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Research article

The systematic significance of floral morphology, nectaries, and nectar concentration in epiphytic cacti of tribes Hylocereeae and Rhipsalideae (Cactaceae)

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ABSTRACT

A long-standing interest in cactus taxonomy has existed since the Linnaean generation, but an appreciation of the reproductive biology of cacti started early in the 1900s. Numerous studies indicate that plant reproductive traits provide valuable systematic information. Despite the extensive reproductive versatility and specializations in breeding systems coupled with the striking floral shapes, the reproductive biology of the Cactaceae has been investigated in approximately 10% of its species. Hence, the systematic value of architectural design and organization of internal floral parts has remained virtually unexplored in the family. This study represents the most extensive survey of flower and nectary morphology in the Cactaceae focusing on tribes Hylocereeae and Rhipsalideae (subfamily Cactoideae). Our objectives were (1) to conduct comparative morphological analyses of flowers and floral nectaries and (2) to compare nectar solute concentration in these two tribes consisting of holo- and semi-epiphytic species. Flower morphology, nectary types, and sugar concentration of nectar have strong taxonomic implications at the tribal, generic and specific levels. Foremost, three types of nectaries were found, namely chamber nectary (with the open and diffuse subtypes), furrow nectary (including the holder nectary subtype), and annular nectary. All Hylocereeae species possess chamber nectaries, in which the nectarial tissue has both trichomes and stomata. The Rhipsalideae are distinguished by two kinds of floral nectaries: furrow and annular, both nectary types with stomata only. The annular nectary type characterizes the genus *Rhipsalis*. Nectar concentration is another significant taxonomic indicator separating the Hylocereeae and Rhipsalideae and establishing trends linked to nectar sugar concentration and amount of nectar production in relation to flower size. There is an inverse relationship between flower size and amount of nectar production in the smaller Rhipsalideae flowers, in which nectar concentration is more than two-fold higher despite the smaller volume of nectar produced when compared to the large Hylocereeae flowers. Variability of nectary morphology and nectar concentration was also evaluated as potential synapomorphic characters in recent phylogenies of these tribes. In conclusion, our data provide strong evidence of the systematic value of floral nectaries and nectar sugar concentration in the Cactaceae, particularly at different taxonomic levels in the Hylocereeae and Rhipsalideae.

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Introduction

A long-standing interest in cactus taxonomy has existed since the Linnaean generation, but an appreciation of the reproductive biology of cacti started early in the 1900s with studies by Beutler (1930), Buxbaum (1953), and Boke (1963, 1964, 1966, 1968), to name a few. The last two decades have seen an increase in various

research areas due in part to the advent of more sophisticated and affordable microscopic and molecular techniques. In addition, the fact that the family has numerous threatened species has boosted research interest in areas of conservation and reproductive biology, e.g., Scogin (1985), Silva and Sazima (1995), Nassar et al. (1997), Valiente-Banuet et al. (2007), Fuentes-Pérez et al. (2009), Rojas-Sandoval and Meléndez-Ackerman (2009), Almeida et al. (2010, 2012), among others. Remarkably, in 2008 the red list of threatened species of the International Union for Conservation of Nature included 157 Cactaceae species (Rojas-Sandoval and Meléndez-Ackerman, 2009), but in 2012 the number of cacti on this list

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climbed to 189 (IUCN, 2012), a significant increase of more than 20% of taxa added to the endangered category in a four-year period.

The extensive reproductive versatility and specializations in breeding systems coupled with the striking floral shapes act as a mechanism for cactus diversification by promoting genetic variability reinforced by outcrossing (Pimienta-Barríos and Del Castillo, 2002; Cota-Sánchez and Crouch, 2008). Despite the ample evidence dealing with the assortment of reproductive systems (Cota-Sánchez, 2004; Reyes-Agüero et al., 2006 and references therein) and the evolution of floral sexual dimorphism in the cactus family (Toivonen and Mutikainen, 2012; Orozco-Arroyo et al., 2012), limited studies impede an inclusive understanding of the breeding systems, pollination mechanisms, seed dispersal, and structures associated with cactus flowers. While plant reproductive traits provide valuable comparative morphological, systematic, and evolutionary information, the reproductive biology of the Cactaceae has been investigated in less than 10% of its species (Cota-Sánchez and Abreu, 2007). The systematic value of architectural design and organization of flowers and its parts has been demonstrated in several plant groups in combination with the evolution of breeding or pollination systems and the intrinsic and extrinsic factors influencing the reproductive success in the form of seed output and recruitment (Armbruster, 1996; Bernardello, 2007).

Pollen, nectar rewards, and animal pollination are attributes common to all members of the Cactaceae, and nectar is one of the most important incentives for pollinating agents. The occurrence, position, characteristics, and types of floral nectaries are sources of valuable morpho-anatomical comparative data to infer phylogenetic relationships (Proctor et al., 1996; Bernardello, 2007; Nepi, 2007), assist in explaining aspects of pollination biology and reproductive mechanisms in plants (Richards, 1986), and lead to robust phylogenies (Endress et al., 2000) in combination with molecular data (Endress, 2002). Also, floral nectaries provide insights in relation to reproductive trends (Bernardello, 2007) because nectaries have different origins, e.g., perianth, receptacle, stamens, ovary wall, and base of style (Fahn, 1952, 1990), and play a vital role in pollination, usually enhancing output in reproductive success and seed set. Even so, to date, the systematic significance of floral and extrafloral nectaries has been underutilized (Brown, 1938; Cronquist, 1981; Chesselet et al., 2002; Bernardello, 2007; Nepi, 2007), despite the fact that nectary information and the reproductive and evolutionary biology of angiosperms is known in a large number of plant families, including the Aizoaceae (Chesselet et al., 2002), Iridaceae (Rudall et al., 2003), Malvaceae (Vogel, 2000), Solanaceae (Bernardello, 1987), among others. Nonetheless, the information dealing with the taxonomic distribution of nectaries and their structures across different angiosperm lineages is limited and is in part correlated with the problematic *ex-situ* examination of internal and external floral structures, i.e., nectaries and associated parts, from preserved herbarium specimens, as opposed to analyses using fresh flowers either *in-situ* in the field or in living collections. Although these studies are challenging, the area of reproductive biology is a rich research field with an ample spectrum of applications.

Notwithstanding the popularity of the Cactaceae, there is still a shortcoming in understanding the floral morphology and reproductive biology of epiphytic cacti, to some extent because of the ephemeral nature of the flowers, the poorly known floral phenology, and the difficulty of conducting field studies in these tree canopy-dwelling plants. In this study we focus on a survey of flowers and nectary morphology in members of tribes Hylocereeae and Rhipsalideae (subfamily Cactoideae). These two lineages represent counterpart assemblages exhibiting a broad spectrum of floral and stem morphological attributes, the Rhipsalideae (with strict or holo-epiphytic species) primarily distributed in South America

and the Hylocereeae (hemi-epiphytic species) mainly in Central America. This investigation involved the morphological examination of a wide array of floral features in representative species of these two tribes, with an emphasis on floral nectaries and nectar sugar concentration. Our inquiries into the morphology and structure of nectaries and nectar sugar concentration provide the largest taxonomic representation ever presented in the Hylocereeae and Rhipsalideae and the Cactaceae as a whole. The objectives of this study were (1) to conduct a comparative morphological analysis of flowers and their nectaries and (2) to determine nectar solute concentration in both holo- and hemi-epiphytic species. We present inclusive information about general floral phenology together with morphological descriptions of major types of nectaries and their distinguishing features, as well as nectar sugar concentration. We compared our data with published records to address taxonomic and phylogenetic inferences at the tribal, generic and specific levels of the Cactaceae. Our data provide substantial evidence of the systematic significance of these characters in the family, particularly the taxonomic distribution of nectary types and sugar concentration in flower nectar at the tribal and generic levels.

Materials and methods

Plant material and taxonomic sampling

Material for floral analyses was collected from living plants acquired from the Montreal Botanic Garden collection and then grown in the greenhouse of the Department of Biology at the University of Saskatchewan (UoFS), Canada. Other flowers were collected at the Parque do Ingá, Maringá-PR, and at the Parque de Lavras, Salto-SP, Brazil. The origin of plant material, species investigated, and herbaria in which voucher specimens were deposited is provided in Table 1. Our taxonomic sampling includes flowers representing the floral diversity of the Hylocereeae and Rhipsalideae, nine taxa (eight species and one cultivated hybrid) in the former and 16 taxa in the latter tribe, for a total of 25 taxa investigated. Overall, this survey encompasses five out of the six genera recognized in the Hylocereeae (*sensu* Anderson, 2001), namely *Disocactus*, *Epiphyllum*, *Hylocereus* (A. Berger) Britton & Rose, *Selenicereus* (A. Berger) Britton & Rose, and *Weberocereus* Britton & Rose. For the Rhipsalideae, we investigated the four genera recognized by Anderson (2001) in this tribe: *Hattoria* (one species), *Lepismium* (three species), *Rhipsalis* (nine species plus two subspecies), and *Schlumbergera* (two species) (Tables 1 and 2).

