UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

Órgãos reprodutivos em Hylocereeae e Rhipsalideae (Cactaceae): morfologia floral e desenvolvimento estrutural do fruto e da semente

ODAIR JOSÉ GARCIA DE ALMEIDA

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Vegetal).

Maio – 2013

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

Órgãos reprodutivos em Hylocereeae e Rhipsalideae (Cactaceae): morfologia floral e desenvolvimento estrutural do fruto e da semente

ODAIR JOSÉ GARCIA DE ALMEIDA

Orientador: Profa. Dra. Adelita Aparecida Sartori Paoli
Co-orientador: Prof. Dr. J. Hugo Cota-Sánchez

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Vegetal).

Maio - 2013

581.4 Almeida, Odair José Garcia de
A447o Órgãos reprodutivos em Hylocereeae e Rhipsalideae (Cactaceae): morfologia floral e desenvolvimento estrutural do fruto e da semente / Odair José Garcia de Almeida. - Rio Claro, 2013
111 f. : il., figs., gráfs., tabs., fots.

Tese (doutorado) - Universidade Estadual Paulista, Instituto de Biociências de Rio Claro
Orientador: Adelita Aparecida Sartori Paoli
Coorientador: Jorge Hugo Cota-Sánchez

1. Anatomia vegetal. 2. Cactoideae. 3. Cactos epífitos. 4. Flor. I. Título.

Ficha Catalográfica elaborada pela STATI - Biblioteca da UNESP
Campus de Rio Claro/SP

Dedico

À minha querida Esposa Alessandra

por todo seu Amor e Companheirismo.

AGRADECIMENTOS

Nas entrelinhas de uma tese estão gravados anos da vida de uma pessoa... Momentos de imensa alegria, imensa tristeza, de superação, de companheirismo, solidão, crescimento pessoal... Enfim, só quem passa por isso é quem sabe!

Agradeço a Minha Esposa Alessandra, pois a nossa história e a história dessa tese se confundem em muitos momentos. Obrigado pelo seu Amor e Companheirismo Constantes. Te amo!

Agradeço a minha Mãe Margarida, bem como minhas irmãs Mari, Fátima e seu marido Antonio Carlos, a meu irmão Luiz Carlos e sua família, pelo apoio e incentivo!

Sou muito grato ao CNPq pela bolsa de doutorado (141861/2009-6)

Sou extremamente grato a Prof. Dra. Adelita A. S. Paoli por sua orientação e sobretudo pela confiança depositada em mim. Muito Obrigado!

Sou profundamente grato a UNESP e ao Programa de Pós-Graduação em Biolgia Vegetal por ter possibilitado e execução desse projeto tão importante em minha vida.

Agradeço aos professores do Departamento de Botânica: Julio Lombardi, Reinaldo Monteiro, Massanori Takaki, Gustavo Habermann, Vera Scatena, Alessandra Coan, Graça Sajo e Vitor Cardoso por seus ensinamentos.

Agradeço aos servidores do Departamento de Botânica que sempre foram muito prestativos: Célia Maria Hebling, Ari R. Pesce e Daniela O. Dinato, e aos servidores da Seção Técnica de Pós-Graduação: Rosemary Cardoso e Vanessa Moruzzi.

Aos amigos que estiveram presentes durante a tese em diferentes momentos, com material, sugestões e conselhos, que apesar da distância mantém viva a amizade. Obrigado a Fábio V. Diniz, Edson Simão, Adriana Nakamura, Rita Pietrobom, André Guaraldo, Henrique Tozzi, Paulo Roberto, Camila Kissmann, Leonardo Biral e Cleto K. Peres. Aos amigos do Laboratório de Anatomia Vegetal: Aline Oriani, Orlando Cavalari, Elaine Lopes, Shirley Martins, Mayra Eichemberg e Rita Andreota. A todos vocês muito obrigado!!!

Agradeço especialmente aos amigos-irmãos Henrique Tozzi e Paulo Roberto de Souza, juntamente com todos os integrantes da Rep. Nemeless, pelo seu companheirismo e por terem me recebido em sua casa, sempre que foi necessário. Galera muito obrigado! A Leonardo Biral pela sua ajuda com as coletas no Parque Ecológico da Pavuna e por ter me alojado em sua casa, quando estive em Botucatu. Muito obrigado!

Agradeço ao Prof. Dr. Luiz Antonio de Souza, por sua amizade, companheirismo e por ter cedido os laboratórios de Botânica do Departamento de Biologia de UEM sempre que foi preciso.

Agradeço a Univesidade Estadual de Maringá, que através do Prof. Souza disponibilizou o laboratório de histotécnica vegetal e microscopia sempre que foi necessário. Agradeço também aos técnicos: Nelson, Sílvia e Rose pelo auxílio prestado; e aos alunos da graduação e da pós-graduaçao: Rodrigo, Rafael, Luciane, Michelli e Daniel pela ajuda prestada.

Agradeço ao Governo do Canadá CBIE/DFAIT pelo auxílio financeiro recebido (ELAP Program) e pela oportunidade de participar da semana de estudos "Democracy – ELAP tour" em Ottawa.

Minha dívida de gratidão é muito grande com o Dr. Hugo Cota-Sánchez por ter viabilizado as viagens ao Canadá nos anos de 2010 e 2012. Por nos receber tão bem em seu laboratório e em sua casa, por seus ensinamentos e por sua amizade. Agradeço também a Dewey Litwiller por sua amizade, seus ensinamentos e por todos os momentos inesquecíveis que Eu e a Alessandra, Você e o Hugo passamos em Saskatoon.

Agradeço aos amigos do Laboratório de Sistemática Vegetal da Universidade de Saskatchewan (Hugo Cota-Sánchez's Plant Systematics Lab): ao Dr. Hyoek Jae Choi por sua ajuda durante nossa adaptação em 2010, por seus ensinamentos, ajuda no laboratório e por seu companheirismo. Ao mestrando Denver Falconer pelo sua ajuda em tudo o que lhe foi solicitado em 2012 (trabalho de campo, preparo de amostras, revisão de manuscritos) e pela amizade, companhia e por todos os cafés do Tim Hotons... Ao Doutorando Wook Rak Jung pelo companherismo e também por todos os cafés.

Agradeço aos técnicos do departamento de biologia da Universidade de Saskatchewan: Dr. Guosheng Liu e Mr. Marlynn Mierau por todo auxílio técnico com a microscopia de varredura, óptica e por todo aparato fotográfico. Agradeço ao doutorando Kunal Baxi pela ajuda prestada no laboratório do Dr. Carlos Carvalho.

Expresso minha gratidão aos demais professores do departamento de Biologia da Universidade de Saskatchewan, ao Dr. Arthur R. Davis por seus ensinamentos em aula, por ter cedido material para análise de néctar e por ter cedido o seu laboratório de morfologia vegetal para confeção dos lamínarios utilisados nessa tese. Ao Dr. Carlos E. Carvalho por seu companheirismo, por ter cedido seu laboratório para captura de imagens sempre que solicitado, e por todos os encontros agradáveis com sua família. Ao Dr. Cedric Gillott pela simpatia e atenção.

Agradeço ao Centro de Línguas da Universidade de Saskatchewan por possibilitado a excursão à Banff, sem dúvida foi uma viagem maravilhosa, onde muito foi aprendido!

Agradeço a Beverley Tomlin por ter recebido a mim, a Alessandra e a Lisa em sua casa sempre que solicitamos, por toda sua ajuda durante nosso período de adaptação em Saskatoon e, principalmente, por ter atuado como nossa Mãe Canadense (Canadian mom) durante nossa estadia em Saskatoon em 2010 e 2012 e também pelas viagens agradáveis.

Gostaria de agradecer de modo sintétido, mas com muita gratidão os amigos que deixamos no Canadá (Saskatoon, em Edmonton ou qualquer outra cidade), mas estarão para sempre em nossas memórias: Vitor e Fabiana, Thiago e Fernanda, Josinaldo, Jair e Ednéia, Felipe e Daniele, Márcia e Allan, Alessandro e Adriana, Fred e Carol, Francisco e Kerry. Graças à companhia de vocês nossa estadia em Saskatoon foi muito mais agradável.

