rose (2-MB), USA: Florida, *1237*; -, -, -, -, -, -, -. *H. aboriginum* Small ex Britton & Rose (3-BP), USA: Florida (Lee Co.), Jun 2007 *s.n.*; -, -, -, -, -, -, -. *H. aboriginum* Small ex Britton & Rose (4-

BK), USA: Florida, 29 Oct 2007 *s.n.* ; JX096553, JX112047, JX135008, JX135022, JX136754, JX136778, JX162216. *H. brookii* Britton, Bahamas: Long Island, *Correll 51278* (NY); JN166812, JN166837, JN166874, JN166928, JX136755, JX136779, JX162217. *H. caymanensis* A.R. Franck, Cayman Islands, *2370*; JX096558, JX112048, JX135009, JX135023, JX136756, JX136780, JQ898105. *H. divaricata* (Lam.) Backeb. (1,2), Dominican Republic: Monte Cristi Prov., *2307*; JX096559, JX112051, JX135010, JX135026, JX136757, JX136782, JX162218. *H. divaricata* (Lam.) Backeb. (3), Dominican Republic: Azua Prov., *2306*; JX096556, JX112050, JX135012, JX135025, JX136759, JX136783, JX162220. *H. divaricata* (Lam.) Backeb. (4), Dominican Republic, *2975*; JX096554, JX112049, JX135011, JX135024, JX136758, JX136781, JX162219. *H. divaricata* (Lam.) Backeb. (5), Dominican Republic: Azua Prov., *Zanoni 31122*; -, -, -, -, JX173478, -, -. *H. eriophora* (Pfeiff.) Britton, Cuba: Havana Prov., *León 7179* (NY); -, -, -, -, JX173479, -, -. *H. fernowii* Britton, Cuba: Holguin, *Dice s.n.* (HNT); JN166809, JN166864, JN166875, JN166929, JX136760, JX136784, JX162221. *H. fragrans* Small ex Britton & Rose (1,2), USA: Florida (Volusia Co.), *473*; JN166811, JN166836, JN166876, JN166930, JQ664555, JX136785, JX162222. *H. fragrans* Small ex Britton & Rose (3,4,5), USA: Florida (St. Lucie Co.), *472*; JX096561, JX112053, JX135014, JX135028, JX136761, JX136786, JX162224. *H. fragrans* Small ex Britton & Rose (6), USA: Florida (Indian River Co.), *2974*; JX096557, JX112052, JX135013, JX135027, JX136762, JX136787, JX162223. *H. gracilis* (Mill.) Britton (1), Jamaica: Clarendon Par., *2661*; JX096564, JX112054, JX135015, JX135029, JX136763,

JX136788, JX162225. *H. gracilis* (Mill.) Britton (2), Jamaica: St. Andrew Par., *2265*; JQ889318, JQ889324, JQ889307, JQ889314, JX136764, JX136789, JX162226. *H. portoricensis* Britton (1), Puerto Rico: Mona Island, *Fumero-Cabán & Meléndez-Ackerman s.n.*; JX096560 , JX112055, JX135016, JX135030, JX136765, JX136793, JX162227. *H. portoricensis* Britton (2), Dominican Republic: Santiago Prov., *Liogier 11241* (NY); -, -, -, -, JX173480, -, -. *H. simpsonii* Small ex Britton & Rose (1), USA: Florida, *2627*; JX096552, JX112056, JX135017, JX135031, JX136768, JX136790, JX162228. *H. simpsonii* Small ex Britton & Rose (2), USA: Florida, *1279*; -, -, -, -, -, -, -. *H. simpsonii* Small ex Britton & Rose (3), USA: Florida, *1278*; -, -, -, -, -, -, -. *H. simpsonii* Small ex Britton & Rose (4), USA: Florida, *1236*; JN166808, JN166838, JN166873, JN166931, JX136767, JX136792, JX162230. *H. simpsonii* Small ex Britton & Rose (5), USA: Florida, *1277*; JX096555, JX112057, JX135018, JX135032, JX136766, JX136791, JX162229. *H. taetra* Areces, Cuba: Pinar del Rio Prov., *ZSS Staff s.n.* (ZSS); JX096562, JX112058, JX135019, JX135033, JX136769, JX136794, JX162231. *H. taylorii* Britton, Cuba: Guantanamo Prov., *2971*; JX096563, JX112059, JX135020, JX135034, JX136770, JX136795, JX162232. **HARRISIA series Earlei**: *H. earlei* Britton & Rose (1), Cuba: Pinar del Río, *Ardisson 99.15*; JN166810, JN166839, JN166872, JN166932, JQ664556, JX136776, JX162233. *H. earlei* Britton & Rose (2), Cuba: Pinar del Río, *ZSS Staff s.n.* (ZSS); JQ889319, JQ889325, JQ889306, JQ889313, JX136752, JX136777, JX162234. **HARRISIA sect. Adscendens**: *H. adscendens* (Gürke) Britton & Rose, xc, *2641*; JN166835, JN166840, JN166871, JN166899,

JQ664554, JX136775, JX162235. **HARRISIA subg. Eriocereus**: *H. regelii* (Weing.) Borg, Argentina, 23 Sep 2008 *s.n.*; JN166807, JN166842, JN166867, JN166902, JQ664557, JX136773, JX162236. *H. tetracantha* (Labour.) D. R. Hunt, xc, *2262*; JN166813. JN166843, JN166866, JN166900, JQ664558, JX136774, JX162237.

APPENDIX E

List of oft confused taxa. Several genera of cacti are confused with *Harrisia*. Besides seed morphology, the easiest characters to discern them are the combination of the dorsal stamen cluster, areolate fruits, and lack of aerial roots in *Harrisia*.

Acanthocereus (Engelm. ex A. Berger) Britton & Rose, stamens arranged radially symmetrical.

Hylocereus (A. Berger) Britton & Rose, stems with aerial roots.

Leptocereus (A. Berger) Britton & Rose, stamens arranged radially symmetrical

Monvillea Britton & Rose (=*Praecereus* Buxb.), stamens arranged radially symmetrical, fruit naked.

Peniocereus (A. Berger) Britton & Rose, (=*Nyctocereus* (A. Berger) Britton & Rose), stamens arranged radially symmetrical.

Selenicereus (A. Berger) Britton & Rose, stems with aerial roots.

Stenocereus (A. Berger) Riccob., e.g. Christenhusz 2008, stamens arranged radially symmetrical.

APPENDIX F

Herbarium specimen preparation recommendations. Healthy, vigorous shoots are often the most diagnostic for exemplifying stem morphology. Length-wise sections of the stem help to retain their shape by decreasing rib contraction during pressed drying. A cross-section of the stem enables the easiest determination of rib number. Areoles from basal portions of the stems or trunk should be collected as they often contain the longest and thickest spines. Stems, flowers, and fruits should be cut length-wise so that both sides can be viewed. The exterior of the stem, flower, and fruit is most informative for species identification. Some pollen can be packeted before mounting and the stigma can be protruded from the flower. The flower petals and pollen are extremely susceptible to insect damage, e.g. by book lice (*Liposcelis* Motschulsky). To attempt to retain fruit dimensions and shape, seeds and pulp should be removed from a halved fruit. Ideally, photographs should be included with the specimen as they are quite informative of plant habit as well as flower and fruit color.