The number of flowers examined per species was in line with flowers formed per plant, e.g., two in *Epiphyllum guatemalense*, *E. oxypetalum*, *E. phyllanthus*, and *Weberocereus panamensis* to 23 in *Rhipsalis grandiflora*. Normally, the Hylocereeae representatives produced fewer (two to six flowers/plant) but larger flowers compared to three to 23 smaller flowers in the Rhipsalideae. Floral attributes, such as symmetry, length, diameter of perianth and floral tube, were measured for each fresh flower as per sample size in Table 2 with a digital caliper (0.01 mm precision) and recorded immediately after collection. The flower length was taken from the ovary base (external wall) to the top (including terminal parts of pericarpel, tube and perianth). Considering the lack of a standard terminology for the description of floral morphology, including the nectary shape and structure, our descriptions follow Buxbaum's (1953) terminology and are complemented with Bernardello (2007) and Leins and Erbar (2010). High-resolution digital pictures were taken for all species, and representative taxa were included in figures. The images and plates were labeled and assembled using Adobe Photoshop CS3 and Corel Photo-Paint X3 version 13 software.

Table 1

List of plant material used in this study. Taxonomic authorities follow Tropicos electronic database of the Missouri Botanical Garden (Tropicos.org, 2012). Plant habit and geographic distribution based on data from botanical collections, Hunt (1999), and Anderson (2001). MBG = Montreal Botanic Garden; UofS = University of Saskatchewan. Herbarium acronyms are according to Index Herbariorum (Thiers continuously updated (Thiers, 2012)). HRCB = Herbário Rioclarense, Universidade Estadual Paulista, Rio Claro; HUEM = Herbário da Universidade Estadual de Maringá; SASK = University of Saskatchewan.

Taxon	Plant habit	Geographic distribution	Source of live specimens	Herbarium acronym and accession no.
Hyllocereae				
<i>Disocactus ackermannii</i> (Haw.) Ralf Bauer	Holo-epiphyte	Mexico	MBG 11-1961	SASK 180688
<i>Epiphyllum guatemalense</i> Britton & Rose	Holo-epiphyte	Mexico, Guatemala and Honduras	MBG 1935-1958	SASK 180689
<i>E. oxypetalum</i> (DC.) Haw.	Holo-epiphyte	Mexico, Guatemala, Honduras, Nicaragua, El Salvador, and Costa Rica	MBG 141-1972B	SASK 180690
<i>E. phyllanthus</i> (L.) Haw.	Holo-epiphyte	From southern Mexico to S. America	MBG 3253-1987B	HUEM 12673, HRCB 48936 and 48937
<i>Epiphyllum</i> X <i>Fern la Borde</i>	Holo-epiphyte	Commercial hybrid	MBG 2-1998A	SASK 180691
<i>Hyllocereus undatus</i> (Haw.) Britton & Rose	Hemi-epiphyte	C. America—widely cultivated	“Parque do Ingá”	HUEM 21152
<i>H. setaceus</i> (Salm-Dyck) Ralf Bauer	Holo-epiphyte	Argentina, Brazil, Bolivia, Paraguay		SASK 180692
<i>Selenicereus anthonyanus</i> (Alexander) D.R. Hunt	Hemi-epiphyte	Mexico	“Parque do Ingá”	HUEM 22576
<i>Weberocereus panamensis</i> Britton & Rose	Holo-epiphyte	Panama	MBG 710-1956	SASK 180693
Rhipsalideae				
<i>Hatiora gaertneri</i> (Regel) Barthlott	Holo-epiphyte	Brazil	UofS 15-2004	SASK 180676
<i>Lepismium bolivianum</i> (Britton) Barthlott	Holo-epiphyte	Bolivia	MBG 3277-1987 and MBG 3277-1977	SASK 180677
<i>L. cruciforme</i> (Vell.) Miq.	Holo-epiphyte	Argentina, Brazil and Paraguay	MBG 1872-1992	HRCB 54227
<i>L. warmingianum</i> (K. Schum.) Barthlott	Holo-epiphyte	Argentina, Brazil and Paraguay	“Parque do Ingá”	HUEM 18986 and 18987
<i>Rhipsalis baccifera</i> (J.S. Muell.) Stearn subsp. <i>baccifera</i>	Holo-epiphyte or lithophyte	Caribbean, eastern Mexico, Florida, C. America, and northern S. America	MBG 2610-1992B	SASK 180678
<i>R. baccifera</i> subsp. <i>horrida</i> (Baker) Barthlott	Holo-epiphyte, or lithophyte	Madagascar	MBG 3357-1987 ^C	SASK 180679
<i>R. cereuscula</i> Haw.	Holo-epiphyte	Argentina, Brazil, Bolivia, Paraguay, and Uruguay	“Parque de Lavras”	HRCB 54226 HUEM 18985
<i>R. floccosa</i> Salm-Dyck ex Pfeiff. subsp. <i>tucumanensis</i> (F.A.C. Weber) Barthlott & N.P. Taylor	Holo-epiphyte	Argentina, Bolivia and Peru	MBG 1346-60	SASK 180680
<i>R. grandiflora</i> Haw.	Holo-epiphyte	Brazil	MBG 1321-1960B	SASK 180681
<i>R. mesembryanthemoides</i> Haw.	Holo-epiphyte	Brazil	MBG 7313-1939D	SASK 180682
<i>R. micrantha</i> (Kunth) DC.	Holo-epiphyte	Costa Rica, western Venezuela, Ecuador, and northern Peru	MBG 1349-1960	SASK 180683
<i>R. neves-armondii</i> K. Schum. f. <i>megalantha</i> (Loefgr.) Barthlott & N.P. Taylor	Holo-epiphyte or lithophyte	Brazil	MBG 7312-1939	SASK 180684
<i>R. puniceodiscus</i> G. Lindb.	Holo-epiphyte	Brazil	MGB 7314-1939	SASK 180685
<i>R. teres</i> (Vell.) Steud.	Holo-epiphyte or lithophyte	Brazil	MGB 993-1995	SASK 180582
<i>Schlumbergera russelianum</i> (Hook.) Britton & Rose	Holo-epiphyte	Brazil	UofS 44-2006	SASK 180686
<i>S. truncata</i> (Haw.) Moran	Holo-epiphyte or lithophyte	Brazil	MBG 1677-1989B	SASK 180687

Scanning electron microscopy (SEM)

For the micromorphological analyses of the nectaries, the flowers of each species were dissected in small (longitudinal and transversal) sections, fixed in 2.5% glutaraldehyde in buffer solution (0.05 M sodium phosphate, pH 7.2) for 48 h, dehydrated in a graded acetone series to 100%, critical-point dried with liquid CO₂ (Polaron Instruments E3000), and then affixed on aluminum stubs. After gold coating (Edwards Sputter Coater S150B), nectary sections were examined with a Philips SEM 505 at 30 kV, and micrographs were taken using Polaroid 665 positive/negative film and the Animator DV (image capture) program. For consistency, the structures were observed in three different flowers per species in different angles to verify the anatomical characteristics of the nectaries. Overall nectary size was estimated based on the scale bar obtained from the SEM.

Measurement of nectar sugar concentration

Nectar collection took place after a preliminary exploration of the flowers to locate the nectary and nectar. The nectar was collected by gently touching the floral nectary with a micropipette of known volume (1.0, 5.0 and 10.0 µL) and/or Drummond Scientific Microcaps (1.0 µL) and, whenever possible, at different times and different days, always in virgin flowers following Bolten et al. (1979) and Almeida et al. (2012). In order to prevent nectar concentration changes due to the relative atmospheric humidity and temperature conditions prevailing in the greenhouse, the nectar was immediately expelled onto the prismatic surface of a hand refractometer (0%–50% and 40%–85%; Bellingham and Stanley, Tunbridge Wells, Kent) to determine solute concentrations, measured as percent nectar concentration by weight (% NCW). Nectar readings were performed in all the flowers produced by each plant

Table 2

Floral attributes, nectary type, secreting structures, and nectar sugar concentrations in representative species of the Hylocereeae and Rhipsalideae. Flower measurements represent mean values; solute concentration of floral and extrafloral nectar expressed as % NCW. Nectary type follows Buxbaum's (1953) classification. Deduction of putative pollinators in the species investigated was based on literature review and morphological, phenological, and nectarial datasets.