"... aqueles que descobriram a sua vocação e a seguiram, sem se deixarem influenciar por opiniões alheias ou por situações de momento, são os que conseguiram se realizar e ser felizes, mesmo enfrentando adversidades."

Berta Lange de Morretes
(Revista Ciência Hoje - Dez 2002)

ÍNDICE

1. RESUMO

Foram realizadas análises macro e micromorfológicas com auxílio de microscopia óptica e eletrônica de varredura em órgãos reprodutivos de espécies epífitas de duas linhagens diferentes: Hylocereeae e Rhipsalideae, buscando ampliar o conhecimento morfo-anatômico e contribuir com a taxonomia e sistemática de Cactaceae, por meio de estudos comparativos; além de elucidar inconstâncias na tipologia do fruto. Foram realizados o estudo sobre a morfologia floral com ênfase nos nectários e o estudo ontogenético dos frutos e sementes em três espécies de cactos epífitos: *Hylocereus undatus* (Hylocereeae), *Lepismium warmingianum* e *Rhipsalis cereuscula* (Rhipsalideae). Os resultados mostram evidências úteis para a taxonomia do grupo, como a separação distinta em relação à estrutura floral e à concentração do néctar entre espécies de Hylocereeae e de Rhipsalideae. Com base na estrutura dos nectários é possível separar três de quatro gêneros reconhecidos para Rhipsalideae. A ontogenia dos frutos das espécies estudadas permitiu o reconhecimento do fruto *cactidium* para a família. As características estruturais e o desenvolvimento das sementes mostraram caracteres importantes para a taxonomia, destacando-se o tipo de óvulo, a morfologia, o tamanho e a forma da semente, bem como, a morfologia do embrião. Os resultados apresentados nesse estudo demonstram a importância do conhecimento morfoanatômico de órgãos reprodutivos para entendimento da classificação e evolução das espécies de Hylocereeae e Rhipsalideae.

Palavras-chave: anatomia, Cactoideae, cactos epífitos, flor, fruto, néctar, nectário, ontogenia, semente

2. ABSTRACT

Macro- and micro-morfological analyses were performed in two lineages of epiphytic species of Hylocereeae and Rhipsalideae, through optical and scanning microscopy, in order to amplify the anatomical knowledge and to contribute to the taxonomy and systematics of this group of plants; furthermore, to elucidate inconstancies concerning the fruit typology within Cactaceae. This study provides the first and most extensive family survey on flower and nectary morphology in the Cactaceae, in addition to an ontogenetic study of fruit and seed in three species of epiphytic cacti *Hylocereus undatus* (Hylocereeae), *Lepismium warmingianum* and *Rhipsalis cereuscula* (Rhipsalideae). The results provide evidences of the systematic utility of floral nectaries and nectar sugar concentration amongst the Hylocereeae and Rhipsalideae. It was found that in the Rhipsalideae tribe it is possible to separate three of the four recognized genera for the tribe based on the nectary structure. The fruit ontogeny of the studied species has allowed the recognition of the cactus fruit as cactidium for the family. The structural features of the seed as well as its development are of taxonomical importance with key features being the type of ovule and its morphology, the size and shape of the seed, and the morphology of the embryo. The results presented show that both morphological and anatomical knowledge of the reproductive organs are of interest in understanding the classification and evolution of species of Hylocereeae and Rhipsalideae.

Key-words: anatomy, Cactoideae, epiphytic cacti, flower, fruit, nectar, nectary, ontogeny, seed

3. INTRODUÇÃO GERAL

Cactaceae distribui-se por todo o continente americano, do sul e oeste do Canadá até o sul da Patagônia na Argentina e Chile (Anderson 2001; Cota-Sánchez 2002), com exceção da espécie *Rhipsalis baccifera* (J. S. Muell) Stearn dispersa para a África Tropical, Madagascar, Sri Lanka e sul da Índia (Barthlott & Hunt 1993; Cota-Sánchez & Bomfim-Patrício 2010). A família é composta por 124 gêneros e 1438 espécies (Hunt et al. 2006) que estão frequentemente relacionadas a ambientes áridos. Entretanto, cerca de 130 espécies (aproximadamente 10%) ocorrem em florestas neotropicais e tropicais como cactáceas epífitas (Barthlott & Hunt 1993; Wallace & Gibson 2002). Cactaceae insere-se em Caryophyllales *sensu* APG III (*Angiosperm Phylogeny Group* 2009), e atualmente está dividida em quatro subfamílias: Pereskioideae, Opuntioideae, Cactoideae e Maihuenioideae (Anderson 2001; Hunt et al. 2006).

Cactoideae é a maior subfamília, possui mais de 100 gêneros agrupados em nove tribos: Browningieae, Cacteae, Calymmantheae, Cereeae, Notocacteae, Pachycereeae, Trichocereeae, Hylocereeae e Rhipsalideae, nas quais, as duas últimas são compostas exclusivamente por espécies epífitas ou lifófitas (Anderson 2001). Hylocereeae e Rhipsalideae representam dois centros de diversidade para cactos epífitos: Hylocereeae é característica de florestas da América Central com poucas espécies ocorrentes na América do Sul, ao passo que Rhipsalideae ocorre na América do Sul, principalmente, na Mata Atlântica brasileira (Barthlott & Hunt 1993).

O estudo da biologia reprodutiva, bem como das estruturas de reprodução em Cactacea iniciou-se no século XX com os estudos de Beutler (1930), Buxbaum (1953, 1955), Engleman (1960) e Boke (1964, 1966, 1968), dentre outros. Mas foi nas últimas décadas que houve um significativo aumento em várias áreas de pesquisas, em parte, devido ao advento e à disponibilização de sofisticadas técnicas de microscopia e de estudos moleculares, inclusive

investigações sobre a estrutura de órgãos reprodutivos e suas interações com o ambiente. Aliado ao interesse em entender o desenvolvimento estrutural e os padrões dentro dos diferentes grupos, Cactaceae possui muitas espécies ameaçadas de extinção, e a necessidade da conservação tem atraído a atenção de muitos pesquisadores. Em 2008 havia 157 espécies incluídas na lista vermelha de espécies ameaçadas da União Internacional para Conservação da Natureza - IUCN (Rojas-Sandoval & Meléndez-Ackerman 2009), já em 2012 esse número aumentou para 189 segundo a IUCN (2012).

Entre os trabalhos envolvendo o estudo da biologia reprodutiva e da estrutura floral, encontram-se os de Scogin (1985), Silva & Sazima (1985), Nassar et al. (1997), Valiente-Banuet et al. (2007), Fuentes-Pérez et al. (2009), Rojas-Sandoval & Meléndez-Ackerman (2009), e Almeida et al. (2010, 2012). Referente a estudos sobre frutos e sementes destacam-se os trabalhos de Barthlott & Hunt (2000), no qual os autores analisaram a morfologia externa da semente em MEV de mais de 100 gêneros da subfamília Cactoideae; o de Rosa & Souza (2003) que descreveram o desenvolvimento do fruto e da semente de uma espécie do gênero basal *Pereskia* Mill., o de Arias & Terrazas (2004) que investigaram as variações na morfologia externa da semente em *Pachycereus* Britton & Rose e, o de Cota-Sánchez & Bomfim-Patrício (2010) que analisaram a morfologia externa das sementes em MEV, além de outras características para encontrar padrões de variações intraespecíficas em *Rhipsalis baccifera.*

Cactaceae apresenta interessantes particularidades em suas estruturas reprodutivas. As flores são altamente derivadas, apresentam ovário ínfero, unilocular, com quatro a mais de 20 carpelos, possui tubo floral ou hipanto de tamanhos variados, o perianto raramente é bisseriado, e o tamanho das flores varia de 6 mm (*Rhipsalis*) a 40 cm (*Hylocereus*). A natureza da flor como um ramo modificado é uma característica presente na maioria dos gêneros, devido à presença de bractéolas, cerdas e/ou espinhos nas aréolas que ocorrem no

pericarpelo e no hipanto (Barthlott & Hunt 1993). Pericarpelo, por sua vez, representa o tecido de origem caulinar que envolve os carpelos (Buxbaum 1953). O fruto é bastante complexo, pois várias peças florais participam da sua formação. A polpa pode originar-se do receptáculo, da própria parede do ovário, dos funículos e de tricomas. No exterior do receptáculo podem ser encontradas brácteas e aréolas, de natureza axial (Roth 1977; Barroso et al. 1999).