Taxon	Sample size (n)	Flower shape, symmetry, color, and scent	Flower length (cm)	Flower diameter (cm)	Anthesis (duration in days)	Nectary type	Secretion structure	Nectar amount ($\mu\text{L}/\text{flower}$)	%NCW (mean \pm s.d.)	Putative pollinator
Tribe Hylocereeae										
<i>Disocactus ackermannii</i>	4	Salverform Radial Red Scentless	11.6	7.0	4	Open chamber	Trichomes, mesophyll and stomata	>90	25.4 \pm 3.3	Hummingbird
<i>Epiphyllum guatemalense</i>	2	Salverform Radial White-yellowish Sweet fragrance	21.1	*	1 night	Diffuse chamber	Trichomes, mesophyll and stomata	*	30.3 \pm 2.1	Hawkmoth
<i>E. oxypetalum</i>	2	Salverform Zygomorphic, curved Strongly fragrant	32.6	21.5	1 night	Diffuse chamber	Trichomes, mesophyll and stomata	Ca. 20	22.4 \pm 2.3	Hawkmoth
<i>E. phyllanthus</i>	2	Salverform Long tubular Radial Sweet fragrance	22.0	1.30	1 night	Open chamber	Trichomes, mesophyll and stomata	Ca. 5	16.6 \pm 1.6	Hawkmoth
<i>E. phyllanthus</i> (ENN)	4 ^A	n/a	n/a	n/a	n/a	n/a	Unknown	<1	77.0 \pm 0.8	Ant
<i>Epiphyllum X Fern la Borde</i>	2	Salverform Slightly bilateral Purple-red Scentless	7.8	4.0	2	Open chamber	Trichomes, mesophyll and stomata	Ca. 25	22.7 \pm 0.6	Bird/butterfly
<i>Hylocereus setaceus</i>	3	Salverform Radial White-cream Strongly fragrant	27.3	14.6	1 night	Diffuse chamber	Trichomes, mesophyll and stomata	Ca. 160	27.7 \pm 0.7	Bat and Hawkmoth
<i>H. setaceus</i> (ENN)	3 ⁺	n/a	n/a	n/a	n/a	n/a	Stomata (abaxial surface)	<1	73.4 \pm 1.1	Ant
<i>H. undatus</i>	3	Salverform Radial White-cream Strongly fragrant	28.9	26.2	1 night	Open chamber	Trichomes, mesophyll and stomata	*	*	Bat and Hawkmoth
<i>Selenicereus anthonyanus</i>	6	Salverform Radial Pinkish-cream Strongly fragrant	13.6	9.3	1 night	Open chamber	Trichomes, mesophyll and stomata	*	*	Hawkmoth

Table 2 (Continued)

Taxon	Sample size (n)	Flower shape, symmetry, color, and scent	Flower length (cm)	Flower diameter (cm)	Anthesis (duration in days)	Nectary type	Secretion structure	Nectar amount ($\mu\text{L}/\text{flower}$)	%NCW (mean \pm s.d.)	Putative pollinator
<i>Weberocereus panamensis</i>	2	Funnelform, Radial Sweet light fragrant	20.3	13.8	1 night	Open chamber	Trichomes, mesophyll and stomata	Ca. 20	18.2 \pm 1.0	Hawkmoth
Tribe Rhipsalideae										
<i>Hatiora gaertneri</i>	5	Bowl-shaped Radial Scarlet-red Scentless	3.5	4.7	9	Furrow	Stomata	Ca. 1.0	76.5 \pm 2.1	Bee
<i>Lepismium bolivianum</i>	6	Bell-shaped Radial Red-yellowish Scentless	1.5	1.7	2	Furrow	unknown	<1.0	51.0 \pm 7.3	Bee
<i>L. cruciforme</i>	20	Bell-shaped Radial Cream-pinkish Scentless	0.9	1.1	2	Furrow	Stomata	0.6–0.8	59.4 \pm 6.7	Bee
<i>L. warmingianum</i>	5	Bell-shaped Radial White Scentless	1.7	1.4	2	Furrow	Stomata	.	.	Bee
<i>Rhipsalis baccifera</i> subsp. <i>baccifera</i>	6	Rotate Radial Green-whitish Scentless	0.4	0.4	2	Annular	unknown	Ca. 1.0	50.0 \pm 6.3	Bee
<i>R. baccifera</i> subsp. <i>horrida</i>	4	Rotate Radial Green-whitish Scentless	0.7	0.7	2	Annular	unknown	Ca. 1.0	55.3 \pm 3.0	Bee
<i>R. cereuscula</i>	9	Bell-shaped Radial White Scentless	1.4	1.4	2	Embedded Ring	Stomata	6.0–7.0	62.4 \pm 4.5	Bee
<i>R. floccosa</i> subsp. <i>tucumanensis</i>	9	Rotate Radial Cream Scentless	0.7	1.7	2	Annular	Stomata	1.0–2.5	71.5 \pm 2.7	Bee
<i>R. grandiflora</i>	23	Rotate Radial Cream-yellowish Scentless	1.1	1.6	2	Annular	Stomata	4.2–7.0	70.5 \pm 4.4	Bee
<i>R. mesembryanthemoides</i>	4	Rotate Radial White Scentless	1.4	1.2	2	Annular	unknown	1.0–2.0	60.7 \pm 5.2	Bee
<i>R. micrantha</i> f. <i>micrantha</i>	9	Rotate Radial White Scentless	0.6	0.9	2	Annular	unknown	Ca. 10.0	69.2 \pm 1.4	Bee

Table 2 (Continued)

Taxon	Sample size (n)	Flower shape, symmetry, color, and scent	Flower length (cm)	Flower diameter (cm)	Anthesis (duration in days)	Nectary type	Secretion structure	Nectar amount ($\mu\text{L}/\text{flower}$)	%NCW (mean \pm s.d.)	Putative pollinator
<i>R. neves-armondii</i>	8	Rotate Radial White Weakly sweet Fragrant	2.5	1.3	3	Annular	Stomata	Ca. 0.6	72.4 \pm 1.1	Bee
<i>R. puniceodiscus</i>	4	Rotate Radial Cream-pinkish Scentless	1.0	1.6	2	Annular	unknown	Ca. 1.0	72.0 \pm 2.0	Bee
<i>R. teres</i> [‡]	8	Rotate Radial White Scentless	0.9	1.1	3	Annular	Stomata	Ca. 1.0	70.6 \pm 2.2	Bee
<i>Schlumbergera russelianum</i>	6	Tube-like Radial Pink Scentless	6.5	29.9	3	Furrow-holder subtype	unknown	5.0–7.0	34.2 \pm 2.4	Hummingbird
<i>S. truncata</i>	3	Tube-like Zygomorphic Pink-white Scentless	7.6	5.8	4	Furrow-holder subtype	Stomata	8.0	37.0 \pm 1.5	Hummingbird

* Measurements not taken.

^Δ Nectar sampled from four different extranuptial nectaries (ENNs) in two flowers in which the floral nectar was measured.[†] Nectar sampled from three different ENNs in one flower for which the floral nectar was measured, in *H. setaceus*.[‡] *Rhipsalis teres* information from Almeida et al. (2012).

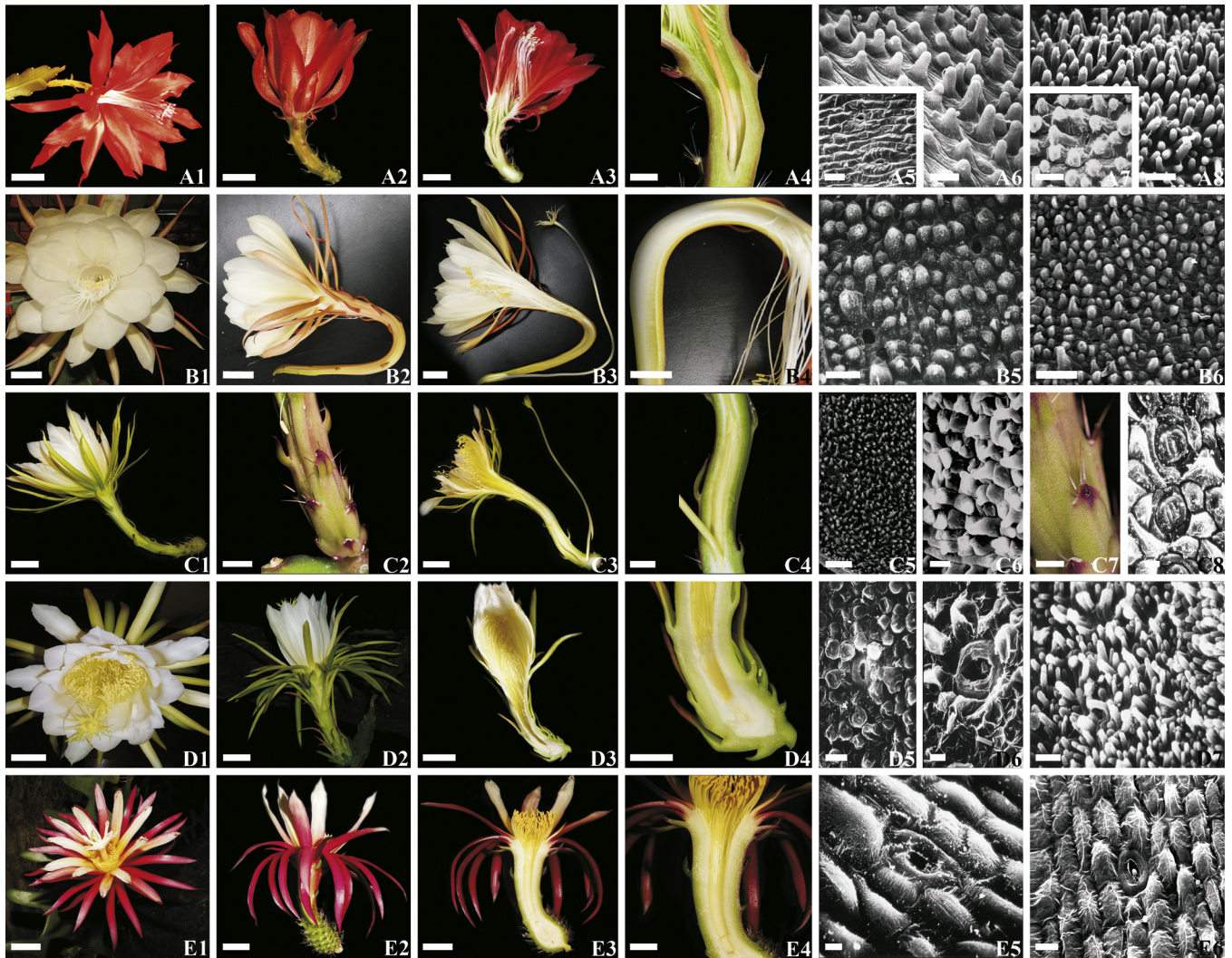


Fig. 1. Floral morphology (photos) and nectary micromorphology (SEM micrographs) in selected Hylocereeae representatives. (A1–A8) *Disocactus ackermannii*. (A1, A2) Flower in frontal and lateral view. (A3) Flower in longitudinal section. (A4) Detail of the open chamber nectary. (A5–A7) SEM of nectary in floral bud (pre-anthesis). (A5, A7) Stomata. (A6) Trichomes. (A8) SEM view of nectarial trichomes at anthesis. (B1–B6) *Epiphyllum oxypetalum*. (B1, B2) Flower in frontal and lateral view. (B3) Flower in longitudinal section. (B4) Detail of the diffuse chamber nectary. (B5, B6) SEM view of floral nectary with stomata and trichomes. (C1–C8) *Hylocereus setaceus*. (C1) Flower in lateral view. (C2) Close-up of the pericarpel region. (C3) Flower in longitudinal section. (C4) Detail of open chamber nectary. (C5, C6) SEM view of floral nectar and detail of trichomes. (C7) Detail of scale with secreting extranuptial nectar on the areole. (C8) SEM view of stomata on abaxial surface of scale. (D1–D7) *Hylocereus undatus*. (D1, D2) Flower in frontal and lateral view. (D3) Flower in longitudinal section. (D4) View of the open chamber nectary. (D5) SEM view of nectary in floral bud. (D6) SEM view of stomata in detail. (D7) SEM view of nectary with trichomes (flower in anthesis). (E1–E6) *Selenicereus anthonyanus*. (E1, E2) Flower in frontal and lateral view. (E3) Flower in longitudinal section. (E4) Detail of open chamber nectary. (E5) SEM view of nectary in floral bud. (E6) SEM view of nectary (flower in anthesis). Scale bars: 1 cm (C2, C4, E4), 2 cm (A1, A2, A3, B4, D4, E1, E2, E3), 3 cm (B1, B2, B3, C3), 4 cm (C1, D1), 5 cm (D2, D3), 5 mm (A4, C7), 50 μm (A5, A7, B5), 100 μm (A6, A8, B6, C5, D7), 25 μm (C6, D5), 30 μm (C8), 10 μm (D6, E5, E6).

(Table 2). Due to the ephemeral nature of some flowers, we were unable to measure nectar concentration in *Hylocereus undatus*, *Lepismium warmingianum*, and *Selenicereus anthonyanus*, as indicated in Table 2.

Results

Flower morphology and phenology in tribe Hylocereeae

The flowers of the Hylocereeae are, in general, large and showy (Fig. 1A1–A3; B1–B3; C1, C3; D1–D3; E1–E3) compared to most lineages of the Cactaceae. Floral symmetry is mostly radial, though some species have flowers slightly zygomorphic and salverform, varying in length from 7.8 cm in the *Epiphyllum X Fern la Borde* hybrid and 11.6 cm in *Disocactus ackermannii*

(Fig. 1A1, A2) to over 32 cm in *E. oxypetalum* (Fig. 1B2) (Table 2). Flower diameter varies from 1.3 cm in *E. phyllanthus* to 26.2 cm in *H. undatus*. The flowers are diurnal, normally lasting more than one day (four in the case of *D. ackermannii*), or nocturnal and ephemeral, opening early in the evening with anthesis onset early the next day (Table 2), and in most cases produce relatively large amounts of nectar and are adapted to different pollinators (Table 2). The color spectrum ranges from red, reddish-orange to white-cream.

In the Hylocereeae the stamens are numerous and organized in several series. According to Buxbaum (1953), the innermost series of stamens form first during flower development. These stamens are called primary. The stamens in the outer (upper) series form last, in a centrifugal fashion, and are called secondary stamens. The filaments of the primary stamens in Hylocereeae

flowers are uniformly inserted at the same height of the floral tube and become free at about the same level in the upper part of the tube, marking the upper portion of the nectar chamber (Fig. 1A4; C4; D4; E4). Exceptions to this pattern were observed in flowers of *E. guatemalense*, *E. oxypetalum* (Fig. 1B4) and *Hylocereus setaceus* (Fig. 1C4), species in which the filaments of the primary stamens become free at different levels on the floral tube.

Flower morphology and phenology in tribe Rhipsalideae

Generally, the flowers in members of the Rhipsalideae have a naked pericarpel. Floral diversity is broad (Figs. 2 and 3), from very small (*Lepismium* spp. and *Rhipsalis* spp.) (Fig. 2B1, B2; C1, C2) to medium (*Hattiora gaertneri*) (Fig. 2A1, A2) to relatively large (*Schlumbergera* spp.) (Fig. 3D1, D2), usually with actinomorphic symmetry, either bowl- (Fig. 2A1, A2) to bell-shaped (Figs. 2B1, B2; C1, C2 and 3A1, A2) to rotate (Fig. 3B1, B2; C1, C2), except in *Schlumbergera truncata* (Fig. 3D1, D2), a species with tube-like zygomorphic flowers (Table 2). The flower length ranges from 0.4 cm in *Rhipsalis baccifera* subsp. *baccifera* to 7.6 cm in *S. truncata* (Table 2). Rhipsalideae flowers vary from bright red–purplish to white and have fewer stamens than those of the Hylocereeae (Table 2). With the exception of *Schlumbergera* (as explained in the next section), the primary and secondary stamens are not relevant in nectary classification of Rhipsalideae because the nectary chamber is exclusive to Hylocereeae. In general, Rhipsalideae flowers are diurnal with anthesis lasting from two (*Lepismium* and *Rhipsalis* species) to four (*S. truncata*) to nine days (*H. gaertneri*) and adapted to insect and bird pollination (Table 2). The flowers produce significantly lower amounts of nectar, which varies among species (Table 2).

Types of nectaries in Cactaceae

To put in perspective earlier research on Cactaceae nectaries, we give a short prologue dealing with nectary categorization in the family and other angiosperms, mainly due to the lack of an all-inclusive classification of nectaries in the Cactaceae. Foremost, Buxbaum's (1953) influential studies on floral characters in the cactus family described three basic nectary types, namely (1) furrow, (2) disc, and (3) chamber, all of which fall within the hypanthial nectary type proposed by Bernardello (2007) for other angiosperms. According to Barthlott and Hunt (1993), the floral nectar in the Cactaceae is secreted by a disc-like structure or along the basal portion of the hypanthium, but Bernardello (2007) discourages the use of the term “disc” or “disk” because of the difficulty in defining such a structure and given that it can be confused with other floral parts. Thus, we use the term annular when referring to the floral nectary of the disc type in *Rhipsalis*, bearing in mind that this annular nectary should be considered homologous to the disc-like structure described earlier in this genus. For the most part, we follow Buxbaum (1953) to maintain consistency with existing terminology in the Cactaceae (see Table 3 for descriptions of the nectary types discussed in this paper).

Nectary types and structure in the Hylocereeae

All the Hylocereeae species investigated possess the chamber nectary type (Table 2). Considering the subdivision of this nectary type into diffuse, open, half-open, closed, and wool-covered chamber (*sensu* Buxbaum, 1953), most Hylocereeae species examined have the open chamber nectary subtype (Fig. 1A4, E4), except the flowers of *E. guatemalense*, *E. oxypetalum* (Fig. 1B4),

and *H. setaceus* (Fig. 1C4), which have the diffuse chamber nectary subtype (Table 3). Also, the flowers of all Hylocereeae examined share a characteristic whitish epidermal nectariferous region on the inner surface of the floral tube (hypanthium) (Fig. 1A4; B4; C4; D4; E4). The nectar produced in hypodermal tissues of Hylocereeae nectaries is released through trichomes (Fig. 1A6, A8; B6; C5, C6; D7) and stomata (Fig. 1A5, A7; B5; D5, D6; E5, E6), which are distributed on the surface of nectary tissue (see Table 2 for the taxonomic distribution of secretory parts).