Na literatura, a classificação e nomenclatura do fruto de Cactaceae são relativamente divergentes entre os autores. A morfologia da semente, particularmente a configuração do hilo e da micrópila (região hilo-micropilar), e a micromorfologia da testa exibem alto grau de diversidade que é de importância taxonômica (Barthloth & Hunt 2000). A semente varia de formato orbicular-lenticular a formato de concha, apresenta testa de coloração preta a marrom-escura, rugosa ou lisa, de tamanho entre 0,4 -7,5 mm. Alguns gêneros apresentam hilo e micrópila separados, mas normalmente em Cactoideae eles são unidos em um complexo único, a região hilo-micropilar (RHM) (Barthlott & Hunt 1993).

Apesar de trabalhos como os de Barthlott & Hunt (1993), Arias & Terrazas (2004) e Barthloth & Hunt (2000) ressaltarem a importância da morfologia de estruturas reprodutivas tanto para a taxonomia como em estudos filogenéticos, a biologia reprodutiva em Cactaceae foi observada em menos de 10% dos táxons. Esta limitada quantidade de dados dificulta o entendimento dos mecanismos reprodutivos na família (Cota-Sánchez & Abreu 2007), visto que mesmo os padrões básicos da morfologia externa e interna dos órgãos reprodutivos são pouco conhecidos na família. Sendo assim, à luz de modernas filogenias moleculares para cactos epífitos de Rhipsalideae (Calvente et al 2011; Korotkova et al 2011) fica claro a necessidade da combinação de dados morfológicos com moleculares para a produção de classificações capazes de incorporar relações evolucionárias, que auxiliem o entendimento da evolução e diversificação desse grupo de plantas.

Diante do exposto, o objetivo dessa tese foi estudar a morfoanatomia dos órgãos reprodutivos de espécies de cactos epífitos, aspectos da biologia reprodutiva e relações taxonômicas do fruto e da semente deste grupo peculiar de plantas. No capítulo 1, o objetivo foi estudar a morfologia floral, dos nectários e concentração de açúcar no néctar de espécies epífitas de Hylocereeae e Rhipsalideae, as relações taxonômicas e aspectos da biologia reprodutiva; no capítulo 2 foi analisado o desenvolvimento estrutural do fruto de *Hylocereus undatus* (Hylocereeae), *Lepismium warmingianum* e *Rhipsalis cereuscula* (Rhipsalideae), a fim de entender a estrutura e as implicações na nomenclatura de fruto dentro de Cactaceae; e no capítulo 3 foiam investigado a estrutura e desenvolvimento seminal de *Hylocereus undatus* (Hylocereeae), *Lepismium warmingianum* e *Rhipsalis cereuscula* (Rhipsalideae) com inferências taxonômicas.

LITERATURA CITADA

Almeida, O. J. G., Paoli, A. A. S., Cota-Sánchez, J. H. 2012. A macro- and micromorphological survey of floral and extrafloral nectaries in the epiphytic cactus *Rhipsalis teres* (Cactoideae: Rhipsalideae). *Flora* 207: 119-125. doi: 10.1016/j.flora.2011.11.004.

Almeida, O. J. G., Paoli, A. A. S., Souza, L. A. 2010. Flower morpho-anatomy in *Epiphyllum phyllanthus* (Cactaceae). *Revista Mexicana de Biodiversidad* 81:65-80

Anderson E. F. 2001. *The cactus family*. Timber Press, Portland, OR

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of Linnean Society* 161: 105-121. doi: 10.1111/j.1095-8339.2009.00996.x.

Arias, S., Terrazas, T. 2004. Seed morphology and variation in the genus *Pachycereus* (Cactaceae). *Journal of Plant Research* 117: 277-89. doi: 10.1007/s10265-004-0156-4.

Barroso, G. M., Morin, M. P., Peixoto, A. L., Ichaso C. L. F. 1999. *Frutos e sementes. Morfologia aplicada à sistemática de dicotiledôneas*. Editora UFV, Viçosa.

Barthlott, W., Hunt, D. 2000. Seed-diversity in the Cactaceae. *Succulent Plant Research* 5:1-173

Barthlott, W., Hunt, D. R. 1993. Cactaceae. In: Kubitzki, K. R., Bittrich, J. G. (Ed.) *The Families and genera of vascular plants*, 3rd ed. Springer, Berlin, pp 161-197.

Beutler, R. 1930. Biologisch-chemische untersuchungen am nektar von immenblumen. *Zeitschrift für Vergleichende Physiologie* 12: 72-176.

Boke, N. H. 1964. The cactus gynoecium: a new interpretation. *American Journal of Botany* 51: 598-610.

Boke, N. H. 1966. Ontogeny and structure of the flower and fruit of *Pereskia aculeata. American Journal of Botany* 53: 534-542.

Boke, N. H. 1968. Structure and development of the flower and fruit of *Pereskia diaz-romeroana*. *American Journal of Botany* 55: 1254-1260.

Buxbaum, F. 1953. *Morphology of cacti, Section II. The flower*. Abbey Garden Press, Pasadena.

Buxbaum, F. 1955. *Morphology of cacti. Section III. Fruits and seeds*. Abbey Garden Press, Pasadena

Calvente, A., Zappi, D. C., Forest, F., Lohmann, L. G. 2011. Molecular phylogeny of tribe Rhipsalideae (Cactaceae) and taxonomic implications for *Schlumbergera* and *Hatiora*. *Molecular Phylogenetics and Evolution* 58:456-468. doi:10.1016/j.ympev.2011.01.001

Cota-Sánchez, H., Bomfim-Patrício, C. 2010. Seed morphlogy, polyploidy and the evolutionary history of the epiphitic cactus *Rhipsalis baccifera* (Cactaceae). *Polibotánica* 29:107-129

Cota-Sánchez, J. H., 2002. Taxonomy, distribution, rarity status and uses of Canadian cacti. *Haseltonia* 9: 17-25.

Cota-Sánchez, J. H., Abreu, D. D. 2007. Vivipary and offspring survival in the epiphytic cactus *Epiphyllum phyllanthus* (Cactaceae). *Journal of Experimental Botany* 58: 3865-3873. doi:10.1093/jxb/erm232

Engleman, E. M. 1960. Ovule and seed development in certain cacti. *American Journal of Botany* 47:460-467

Fuentes-Perez, M., Terrazas, T., Arias, S. 2009. Anatomía floral de cinco especies de *Opuntia* (Opuntioideae, Cactaceae) de México. *Polibotánica* 27: 87-100.

Hunt, D., Taylor, N., Charles, G. 2006. *The new cactus lexicon*. Dh books, London

IUCN [International Union for Conservation of Nature], 2012. *IUCN Red List of Threatened Species*. Version 2011.2. http://www.iucnredlist.org. Acessado em 16 de maio de 2012.

Korotkova, N., Borsch, T., Quandt, D., Taylor, N. P., Müller, K. F., Barthlott, W. 2011. What does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic Rhipsalideae (Cactaceae). *American Journal of Botany* 98:1549-1572. doi: 10.3732/ajb.1000502

Nassar, J. M., Ramírez, N., Linares, O. 1997. Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. *American Journal of Botany* 84: 918-927.

Rojas-Sandoval, J., Meléndez-Ackerman, E. 2009. Pollination biology of *Harrisia portoricensis* (Cactaceae), an endangered Caribbean species. *American Journal of Botany* 96: 2270-2278. doi:10.3732/ajb.0900026.