This study revealed additional secretory structures in the scales of the pericarpel and floral tube in flowers of *Epiphyllum phyllanthus* and *H. setaceus* (Table 2; Fig. 1C2, C7), in which nectar was detected on the abaxial surface of these scales. The secretion starts during the floral bud stage and lasts until the onset of anthesis. SEM analyses of these scales in *H. setaceus* unveiled stomata only on the abaxial side (Fig. 1C8), the surface secreting nectar. These nectaries will be referred to hereafter as extranuptial nectaries (ENN). For an explanation of this term, see Vogel (1977).

Nectary types and structure in the Rhipsalideae

Members of the Rhipsalideae are distinguished by two kinds of floral nectaries, namely furrow nectary (Figs. 2A3–A6; B3, B4; C3, C4; 4B, C), including the subtype holder nectary (Fig. 3D3–D6; 4D), and annular (donut-like) nectary (Tables 2 and 3; Figs. 3A3, A4, A6; B3–B6; C3, C4; 4A). Whereas the nectaries of Hylocereeae release nectar by means of trichomes and stomata (Fig. 1), in the Rhipsalideae the nectar is released only through stomata (Table 2; Figs. 2 and 3).

The nectary tissue in *Hattiora gaertneri* covers the short floral tube (Fig. 2A3, A4), forming the furrow nectary type, a structure roughly bowl-shaped (Fig. 2A4–A6). The nectariferous tissue in this nectary is distributed throughout the floral tube and has stomata but lacks trichomes (Fig. 2A7). Similarly, the flowers of *Lepismium* species also exhibit the furrow type with stomata only (Fig. 2B3–B6; C3–C5), as in *H. gaertneri*.

The characteristic annular nectary in flowers of most *Rhipsalis* species shares similar position and morphological structures. That is, it is typically located at the base of the short floral tube and has an annular (donut-like) shape (Table 2; Fig. 3B5, B6; C3, C4). The nectary surface is made of cells of irregular shape, lacks trichomes but has stomata scattered throughout (Fig. 3A5, A7; B7, B8; C5). Slight variations of the annular nectary were observed in *Rhipsalis*, quite likely in relation to flower shape. For instance, in *R. cereuscula* the annular nectary is set apart because the donut-like structure is embedded in the somewhat longer floral tube, making this circular structure look higher (elevated) (Fig. 3A3, A4, A6). *R. grandiflora* has the largest annular nectary within the genus (Fig. 3B3–B6), whereas the smallest nectary (based on SEM scale bars and floral measurements) is found in *R. neves-armondii*, despite the fact that the latter taxon bears one of the largest flowers among the *Rhipsalis* species investigated (Fig. 3C3, C4).

Within the Rhipsalideae, the nectary in *Schlumbergera* species has rather different features compared to other tribal members. At the base of the floral tube and just above the ovary, the nectary tissue covers the wall of the floral tube and surrounds the style (Fig. 3D4–D7). The tip of the nectary resembles a vault-roof structure made of primary stamens basally fused (Fig. 3D4–D7) enclosing the style (Fig. 3D4, D6). The secondary stamens are inserted on the floral tube wall (Fig. 3D4, D5). Stomata are conspicuous on the nectary tissue (Fig. 3D8, D9) but not on the vault-roof area.

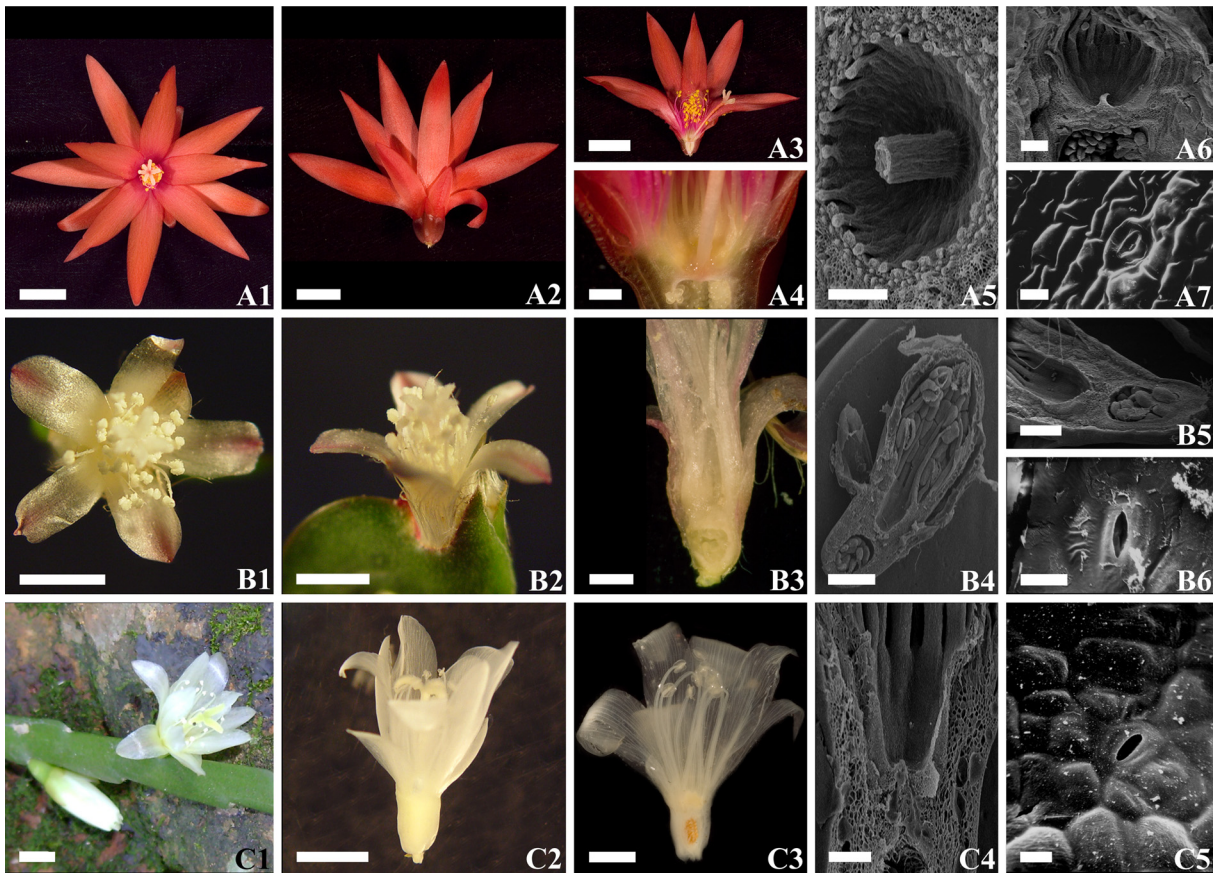


Fig. 2. Floral morphology (photos) and micromorphology (SEM micrographs) of nectary in *Hattiora* and *Lepismium* (Rhipsalideae). (A1–A7) *Hattiora gaertneri*. (A1, A2) Flower in frontal and lateral view. (A3) Flower in longitudinal section. (A4) Detail of the furrow nectary. (A5–A7) SEM view of nectary region. (A5) Furrow nectary in frontal view (bowl-shaped). (A6) Nectary in longitudinal section. (A7) Stomata in nectary epidermis. (B1–B5) *Lepismium cruciforme*. (B1, B2) Flower in frontal and lateral view. (B3) Flower in longitudinal section. (B4) SEM view of floral bud in longitudinal section. (B5) SEM view of lower region of flower in longitudinal section. (B6) SEM of stomata from nectary epidermis, flower in anthesis. (C1–C5) *Lepismium warmingianum*. (C1) View of flower and lateral floral bud. (C2) Flower in lateral view. (C3) Flower in longitudinal section. (C4) SEM of furrow nectary. (C5) SEM view of stomata from nectary epidermis. Scale bars: 1 cm (A1–A4, C1, C2), 1 mm (A5, B4), 500 μm (A6, C4), 5 mm (B1, B2), 2 mm (B3), 6 mm (C3), 10 μm (A7, B6, C5).

Table 3

Explanation of nectary types and their taxonomic occurrence in species investigated of tribes Hylocereeae and Rhipsalideae.

Tribe	Genus and/or Species	Nectary type	Nectary description	Picture
Hylocereeae	<i>Disocactus ackermannii</i> , <i>Epiphyllum phyllanthus</i> , <i>Epiphyllum X Fern la Borde</i> , <i>Hylocereus undatus</i> , <i>Selenicereus</i> , <i>Weberocereus</i>	Open chamber	Nectary type with nectarial tissue surface extending beyond the floral tube. The primary stamens are inserted at the same height of the floral tube wall	(Fig. 1A4, E4)
	<i>Epiphyllum guatemalensis</i> , <i>E. oxypetalum</i> , <i>Hylocereus setaceus</i>	Diffuse chamber	Nectary type with nectarial surface extending beyond the floral tube. The primary stamens are inserted at different heights of the floral tube	(Fig. 1B4, D4)
Rhipsalideae	<i>Hattiora gaertneri</i> , <i>Lepismium</i>	Furrow	Bowl- or bell-shaped nectary in which the bases of the stamen filaments create undulations (furrows) in the nectariferous tissue	(Fig. 2A3–A6, B3, B4, C3, C4)
	<i>Rhipsalis</i>	Annular	Nectary with a ring or donut-like structure around the style at the base of the floral tube	(Fig. 3A3, A4, A6; B3–B6; C3, C4)
	<i>Schlumbergera</i>	Furrow (holder subtype)	Nectary located at the base of floral tube. The nectarial tissue covers the wall of the floral tube and surrounds the style, which is more or less bell-shaped (furrow type). The top surface of the nectary resembles a vault-roof structure formed by the fusion of the base of the primary stamens surrounding the style (holder subtype)	(Fig. 3D4–D6)

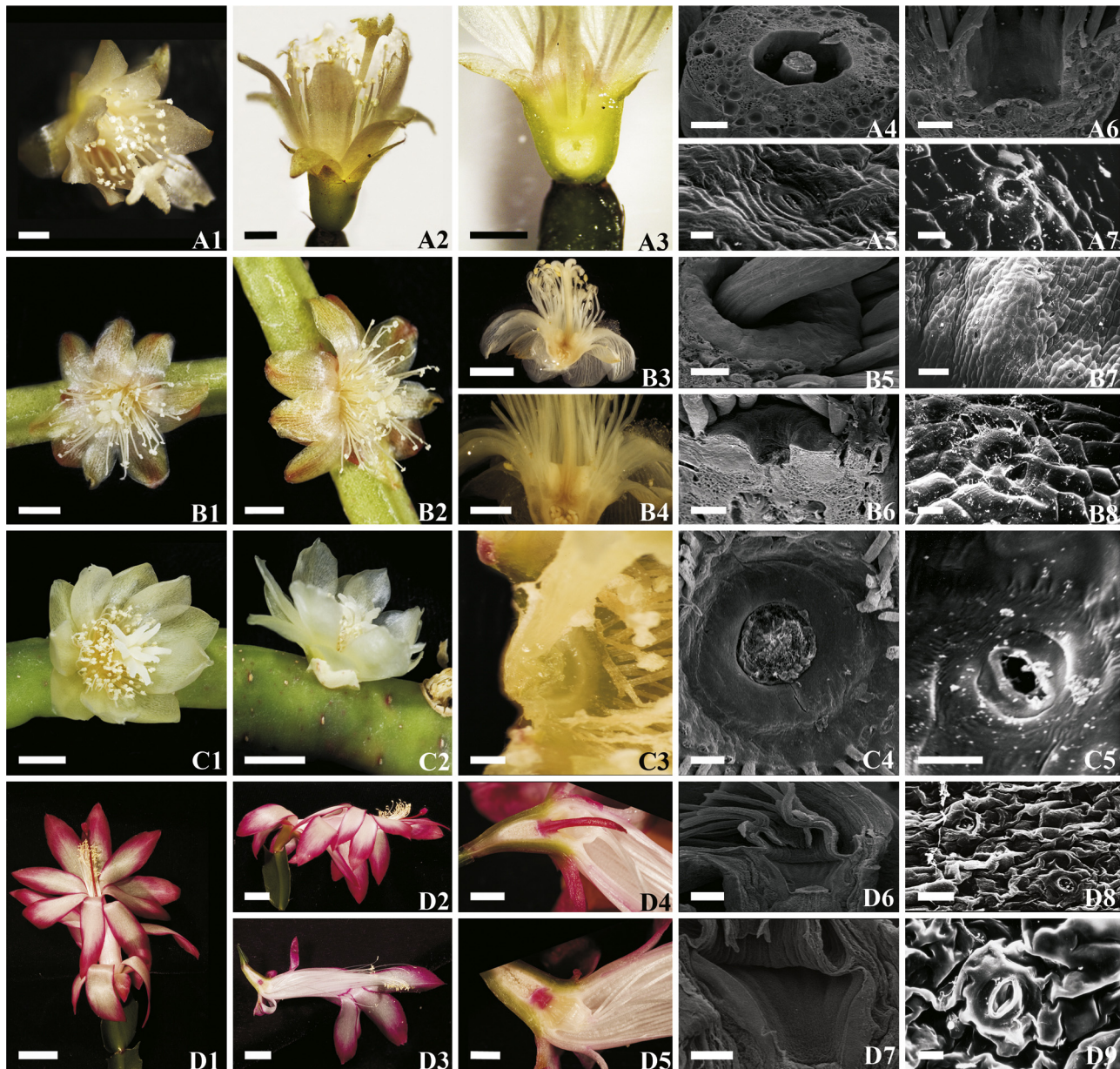


Fig. 3. Floral morphology (photos) and micromorphology (SEM micrographs) of nectary in selected species of *Rhipsalis* and *Schlumbergera truncata* (Rhipsalideae). (A1–A7) *Rhipsalis cereuscula*. (A1, A2) Flower in frontal and lateral view. (A3) Flower in longitudinal section. (A4–A7) SEM view of annular nectary. (A4, A6) Floral annular nectary in cross- and longitudinal sections. (A5, A7) Stomata from nectary epidermis. (B1–B8) *R. grandiflora*. (B1, B2) Flower in frontal and lateral view. (B3–B4) Flower in longitudinal section and detail of nectary region. (B5–B8) SEM view of annular nectary. (B5) Annular nectary. (B6) Annular nectary in longitudinal section. (B7) Nectary epidermis. (B8) Stomata from nectary epidermis. (C1–C5) *R. neves-armondii* f. *megalantha*. (C1, C2) Flower in frontal and lateral view. (C3) Annular nectary in longitudinal section. (C4) SEM view of annular nectary in frontal view. (C5) SEM view of stomata on nectary epidermis. (D1–D9) *Schlumbergera truncata*. (D1, D2) Flower in frontal and lateral views. (D3) Flower in longitudinal section. (D4, D5) Flower in longitudinal section, detail of the furrow nectary, holder subtype. (D6) SEM view of furrow nectary, holder subtype region. (D7) SEM of the furrow nectary, holder subtype in detail. (D8) SEM view of nectary epidermis. (D9) SEM view of stomata on nectary epidermis. Scale bars: 2 mm (A1–A3, B4, C3), 500 μ m (A4, A6, D7), 20 μ m (A5, D8), 10 μ m (A7, B8, C5, D9), 5 mm (B1, B2, C1, D4), 4 mm (B3), 300 μ m (B5, B6, C4), 50 μ m (B7), 10 mm (C2, D2, D3), 15 mm (D1), 1 mm (D6), 3 mm (D5).

Nectar volume and solute concentration in flowers of Hylocereeae and Rhipsalideae

The floral nectar in members of the Hylocereeae and Rhipsalideae varies among species, from very small volumes (<0.6–1.0 μ L) in *Lepismium* species, *Rhipsalis baccifera* and *R. neves-armondii*, to moderate quantities (4.0–7.0 to 8.0 μ L) in *R. cereuscula* and *R. grandiflora*, and *Schlumbergera* species, to rather larger amounts (160 μ L) in the flowers of *Hylocereus setaceus* (Table 2). On average, nectar amount in Hylocereeae and Rhipsalideae

flowers was estimated at 50.0 and 3.20 μ L, respectively. Also, nectar production is linked to flower size: The larger flowers of the Hylocereeae produce more nectar than the smaller flowers of the Rhipsalideae (Table 2; Fig. 4). The nectar is typically transparent and odorless (to human olfactory sense) in all species. It is relatively diluted or aqueous in flowers of Hylocereeae and *Schlumbergera* species and relatively thicker and viscous in flowers of *Hatiora*, *Lepismium*, and *Rhipsalis*.