Rosa, S. M., Souza, L. A. 2003. Morfo-anatomia do fruto (hipanto, pericarpo e semente) em desenvolvimento de *Pereskia aculeata* Miller (Cactaceae). *Acta Scientiarum, Biological Sciences* 25:415-428.

Roth, I. 1977. Fruit of Angiosperms. In: K. Linsbauer (Ed.) *Encyclopedia of plant anatomy*. Gebrüder Borntraeger, Berlin, pp. 1-675

Scogin. R., 1985. Nectar constituents of the Cactaceae. Southwest. *Naturalist* 30: 77-82.

Silva, W.S., Sazima, M. 1995. Hawkmoth pollination in *Cereus peruvianus,* a columnar cactus from southeastern Brazil. *Flora* 190: 339-343.

Valiente-Banuet, A., Gally, R. S., Arizmendi, M. C., Casas, A. 2007. Pollination biology of the hemiepiphytic cactus *Hylocereus undatus* in the Tehuacán Valley, Mexico. *Journal of Arid Environments* 68: 1-8. doi:10.1016/j.jaridenv.2006.04.001.

Wallace, R. S., Gibson, C. 2002. Evolution and Systematics. In: Nobel, P.S., (Ed.) *Cacti: biology and uses.* University of California Press, Berkeley, pp 1-21.

CAPÍTULO 1

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

Odair J.G. Almeida [a, b], J. Hugo Cota-Sánchez [b, *], Adelita A.S. Paoli [a]

[a]*Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP, 13506-900, Brasil*

[b]*Department of Biology, University of Saskatchewan, Saskatoon, SK, S7N5E2, Canada*

Submetido ao periódico Perspectives in Plant Ecology, Evolution and Systematics em outubro/2012, devolvido com correções e sugestões em abril/2013

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 (Cactoideae). Our objectives were: 1) to conduct comparative morphological analyses of flower 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 subtype nectar holder), and annular nectary. All Hylocereeae species possess chamber nectary, in which the nectarial tissue has both trichomes and stomata as nectar-secreting structures. The Rhipsalideae is 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 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. 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 synapomorhic characters in recent phylogenies of these tribes. In conclusion, our data provide strong evidence of the systematic utility of floral nectaries and nectar sugar concentration in the Cactaceae, particularly at different taxonomic levels in the Hylocereeae and Rhipsalideae.

Key words: Annular nectary, Cactoideae, chamber nectary, floral phenology, flower morphology, furrow nectary, nectar volume, stomata, trichomes

INTRODUCTION

Among other structures, the Cactaceae is characterized by the presence of areoles, which usually develop spines (Taylor, 1997). Nonetheless, the areoles of numerous cacti, in particular epiphytic species, form long to short hairs or bristle-like structures, and in extreme cases are very minute or absent, such as in species of *Disocactus* Lindl., *Epiphyllum* Haw, *Lepismium* Pfeiff., *Hatiora* Britton & Rose, *Rhipsalis* Gaertn., and *Schlumbergera* Lem. (Barthlott and Taylor, 1995; Anderson, 2001).

With the exception of the widely distribution of *Rhipsalis baccifera* in the Paleotropics (Barthlott and Taylor, 1995), a species that radiated to central Africa, Madagascar, Sri Lanka and southern India via successive polyploidy events (Cota-Sánchez and Bomfim-Patrício, 2010), the family is native to the New World. It occurs from southern and central Canada (Cota-Sánchez, 2002) to southern Patagonia in Argentina and Chile (Taylor, 1997; Anderson, 2001). The vast majority of species are adapted to dry, xerophytic terrestrial environments, but ca. 10% of cactus diversity (about 130 species) includes epiphytic and semiepiphytic lineages inhabiting the tree canopies of the Neotropical forests and woodlands (Benzing, 1990; Wallace and Gibson, 2002). In fact, the Cactaceae is the

tenth most species-rich epiphytic angiosperm plant family in the Neotropics (Gentry and Dodson, 1987).

A long-standing interest in cactus taxonomy has existed since the Linnaean generation, but an appreciation of the floral morphology 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 interest in areas of conservation and reproductive biology, e.g., Scogin (1985), Silva and Sazima (1985), 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 climbed to 189 (IUCN, 2011), a significant increase of more than 20% of táxons 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 attracting a wide range of pollinators, act as a mechanism for cactus diversification by promoting genetic variability reinforced by outcrossing (Pimienta-Barrios and Del Castillo, 2002; Cota-Sánchez and Croutch, 2008). Despite the ample evidence dealing with the diversity of reproductive systems (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, mechanisms of pollination, 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 numerous plants 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).

The animal pollination, and pollen and nectar rewards are common attributes to all members of the Cactaceae. Indeed, nectar is one of the most important incentives for pollinating agents, and the occurrence, position, characteristics, and types of floral nectaries are a source of valuable morpho-anatomical comparative data to infer phylogenetic relationships (Proctor et al., 1996; Bernardello, 2007) and may explain aspects of pollination biology and reproductive mechanisms in plants (Richards, 1986). In addition, floral nectaries provide insight regarding evolutionary reproductive trends in plants (Bernardello, 2007). These secreting organs have different origins, e.g., perianth, receptacle, stamens, ovary wall, and base of style (Fahn, 1952, 1990), and may be present in all floral parts 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), despite the fact that the utility of nectary information in the reproductive and evolutionary biology of angiosperms is known in a reasonable number of plant families, e.g., Aizoaceae (Chesselet et al., 2002), Iridaceae (Rudall et al., 2003), Malvaceae (Vogel, 2000), Solanaceae (Bernardello, 1987), among others. 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 voucher herbarium specimens, as opposed to analyses using fresh flowers either *in-situ* in the field or living collections maintained in greenhouses. Although these studies offer certain challenges, the area of reproductive biology is an open and 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-living plants. In this study we focus on a survey of flower 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 (semi- or hemi-epiphytic species) mainly in Central America. This investigation involves 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 in relation to pollinators. 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. We provide substantial data as evidence of the systematic significance of these characters in the family, particularly the description and taxonomic distribution of nectary types and sugar nectar concentrations with concomitant phylogenetic implications at the tribal and generic levels.

The objectives of this study were twofold: 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 regarding the general floral and phenological features together with morphological descriptions, major types of

nectaries, their distinguishing features, and nectar sugar concentrations. Within this context, we compared our data with published records to address taxonomic and phylogenetic inferences in relation to the variability of nectary morphology and nectar concentration at the tribal, generic and specific levels of the Cactaceae.

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 samples of flowers were collected at the Parque do Ingá, Maringá-PR and at the Parque of 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 because *R. baccifera* includes the type subspecies and the subsp. *horrida*, for a total of 25 taxa investigated. 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) Briton & Rose, and *Weberocereus* Britton & Rose. For the Rhipsalideae, we surveyed the four genera recognized by Anderson (2001) in this tribe: *Hatiora* (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 base to the top (including pericarpel, tube and perianth). Considering the lack of a standard terminology for the description of floral morphology, including the nectary shape and structure, the descriptions presented here are based on Buxbaum's (1953) terminology and 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. Drawings of representative flowers were performed with a Pen Tablet (Wacom, Bamboo Capture) and the Adobe Photoshop CS3 program using digital photos as models. The images and plates were labeled and assembled using Adobe Photoshop CS3 and Corel Photo-Paint X3 version 13 software.

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 CO_2 (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 each nectary.

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 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). Due to the ephemeral nature of some flowers, we were unable to measure nectar concentration in some species (*Hylocereus undatus*, *Selenicereus anthonyanus*, and *Lepismium warmingianum*), 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 7.81 cm in the *Epiphyllum X Fern la Borde* hybrid, to 11.57 cm in *Disocactus ackermannii* (Fig. 1A1, A2), to over 32 cm in *Epiphyllum oxypetalum* (Fig. 1B2) (Table 2). Flower diameter varies from 1.3 cm in *E. phyllanthus* to 26.25 cm in *Hylocereus undatus*. The flowers may be 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, including hummingbirds, hawkmoths, and possibly bats (Table 2). The color spectrum ranges from red, reddish-orange to white-cream. The stamens are numerous and organized in several whorls. According to Buxbaum (1953) the innermost stamen whorls (primary or lower stamens) form first and the stamens in the outer (upper) whorls form last, in a centrifugal fashion. The primary stamens in Hylocereeae flowers are uniformly inserted at the same height of the floral tube, and the filaments 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* and *E. oxypetalum* (Fig. 1B4), two species in which the filaments of the primary stamens become free at different levels on the floral tube. The areoles of the pericarpel (stem tissue enclosing the characteristic inferior ovary) are located outside the flower and vary from naked, spiny or with long bristles or hairs in the members of this tribe. Pollen production, though not measured, is seemingly high and directly related to the numerous stamens and relatively large anthers characteristic of the Hylocereeae flowers.