The nectar sugar concentration (as percent nectar concentration by weight – % NCW) varies from 16.6% to 30.3% in the Hylocereeae

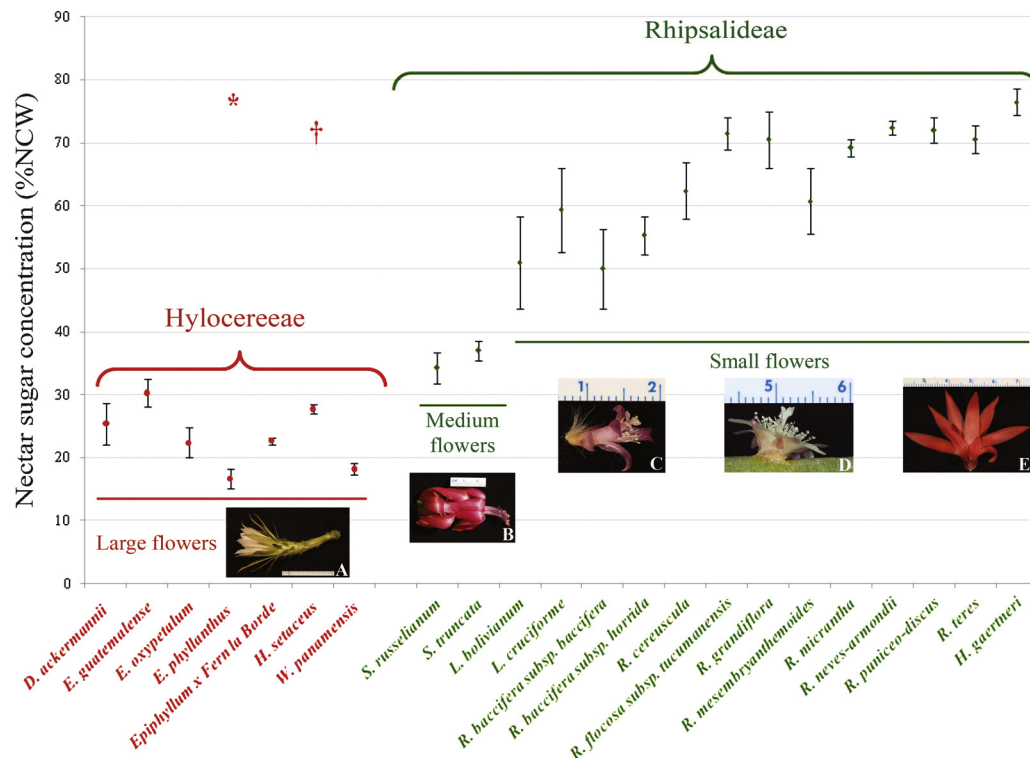


Fig. 4. Mean values of floral and extranuptial nectar solute concentration (% NCW) in epiphytic and semi-epiphytic cacti of tribes Hylocereeae and Rhipsalideae. Mean value of the extranuptial nectar of the floral scale of (*) *Epiphyllum phyllanthus* (77.00) and (†) *Hylocereus setaceus* (73.40). (A) *Weberocereus panamensis*. (B) *Schlumbergera russelianum*. (C) *Lepismium cruciforme*. (D) *Rhipsalis floccosa* subsp. *tucumanensis*. (E) *Hatiora gaertneri*.

and from 34.2% to 76.5% in the Rhipsalideae. The lowest concentration was recorded in *Epiphyllum phyllanthus* (16.6%) and the highest in *Hatiora gaertneri* (76.5%) (Table 2; Fig. 4). Remarkably, the large Hylocereeae flowers exhibit the lowest nectar sugar concentration mean values, varying from 16.6% (*E. phyllanthus*) to 27.7% (*Hylocereus setaceus*) and 30.3% (*E. guatemalense*) (Table 2; Fig. 4). Furthermore, the mean values for solute concentration of nectar in Hylocereeae flowers contrast significantly with those measurements obtained from nectar secreted by the scales of the floral tube of *E. phyllanthus* (77.0%) and *H. setaceus* (73.4%), an up to three-fold higher sugar concentration (Table 2; Fig. 4).

The mean values of nectar sugar concentration range from 34.2% in *Schlumbergera russelianum* to 76.5% in *Hatiora gaertneri* (Table 2; Fig. 4) in the Rhipsalideae, which represents a two-fold difference within the tribe, something not observed in the Hylocereeae. It is worth noting that these two species bear the largest flowers among the Rhipsalideae species examined, but the former has a relative longer floral tube. In contrast, the nectaries in the characteristic smaller flowers of *Lepismium* and *Rhipsalis* produce nectar with consistently higher mean values of nectar solute concentration, e.g., 51.0% in *L. bolivianum*, 59.4% in *L. cruciforme*, 62.4% in *R. cereuscula*, 71.5% in *R. floccosa*, 70.5% in *R. grandiflora*, and 72.4% in *R. neves-armondii* (Table 2; Fig. 4). The nectaries in small to medium-sized flowers of the Rhipsalideae produce nectar with at least twice or more the sugar concentration produced in nectaries of the large Hylocereeae flowers, except the nectaries in *Schlumbergera* (Table 2). The average nectar sugar concentration for the Rhipsalideae is 60.7% (NCW), a concentration around three times higher than in the Hylocereeae.

Discussion

Phylogenetic implications of nectary types in the Hylocereeae and Rhipsalideae

The Hylocereeae and Rhipsalideae, two convergent evolutionary lineages sharing similar vegetative and reproductive traits, exhibit contrasting geographic areas of putative origin and species diversity, the former mainly in Central America and the latter in South America. In spite of the vegetative phenotypic resemblance, the flowers of these tribes have different internal structures of systematic value. One of these attributes with taxonomic implications at the tribal and generic levels is the type and morphology of floral nectary. The nectary in Hylocereeae representatives corresponds to the chamber nectary type, with prevalence of the open nectar chamber (Tables 2 and 3), but in Rhipsalideae to the furrow and annular types (Tables 2 and 3). The nectaries are typically found at the base or lowermost to middle part of the floral tube, which includes parts of stamen filaments and gynoecium. This placement of nectaries between the androecium and gynoecium in connection with filament bases is common in core eudicots (Bernardello, 2007).

Within the Rhipsalideae the genus *Rhipsalis* is set apart by the annular nectary (Tables 2 and 3), distinguished by an obvious donut-like structure, except in *R. cereuscula*, a species with a distinct annular nectary embedded in the floral tube (Fig. 3A3, A4, A6). The structure and type of floral nectaries in other genera of the Rhipsalideae is equally useful. For instance, *Hatiora* and *Lepismium* spp. have in common the furrow nectary, whereas the flowers of *Schlumbergera* spp. possess the holder nectary subtype (Tables 2 and 3). Our findings indicate that the structure of floral

nectaries in Hylocereeae and Rhipsalideae is a distinctive tribal feature. This character also has strong taxonomic implications at the generic level in the Rhipsalideae.

In molecular phylogenetic analyses, Calvente et al. (2011) recognized two main clades: one including *Hatiora*, *Lepismium*, and *Schlumbergera*, and the other embracing all species in the genus *Rhipsalis*. The taxonomic distribution of nectary types in our study supports this phylogenetic division of the Rhipsalideae. The three genera *Hatiora*, *Lepismium* and *Schlumbergera* have the furrow nectary, and the largest and more problematical genus *Rhipsalis* is distinguished by the annular nectary. However, morphological differences between the flowers and nectaries of *Hatiora* and *Schlumbergera* do not support Calvente's et al. (2011) taxonomic and nomenclatural changes with the transfer of three species from *Hatiora* to *Schlumbergera* because in this coalition nectary type does not provide a clear-cut distinction and becomes an ambiguous character. The competing phylogeny by Korotkova et al. (2011), does not support such alliance either and maintains the *Hatiora* species [*H. cylindrica* Britton & Rose, *H. salicornioides* (Haw.) Britton & Rose and *H. herminiae* (Porto & A. Cast.) Backeb. ex Barthlott] within genus *Rhipsalidopsis* Britton & Rose, based on differences in floral characters between *Rhipsalidopsis* and *Schlumbergera*. Nonetheless, the distinctive embedded annular nectary in *Rhipsalis cereuscula* could be a potential feature endorsing the classification of subgenus *Erythrothipsalis* A. Berger (sensu Korotkova et al., 2011 and Calvente et al., 2011), but floral analyses in other members of this subgenus, i.e., *R. pulchra* Loefgr., *R. pilocarpa* Loefgr., *R. clavata* A.A. Weber, *R. campos-portoana* Loefgr., and *R. juengeri* Barthlott & N.P. Taylor, are needed to confirm this idea. For the Hylocereeae, the *Hylocereus*–*Selenicereus* clade in the phylogeny by Korotkova's et al. (2010) can be distinguished by the open chamber nectary.

Literature dealing with nectaries consisting of both trichomes and mesophyll (with stomata for nectar release) as secretory structures is scarce, and according to Bernardello (2007), nectar release via stomata is the most common mechanism in flowers. However, the nectar spur of *Tropaeolum majus* L. has both stomata and trichomes (Fahn, 1979, 1990), and our survey revealed both structures in floral nectaries of all taxa investigated in the Hylocereeae but only stomata in the Rhipsalideae (Table 2). We predict that these two prototypes of floral nectaries, one with both trichomes and stomata (Hylocereeae) and the other with stomata only (Rhipsalideae), will be constant in other taxa of these tribes and may provide two additional synapomorphic characters with prospective utility in the taxonomy and phylogeny of these two lineages. It is worth noting that the presence of stomata and trichomes is correlated with larger flowers of the Hylocereeae with larger surface area of secreting tissue, a characteristic of the chamber nectary. Conversely, trichomes are wanting in the smaller flowers of the Rhipsalideae, whose nectaries have smaller secreting surface area with stomata, and less nectar production.