Flower morphology and phenology in tribe Rhipsalideae

As a general rule, the flowers in members of the Rhipsalideae have naked pericarpel. Floral diversity in this tribe is broad (Figs. 2, 3), from very small (*Lepismium* spp. and *Rhipsalis* spp.) (Fig. 2B1, B2; C1, C2) to medium (*Hatiora gaertneri*) (Fig. 2A1, A2) to relatively large (*Schlumbergera* spp.) (Fig. 3D1, D2), usually with actinomorphic symmetry, either bowl-shaped (Fig. 2A1, A2) to bell-shaped (Fig. 2B1, B2; C1, C2 and 3A1, A2), to rotated (Fig. 3B1, B2; C1, C2), except in *Schlumbergera truncata* (Fig. 3D1, D2), a species with tube-like zygomorphic flowers (Table 2). The flower length among the species investigated ranges from 0.43 cm in *Rhipsalis baccifera* subsp. *baccifera* to 7.64 cm in *S.*

truncata (Table 2). Rhipsalideae flowers vary from bright red-purplish to white, have fewer stamens than those of the Hylocereeae, and produce significantly lower amounts of nectar (Table 2). Diurnal flowers with anthesis lasting from two (*Lepismium* and *Rhipsalis* species) to four (*S. truncata*) to nine days (*H. gaertneri*) and adapted for insect and bird pollination are characteristic in this tribe (Table 2). The amount of nectar and pollen produced varies among species and pollen amount is also correlated with flower size and number of stamens. That is, the larger the flower the more numerous the stamens bearing larger anthers with higher amounts of pollen production.

Types of nectaries in Cactaceae

In order to put in perspective earlier research of this area in the Cactaceae and prior to our description of nectaries, we consider necessary to provide, in this section, a short prologue of works dealing with nectary classification in the family and other angiosperms, mainly due to the lack of an all-inclusive classification of nectaries in Cactaceae. Foremost, Buxbaum's (1953) studies on floral characters in the cactus family have been influential in inquiries dealing with the reproductive biology of the family. In addition to implementing useful terminology for floral parts, his work described three basic nectary types in this family based on specific structural details, 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. However, Bernardello (2007) discourages the use of the term "disc" or "disk" because of the difficulty in defining such a structure and because it can be confused with other floral parts. Thus, we opted to 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 structures

described in earlier studies of this genus. The remainder of our work follows Buxbaum (1953) to maintain consistency with existing terminology in the cactus family. Descriptions and illustrations of the nectary types presented and discussed in this paper are found in Table 3 and Figure 4.

Nectary types and structure in the Hylocereeae

All the Hylocereeae species investigated possess the chamber nectary type (Table 2; Fig. 4). 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 (Figs. 1A4, 1E4; 4E, F) except the flowers of *Epiphyllum guatemalense*, *E. oxypetalum* (Fig. 1B4), and *Hylocereus setaceus* (Fig. 1C4; 4G), which have the diffuse chamber nectary subtype (see Table 3 for nectary description and taxonomic distribution within the tribe). Also, the flowers of all Hylocereeae examined share a characteristic whitish epidermal nectariferous region in the inner surface of the floral tube (hypanthium) (Fig. 1A4; B4; C4; D4; E4). The main releasing via for the nectar produced in the nectaries of all Hylocereeae species are 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 also Table 2 for the taxonomic distribution of secretory parts).

This study revealed additional secreting structures in the scales of the pericarpel and floral tube of mature flowers of *Epiphyllum phyllanthus* and *H. setaceu*s (Fig. 1C2, C7; Table 2), in which nectar was detected on the abaxial face of these scales. The secretion starts in 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), which is the surface where nectar is released. These nectaries will be referred to as extranuptial nectaries (ENN). The idea behind this preference is presented in the discussion.

Nectary types and structure in the Rhipsalideae

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

The nectary tissue in *Hatiora gaertneri* covers the short floral tube (Fig. 2A3, A4), forming, in part, the furrow nectary type. This structure is roughly bowl-shaped (Fig. 2A4-A6). The epidermis 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 but not trichomes, as in *H. gaertneri* (Fig. 2B3-B6; C3-C5).

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 annular (donut-like) shape (Fig. 3B5, B6; C3, C4; Table 2). The nectary epidermis is made of irregular-shaped cells, lacks trichomes, but has stomata scattered throughout the surface (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 from other *Rhipsalis* species because the donut-like structure is embedded in the somewhat longer floral tube making this circular structure look higher (Fig. 3A3, A4, A6). *Rhipsalis grandiflora* has, in turn, the largest annular nectary within the genus (Fig. 3B3-B6), whereas the smallest nectary 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 in relation to congeneric members of the tribe. 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). Unlike other Rhipsalideae members described previously, the top of the nectary in *Schlumbergera* resembles a vault-roof structure made of primary stamens fused at their base (Fig. 3D4-D7) and surrounding the style (Fig. 3D4, D6). The insertion of secondary stamens is on the wall of the floral tube (Fig. 3D4, D5) and stomata are conspicuous on the nectary tissue (Fig. 3D8, D9) but not on the vault-roof area.

Nectar volume and solute concentration in flowers of Hylocereeae and Rhipsalideae

The nectar collected in members of the Hylocereeae and Rhipsalideae varied among species, from very small volumes (< 0.6-1.0 µL) in flowers of *Lepismium* species and *Rhipsalis baccifera* and *R. neves-armondii,* to moderate quantities (4.0-7.0 to 8.0 µL) in *R. cereuscula* and *R. grandiflora,* and *Schlumbergera* spp., to rather larger amounts (160 µL) in the flowers of *Hylocereus setaceus* (Table 2). On average, nectar production in flowers of the Hylocereeae and Rhipsalideae was estimated at 50.0 µL and 3.20 µL, respectively. In addition, 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). The nectar is typically transparent and odorless (to human olfactory sense) in all species, but 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 16.64% to 30.32% in the Hylocereeae and from 34.25% to 76.50% in the Rhipsalideae. The lowest concentration was found in *Epiphyllum phyllanthus* (16.64%) and the highest in *Hatiora gaertneri* (76.50 %) (Fig. 5; Table 2). It is remarkable that the large

Hylocereeae flowers have the lowest nectar sugar concentration mean values, varying from 16.64% (*E. phyllanthus*) to 27.71% (*Hylocereus setaceus*) and 30.32% (*E. guatemalense*) (Fig. 5; Table 2). 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* (77.00%) and *H. setaceus* (73.40%), up to three-fold higher sugar concentration (Fig. 5; Table 2).

In the Rhipsalideae, the mean values of nectar sugar concentration range from 34.25% in *Schlumbergera russelianum* to 76.5% in *Hatiora gaertneri* (Table 2; Fig. 5). This represents a two-fold difference within the tribe, something not observed in the Hylocereeae. Interestingly, 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., 51.00% in *L. bolivianum*, 59.40% in *L. cruciforme,* 62.38% in *R. cereuscula,* 71.46% in *R. floccosa,* 70.52% in *R. grandiflora,* and 72.43% in *R. neves-armondii* (Fig. 5; Table 2). With the exception of the nectaries in *Schlumbergera,* the nectaries of small to medium-size flowers of the Rhipsalideae produce nectar with at least twice or more the sugar concentration produced in nectaries of the large Hylocereeae flowers (Table 2). The average nectar sugar concentration for the Rhipsalideae is 60.86% (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, 3). Floral nectary type is also a distinguishing feature in members of the Rhipsalideae, characterized by the furrow and annular types (Tables 2, 3). The nectaries are typically found at the base or lowermost to middle part of the floral tube, which is made up of receptacular tissue associated with stamen filaments and portions of the gynoecium. This placement of nectaries between the androecium and gynoecium in connection with filament bases is common in families of the Eudicot core (Bernardello, 2007), which concords with the phylogenetic position of the Cactaceae within this large clade.

Within the Rhipsalideae the genus *Rhipsalis* is set apart by the annular nectary type (Tables 2, 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 type, whereas the flowers of *Schlumbergera* spp. possess the holder subtype (Tables 2, 3). These findings indicate that the nature of the nectary in flowers of Hylocereeae and Rhipsalideae is a distinctive feature at the tribal level and that this character has also strong taxonomic implications at the generic level in the Rhipsalideae. We hypothesize that flowers of other species not included in this survey will match the general nectary type(s) reported now.

The nectary types discussed here have additional systematic implications in the Rhipsalideae. The usefulness of these data is clear when our data are extrapolated into the recent molecular phylogenies of the tribe, e.g., Calvente et al. (2011) and Korotkova et al.

(2011), but at the same time reinforces the necessity of studies encompassing larger taxonomic sampling of uninvestigated species. 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 found in our study supports this phylogenetic division of the Rhipsalideae because nectary types characterize, and can be used as morphological synapomorphies of the clades recovered in the aforecited phylogeny; that is, the three genera *Hatiora, Lepismium* and *Schlumbergera* have the furrow nectary, and the largest and more conflictive genus *Rhipsalis* is distinguished by the annular nectary. In addition, Calvente's et al. (2011) proposed taxonomic and nomenclatural changes transferring three species from *Hatiora* to *Schlumbergera*. However, the competing phylogeny, i.e., Korotkova's et al. (2011) does not support such alliance, and maintain 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*. It is worth noting that the distinctive embedded annular nectary in *Rhipsalis cereuscula* could be a feature endorsing the classification of subgenus *Erythrothipsalis* A. Berger, (*sensu* Calvente et al., 2011 and Korotkova 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.

Literature dealing with nectaries bearing both trichomes and stomata as secretory structures is scarce, but according to Bernardello (2007), stomatal secretion is the most common mechanism for nectar release in flowers. However, the nectar spur of *Tropaeolum majus* L. has both stomata and trichomes (Fahn, 1979, 1990) and our survey revealed floral nectaries with both nectar-secreting structures: stomata and trichomes. These two secreting elements are present in all taxa investigated in the Hylocereeae, but only stomata were

observed 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 type, but trichomes are absent in the smaller flowers of the Rhipsalideae, which nectaries have smaller secreting surface area with stomata, and correlated with lower nectar volume produced.

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

Flower size in relation to nectar production and nectar sugar concentration in the Hylocereeae and Rhipsalideae offer several valuable clues to infer taxonomic and evolutionary trends. Foremost, nectar volume in representative species of these tribes is directly correlated with flower size, i.e., larger flower produces more nectar (Fig. 5; Table 2). Similar observations have been made in sizeable flowers of tropical woody species (Baker, 1978). Still, there is a general inverse relationship between flower size and the sugar concentration of the nectar produced in the flowers of epiphytic cacti, e.g., in the Rhipsalideae the nectar concentration is more than two-fold higher despite the smaller nectar volume produced in relation to the Hylocereeae flowers (Table 2; Fig. 5).

The disparity between the size flower and the nectar sugar concentration may be explained in terms of flower morphology, specifically the length and depth of the floral tube and surface area of secreting nectarial tissue. In addition, nectar fluids accumulated inside the short floral tube of the Rhipsalideae readily evaporate, as opposed to the largest surface area of nectary tissue associated with production of higher volume but more diluted nectar inside

the long floral tubes of the Hylocereeae. Further, the elongated floral tubes act as a structural mechanism to prevent nectar evaporation. Similar relationships have been reported in flowers with long corolla tubes with characteristically low nectar concentration and in flowers with short or floral tube absent, which have 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, it is well known that 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), involves high consumption levels of daily photosynthates (Southwick, 1984), and its production embraces high energetic costs associated with producing secretory tissues and large nectar volumes (Pyke, 1991; Pacini et al., 2003). It is possible that the relatively small number of large flowers with abundant nectar in members of the Hylocereeae is correlated with a high energetic cost, 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 %) in relation 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 representatives. 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 of hidden nature, with a floral tube of intermediate size between the Hylocereeae and Rhipsalideae (Fig. 5; Table 2 and 3). This indicates that the lower nectar sugar concentration is correlated with the relatively longer

floral 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 are primarily restricted to the Rhipsalideae (Table 1), 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 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 of hummingbirds (and other birds), hawkmoths, and bats (Table 2).

Nectar, pollen and scent are key pollinator rewards in plants (Richards, 1986; Nassar et al., 1997; Fleming, 2002; Pimienta-Barrios and del Castillo, 2002; Cota-Sánchez and Croutch, 2008; Leins and Erbar, 2010). The same premise applies to the flowers examined here. 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 bird and/or moth, whereas the thicker and higher sugar nectar concentration of the Rhipsalideae is correlated with bee-pollinated flowers. In fact, abundant but diluted and rather runny nectar are features facilitating nectar uptake because viscosity tends to remain 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 increasing fidelity, pollination efficiency, and promoting outcrossing.

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 papers, the author pointed out that this classification creates a terminological inconsistency because sometimes the EFNs are located in the flower. As indicated earlier, the floral scales of *Hylocereus setaceus* (Fig. 1C7, C8) and *Epiphyllum phyllanthus* have nectaries (Table 2). Similar structures were observed in *E. guatemalense* and *E. oxypetalum,* but we were unable to measure nectar secreted from these two species. These nectar-secreting scales are not directly involved in pollination. Thus, in order to avoid calling "extrafloral" nectary a secretory structure actually present in the flower, we resort to Delpino's (1875) functional classification, utilizing the term floral extranuptial nectary (ENN) to designate those nectar secreting structures located in the flower but not involved with the pollination process. This idea is also in agreement with that proposed by Paiva (2011) for petaline nectaries in *Swietenia macrophylla* King. We argue that the floral ENNs reported here for the Hylocereeae species are not related to pollination due to the secretion time, i.e., they start to produce nectar much earlier than floral anthesis, during the development of the floral bud, and also because the sugar concentration is quite high (over 70%, see Table 2) compared to floral nectar (16.64% to 30.32%). These traits suggest that this nectar is intended for a different group of visitors, ants for instance, which tend to defend floriferous structures from herbivorous species and have nothing to do with pollination.

Essentially, we claim that epiphytic cacti exhibit relatively ample diversity of nectaries and nectar presentation. *Rhipsalis teres*, for instance, also has two types of nectaries with different nectar sugar concentration solution. One is the annular floral nectary, a characteristic of the genus *Rhipsalis*, and the second type is the bracteolar nectaries found in the stem areoles and shoot meristem, which produce nectar with higher sugar concentration, act as EFNs, and are purportedly related to ant-plant interaction (Almeida et al., 2012). Similarly, the floral ENN in the flowers and floral buds of *Epiphyllum* spp. and *H. setaceous* suggest an insect-plant interaction. Extra floral nectaries in close relationship with ants have also been documented in stem areoles of various species of distantly related 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). Because these structures occur in phylogenetically distant lineages and quite likely have evolved independently, they represent another convergent character in the cactus family.

Concluding remarks and future prospects

This study documents the structural variability and modes of secretion in floral nectaries in the Hylocereeae and Rhipsalideae. Our data provide strong evidence of the systematic utility of floral nectaries and nectar sugar concentration in the cactus family. We show that the Hylocereeae and Rhipsalideae differ in several traits, most importantly, nectary type (morphology) and nectar concentration, two attributes with taxonomic implications that easily 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. The members of these two tribes exhibit a wide pollinator spectrum

and species with large diurnal and nocturnal flowers of the Hylocereeae are characterized by larger nectar volumes lower in 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 various lineages of the Cactaceae, this field remains open to numerous endeavors. Future anatomical studies involving other groups of the cactus family are needed 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 becoming particularly important for new Cactaceae phylogenies in revealing character evolution. We believe that future research should include *in-situ* ecological and field studies, which is are key component to understanding flower phenology, floral rewards, and the relative fitness and reproductive success of plants in relation to pollinator behavior and ecosystem services.