Flower size and morphology in relation to nectar production and sugar concentration

Nectar volume in Hylocereeae and Rhipsalideae is directly correlated with flower size, i.e., larger flowers produce more nectar (Table 2; Fig. 4). Similar observations have been made in sizeable flowers of tropical woody species (Baker, 1978). Nonetheless, there is a general inverse relationship between flower size and the sugar concentration of the nectar in the flowers of epiphytic cacti, e.g., in the Rhipsalideae nectar concentration is more than two-fold higher despite the smaller nectar volume in relation to Hylocereeae flowers (Table 2; Fig. 4).

The disparity between flower size and nectar sugar concentration may be explained in terms of flower morphology, specifically the length and depth of the floral tube and surface area of nectarial tissue. In addition, nectar fluids accumulated inside the short floral tube of the Rhipsalideae readily evaporate, as opposed to the large surface area of nectary tissue associated with production of higher volume but more diluted nectar inside the long floral tubes of the Hylocereeae, which act as a structural mechanism to prevent nectar evaporation. Similar relationships exist in flowers with long corolla tubes with characteristically low nectar concentration and in flowers with short or lacking floral tube and with higher nectar concentration (Corbet, 1978; Bernardello, 2007). All things considered, these attributes contribute to the production of more but diluted nectar in the chamber nectaries of the Hylocereeae. Moreover, nectar is an expensive floral reward and its production entails a trade-off in terms of growth, reproductive success, and seed production (Zimmerman, 1988; Pyke, 1991), it involves high consumption levels of daily photosynthates (Southwick, 1984), high energetic costs associated with producing secretory tissues and large nectar volumes (Pyke, 1991; Pacini et al., 2003), and limits on nectar production are determined by energetic cost (Klinkhamer and Jong, 1993). It is possible that the relatively small number of large flowers with abundant nectar in members of the Hylocereeae is correlated with high energetic costs, whereas in most members of the Rhipsalideae less energy is invested in making more but smaller and less showy flowers producing lower nectar amounts.

The flowers of *Schlumbergera russelianum* and *S. truncata* have intermediate nectar sugar concentration (37.0%) compared to other members of the Rhipsalideae and the Hylocereeae and hence deserve a brief discussion. These two species fall below the typical high concentration pattern observed in the Rhipsalideae; therefore, nectar concentration is more similar to Hylocereeae. The dissimilarity in nectar volume and concentration with most members of the Rhipsalideae is significant and can also be explained considering the nectary type and flower size. The flowers of these two species have nectar furrow (holder subtype) nectary (Fig. 3D3–D5), which is hidden, with a floral tube of intermediate size between the Hylocereeae and Rhipsalideae (Tables 2 and 3; Fig. 4). This indicates that the lower nectar sugar concentration is correlated with the relatively longer floral tube and the undulated morphology of the nectary, which shelters the nectar inside the flower.

The phenotypic spectra of the Hylocereeae and Rhipsalideae differ in terms of flower shape, symmetry, color, size, rewards, time of anthesis, and scent, among other features (Table 2). Nearly all species examined are insect-pollinated and belong to the Rhipsalideae (Tables 1 and 2), a lineage with various floral shapes allowing the visitation of bees and flies of various sizes and taxonomic groups. The flowers of this tribe are generalistic, except those of *Schlumbergera*. Conversely, Hylocereeae flowers tend to be specialized and designed for a limited number of visitors because they exhibit more sophisticated architecture and specific modes of stamen arrangement and anther presentation in relation to the stigma lobes and the position of the nectary chamber. The ephemeral nature and diurnal and nocturnal anthesis of Hylocereeae flowers suggest adaptations to exploit visits by hummingbirds (and other birds), hawkmoths, and bats (Table 2).

Nectar, pollen and scent are key pollinator rewards in plants (Richards, 1986; Endress, 1994; Nassar et al., 1997; Fleming, 2002; Pimienta-Barrios and Del Castillo, 2002; Leins and Erbar, 2010; Cota-Sánchez et al., 2013; Farré-Armengol et al., 2013). In addition to floral phenotypes, floral rewards are also relevant in the Hylocereeae and Rhipsalideae. For instance, the abundant though diluted and less viscous nectar in the Hylocereeae implies a reward linked to pollination by birds and/or moths, whereas the thicker nectar with higher sugar concentration of the Rhipsalideae is

correlated with bee-pollinated flowers. Diluted and fluid nectar are features facilitating nectar uptake, but viscosity tends to make nectar more stable under different environmental conditions (Baker, 1975; Proctor et al., 1996). Also, the production of diluted nectar in hummingbird- and moth-pollinated Hylocereeae flowers probably deter, rather than attract, nectar-robbing insects, such as bees, which are less efficient pollinators of these large flowers. According to Bolten and Feinsinger (1978), diluted nectar discourages bee visits but encourages more foraging bird visits, while promoting outcrossing, increasing fidelity, and pollination efficiency.

Nectaries in the scales of Hylocereeae flowers

The term floral nectary (FN) is linked to the pollination process but not the term extrafloral nectary (EFN) (Bernardello, 2007). Despite the fact that these are two standard terms used in botanical studies, this classification is inconsistent because sometimes the EFNs are located in the flower. As indicated earlier, the scales of the outer surface of the flowers of *Hylocereus setaceus* (Fig. 1C7) and *Epiphyllum phyllanthus* have nectaries (Table 2). Similar structures were observed in *E. guatemalense* and *E. oxypetalum*. These nectar-secreting scales are not directly involved in pollination (see discussion in Vogel (1977) and Endress (1994)). Thus, we use the term “extranuptial” (Delpino, 1875) for nectaries located on the flower but not involved in pollination. This idea is in agreement with Paiva (2011) for petaline nectaries in *Swietenia macrophylla* King. We argue that the floral ENNs reported here for Hylocereeae species are not related to pollination due to the secretion time, i.e., they start to produce nectar before anthesis, during the development of the floral bud, and also because the sugar concentration is quite high (over 70%, see Table 2; Fig. 4) compared to floral nectar (16.6% to 30.3%). These traits suggest that this nectar is intended for a different group of visitors, ants for instance, which defend flowers from herbivores.

Epiphytic cacti exhibit relatively ample diversity of nectaries and nectar presentation. *Rhipsalis teres*, for instance, also has two types of nectaries with different concentrations of nectar sugar solution. One is the annular floral nectary, characteristic of the genus *Rhipsalis*, and the second type are the bracteolar nectaries found in the stem areoles and shoot meristem, which produce nectar with higher sugar concentration, and are purportedly related to ant-plant interaction (Almeida et al., 2012). Extrafloral nectaries in close association with ants also occur in stem areoles of various species of terrestrial cacti, e.g., *Opuntia acanthocarpa* Engelm. & Bigelow var. *major* (Engelm. & Bigelow) Benson (Pickett and Clark, 1979), *Ferocactus gracilis* Gates (Blom and Clark, 1980), and *F. acanthodes* (Lem.) Britton & Rose var. *lecontei* (Engelm.) Lindsay (Ruffner and Clark, 1986). These structures occur in phylogenetically distant lineages, quite likely evolved independently, and represent another convergent character in the cactus family (see also Marazzi et al., 2013, and Weber and Keeler, 2013).

Concluding remarks and future prospects

This study documents the structural variability and modes of secretion in floral nectaries in the Hylocereeae and Rhipsalideae and provides robust evidence of the systematic value of floral nectaries and nectar sugar concentration in the Cactaceae. We demonstrate that the Hylocereeae and Rhipsalideae differ in nectary type (morphology) and nectar concentration, two attributes that clearly delimit tribal and generic boundaries in these two lineages. Nectar sugar concentration is another significant taxonomic indicator separating the Hylocereeae and Rhipsalideae and establishing trends linked to nectar sugar concentration and amount of

nectar production in relation to flower size. These two tribes exhibit a wide pollinator spectrum, and species with large diurnal and nocturnal flowers of the Hylocereeae produce larger nectar volumes with lower sugar concentration, whereas the relatively small to medium-sized diurnal flowers of the Rhipsalideae produce less but more concentrated nectar.

While progress has been made in understanding the structure of floral nectaries in the Cactaceae, future anatomical studies involving other groups of the family will be significant to generate a broader picture of the architecture and taxonomic distribution of floral nectaries and associated structures in connection with floral diversity and evolution of breeding systems. This information is particularly important in new Cactaceae phylogenies combining molecular and morphological data to infer character evolution. Future research should include *in-situ* ecological and field studies, which are key components to understanding flower phenology, floral rewards, and the relative fitness and reproductive success of plants in relation to pollinator behavior and ecosystem services.

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