ACKNOWLEDGEMENTS

We thank A.P. Lonardoni, H.J. Choi, D. Litwiller, D. Falconer and the Cota-Sánchez' Plant Systematics Lab Personnel for comments on early drafts of the manuscript; and G. Liu and M. Mierau for technical assistance. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil, Ph.D. scholarship (Proc. No. 141861/2009-6) to OJGA, grants (Proc. No. 300495/2010-2 and No. 474068/2009-9) to AASP; the Emerging Leaders in the Americas Program (ELAP-Canada) for providing an exchange grant to OJGA; the National Geographic Society (Grant No. 7382-02), and the University of Saskatchewan Tri-Council Bridge (Grant No. 411051) to JHCS.

REFERENCES

Almeida, O.J.G., Paoli, A.A.S., Souza, L.A., 2010. Flower morpho-anatomy in *Epiphyllum phyllanthus* (Cactaceae). Rev. Mex. Biodiver. 81, 65-80.

Almeida, O.J.G., Paoli, A.A.S., Cota-Sánchez, J.H., 2012. A macro- and micromorphological survey of floral and extrafloral nectaries in the epiphytic cactus *Rhipsalis teres* (Cactoideae: Rhipsalideae). Flora 207, 119-125. doi: 10.1016/j.flora.2011.11.004.

Anderson, E.F., 2001. The cactus family. Timber Press; Cambridge.

Armbruster, W.S., 1996. Evolution of floral morphology and function: an integrated approach to adaptation, constraint, and compromise in *Dalechampia* (Euphorbiaceae). In: Lloyd, D., Barrett, S. (Eds.) Floral biology. Chapman and Hall; New York, pp. 241-272.

Baker, H.G., 1975. Sugar concentrations in nectars from hummingbird flowers. Biotropica 7, 37-41.

Baker, H.G., 1978. Chemical aspects of the pollination biology of woody plants in the tropics. In: Nitecki, M.H. (Ed.) Biochemical aspects of evolutionary biology. University of Chicago Press; Chicago, pp. 131-171.

Barthlott, W., Hunt, D.R., 1993. Cactaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.) The families and genera of vascular plants, Vol. 2. Springer; Berlin, pp. 161-197.

Barthlott, W., Taylor, N.P., 1995. Notes towards a monograph of Rhipsalideae (Cactaceae). Bradleya 13, 43-79.

Benzing, D.H., 1990. Vascular epiphytes: general biology and related biota. Cambridge University Press; New York.

Bernardello, G., 2007. A systematic survey of floral nectaries. In: Nicolson, S.W., Nepi, M., Pacini, E. (Eds.) Nectaries and nectar. Springer; Dordrech, pp. 19-128.

Bernardello, L.M., 1987. Comparative floral morphology in *Lycieae* (Solanaceae). Brittonia 39, 112-129.

Beutler, R., 1930. Biologisch-chemische untersuchungen am nektar von immenblumen. Z. Vergl. Physiol. 12, 72-176.

Blom, P.E., Clark, W.H., 1980. Observations of ants (Hymenoptera: Fomicidae) visiting extrafloral nectaries of the barrel cactus, *Ferocactus gracilis* Gates (Cactaceae), in Baja California, Mexico. Southwest. Naturalist 25, 181-196.

Boke, N,H., 1963. Anatomy and development of the flower and fruit of *Pereskia pititache.* Am. J. Bot. 50, 843-858.

Boke, N.H., 1964. The cactus gynoecium: a new interpretation. Am. J. Bot. 51, 598-610.

Boke, N.H., 1966. Ontogeny and structure of the flower and fruit of *Pereskia aculeata.* Am. J. Bot. 53, 534-542.

Boke, N.H., 1968. Structure and development of the flower and fruit of *Pereskia diaz-romeroana*. Am. J. Bot. 55, 1254-1260.

Bolten, A.B., Feinsinger. P., 1978. Why do hummingbird flowers secrete dilute nectar? Biotropica 10, 307-309.

Brown, W., 1938. The bearing of nectaries on the phylogeny of flowering plants. P. Am. Philos. Soc. 79, 549-595.

Buxbaum. F., 1953. Morphology of cacti, Section II. The flower. Abbey Garden Press; Pasadena.

Calvente, A., Zappi, D.C., Forest, F., Lohmann, L.G., 2011. Molecular phylogeny of tribe Rhipsalideae (Cactaceae) and taxonomic implications for *Schlumbergera* and *Hatiora.* Mol. Phylogenet. Evol. 58, 456-468. doi: 10.1016/j.ympev.2011.01.001.

Chesselet, P., Smith, G.F., Wyk, A.E., 2002. A new tribal classification of Mesembryanthemaceae: evidence from floral nectaries. Taxon 51, 295-308.

Corbet, S.A., 1978. Bee visits and the nectar of *Echium vulgare* and *Sinapis alba.* Ecol. Entomol. 3, 25-38.

Cota-Sánchez, J.H., 2002. Taxonomy, distribution, rarity status and uses of Canadian cacti. Haseltonia 9, 17-25.

Cota-Sánchez, J.H., Abreu, D.D., 2007. Vivipary and offspring survival in the epiphytic cactus *Epiphyllum phyllanthus* (Cactaceae). J. Exp. Bot. 58, 3865-3873. doi:10.1093/jxb/erm232.

Cota-Sánchez, J.H., Bomfim-Patrício, M., 2010. Seed morphology, polyploidy and the evolutionary history of the epiphytic cactus *Rhipsalis baccifera* (Cactaceae). Polibotánica. 29, 107-129.

Cota-Sánchez, J.H., Croutch, D.S., 2008. Notes on the floral biology of *Praecereus euchlorus* subsp. *euchlorus* (Cactaceae). Schumannia 5, 102-103.

Cronquist, A., 1981. An integrative system of classification of flowering plants. Columbia University Press; New York.

Delpino, F., 1875. Rapporte tra insetti e tra nettarii estranuziali in alcune piante. Bull. Soc. Entomol. Ital. 7, 69-90.

Fahn, A., 1952. On the structure of floral nectaries. Bot. Gaz. 113, 464-470.

Fahn, A., 1979. Secretory tissues in plants. Academic Press; London.

Fahn, A., 1990. Plant anatomy. Pergamon Press; Oxford.

Fleming, T.H., 2002. Pollination biology of four species of Sonoran Desert columnar cacti. In: Fleming, T.H., Valiente-Banuet, A. (Eds.) Columnar cacti and their mutualists: evolution, ecology, and conservation. University of Arizona Press; Tucson, pp. 207-224.

Fuentes-Perez, M., Terrazas, T., Arias, S., 2009. Anatomía floral de cinco especies de *Opuntia* (Opuntioideae, Cactaceae) de México. Polibotánica 27, 87-100.

Gentry, A.H., Dodson, C.H., 1987. Contribution of nontrees to species richness of a tropical rain forest. Biotropica 19, 149-156.

Hunt, D., 1999. CITES. Cactaceae Checklist, 2nd edn. KEW: Royal Botanic Gardens; London.

IUCN [International Union for Conservation of Nature], 2011. IUCN Red List of Threatened Species. Version 2011.2. http://www.iucnredlist.org. Date accessed: 16 May 2012.

Kaiser, R., Tollsten, L., 1995. An introduction to the scent of cacti. Flavour Frag. J. 10, 153-164. doi:10.1002/ffj.2730100307.

Korotkova, N., Borsch, T., Quandt, D., Taylor, N.P., Müller, K.F., Barthlott, W., 2011. What does it take to resolve relationships and to identify species with molecular markers? An example from epiphythic Rhipsalideae (Cactaceae). Am. J. Bot. 98, 1549-1572. doi:10.3732/ajb.1000502

Leins, P., Erbar. C., 2010. Flower and fruit – Morphology, ontogeny, phylogeny, function and ecology. Schweizerbart Science Pub; Stuttgart.

Nassar, J.M., Ramírez, N., Linares, O., 1997. Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. Am. J. Bot. 84, 918-927.

Orozco-Arroyo, G., Vázquez-Santana, S., Camacho, A., Dubrovsky, J.G., Cruz-García, F., 2012. Inception of maleness: auxin contribution to flower masculinization in the dioecious cactus *Opuntia stenopetla*. Planta, in press. doi 10.1007/s00425-012-1602-5.

Paiva, E.A.S., 2011. Petaline nectaries in *Swietenia macrophylla* (Meliaceae): distribution and structural aspects. Flora 206, 484-490. doi: 10.1016/j.flora.2010.09.009.

Pacini, E., Nepi, M., Vesprini, J.L., 2003. Nectar biodiversity: a short review. Plant Syst. Evol. 238, 7-21. doi: 10.1007/s00606-002-0277-y.

Pickett, C.H., Clark, W.D., 1979. The function of extrafloral nectaries in *Opuntia acanthocarpa* (Cactaceae). Am. J. Bot. 66, 618-625.

Pimienta-Barrios, E., Del Castillo, R., 2002. Reproductive biology. In: Nobel, P.S. (Ed.) Cacti: biology and uses. University of California Press; Berkeley, pp. 75-79.

Proctor, M., Yeo, P., Lack, A., 1996. The natural history of pollination. Harper Collins; London.

Pyke, G.H., 1991. What does it cost a plant to produce floral nectar? Nature 350, 58-59. doi:10.1038/350058a0.

Reyes-Agüero, J.A., Aguirre, J.R., Valiente-Banuet, A., 2006. Reproductive biology of *Opuntia*: a review. J. Arid Environ. 64, 549-585. doi:10.1016/j.jaridenv.2005.06.018

Richards, A.J., 1986. Plant breeding systems. George Allen and Unwin; London.

Rojas-Sandoval, J., Meléndez-Ackerman, E., 2009. Pollination biology of *Harrisia portoricensis* (Cactaceae), an endangered Caribbean species. Am. J. Bot. 96, 2270-2278. doi:10.3732/ajb.0900026.

Rudall, P., Manning, J.C., Goldblatt, P., 2003. Evolution of floral nectaries in *Iridaceae*. Ann. Missouri Bot. Gard. 90, 613-631.

Ruffner, G.A., Clark, W.D., 1986. Extrafloral nectar of *Ferocactus acanthodes* (Cactaceae): composition and its importance to ants. Am. J. Bot. 73, 185-189.

Scogin. R., 1985. Nectar constituents of the Cactaceae. Southwest. Naturalist 30, 77-82.

Silva, W.S., Sazima, M., 1995. Hawkmoth pollination in *Cereus peruvianus*, a columnar cactus from southeastern Brazil. Flora 190, 339-343.

Southwick, E.E., 1984. Photosynthate allocation to floral nectar: a neglected energy investment. Ecology 65, 1775-1779.

Taylor, N.P., 1997. Cactaceae. In: Oldfield, S. (Comp.) Cactus and succulent plants – status survey and conservation action plan. IUCN/SSC Cactus and succulent specialist group – IUCN; Gland, Switzerland, and Cambridge, UK. 17-20.

Thiers, B., [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/. Date accessed: 15 June 2012.

Toivonen, E., Mutikainen, P., 2012. Differential costs of reproduction in females and hermaphrodites in a gynodioecious plant. Ann. Bot. 109, 1159-1164. doi:10.1093/aob/mcs037.

Tropicos.org. Missouri Botanical Garden, 23 June 2012. http://www.tropicos.org. Date accessed: 22 June 2012.

Valiente-Banuet, A., Gally, R.S., Arizmendi, M.C., Casas, A., 2007. Pollination biology of the hemiepiphytic cactus *Hylocereus undatus* in the Tehuacán Valley, Mexico. J. Arid Environ. 68, 1-8. doi:10.1016/j.jaridenv.2006.04.001.

Vogel, S., 2000. The floral nectaries of *Malvaceae* sensu lato. A conspectus. Kurtziana 28, 155-171.

Wallace, R.S., Gibson, C., 2002. Evolution and Systematics. In: Nobel, P.S., (Ed.) Cacti: biology and uses. University of California Press; Berkeley, pp.1-21.

Zimmerman, M., 1988. Nectar production, flowering phenology, and strategies for pollination. In: Doust, J.L., Doust, L.L. (Eds.) Plant reproductive ecology: patterns and strategies. Oxford University Press; New York, pp. 157-176.

TABLE 1. List of plant material used in this study. 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, continually updated). HRCB = Herbário Rioclarense, Universidade Estadual Paulista, Río 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.
Hylocereeae Buxb.				
Disocactus ackermannii (Haw.) Ralf Bauer	Holo-epiphyte	Mexico	MBG 11-1961	SASK 180688
Epiphyllum guatemalense Britton & Rose	Holo-epiphyte	Mexico, Guatemala and Honduras	MBG 1935-1958	SASK 180689
E. oxypetalum (DC.) Haw.	Holo-epiphyte	Mexico, Guatemala, Honduras, Nicaragua, El Salvador, and Costa Rica	MBG 141-1972B	SASK 180690
E. phyllanthus (L.) Haw.	Holo-epiphyte	From southern Mexico to S. America	MBG 3253-1987B	HUEM 12673, HRCB 48936 and 48937
Epiphyllum X *Fern la Borde*	Holo-epiphyte	Commercial hybrid	MBG 2-1998A	SASK 180691
Hylocereus undatus (Haw.) Britton & Rose	Hemi-epiphyte	C. America – widely cultivated	"Parque do Ingá"	HUEM 21152
H. setaceus (Salm-Dyck) Ralf Bauer	Holo-epiphyte	Argentina, Brazil, Bolivia, Paraguay		SASK 180692
Selenicereus anthonyanus (Alexander) D.R. Hunt	Hemi-epiphyte	Mexico	"Parque do Ingá"	HUEM 22576
Weberocereus panamensis Britton & Rose	Holo-epiphyte	Panama	MBG 710-1956	SASK 180693
Rhipsalideae DC.				
Hatiora gaertneri (Regel) Barthlott	Holo-epiphyte	Brazil	UofS 15-2004	SASK 180676
Lepismium bolivianum (Britton) Barthlott	Holo-epiphyte	Bolivia	MBG 3277-1987 and MBG 3277-1977	SASK 180677
L. cruciforme (Vell.) Miq.	Holo-epiphyte	Argentina, Brazil and Paraguay	MBG 1872-1992	HRCB 54227
L. warmingianum (K. Schum.) Barthlott	Holo-epiphyte	Argentina, Brazil and Paraguay	"Parque do Ingá"	HUEM 18986 and 18987
Rhipsalis baccifera (J.S. Muell.) Stearn subsp.	Holo-epiphyte	Caribbean, eastern Mexico, Florida, C.	MBG 2610-1992B	SASK 180678

baccifera	or lithophyte	America, and northern S. America		
R. baccifera subsp. *horrida* (Baker) Barthlott	Holo-epiphyte, or lithophyte	Madagascar	MBG 3357-1987*C	SASK 180679
R. cereuscula Haw.	Holo-epiphyte	Argentina, Brazil, Bolivia, Paraguay, and Unruguay	"Parque de Lavras"	HRCB 54226 HUEM 18985
R. floccosa Salm-Dyck ex. Pfeiff.	Holo-epiphyte	Argentina, Bolivia and Peru	MBG 1346-60	SASK 180680
R. grandiflora Haw.	Holo-epiphyte	Brazil	MBG 1321-1960B	SASK 180681
R. mesembryanthemoides Haw.	Holo-epiphyte	Brazil	MBG 7313-1939D	SASK 180682
R. micrantha (Kunth) DC.	Holo-epiphyte	Costa Rica, western Venezuela, Ecuador, and northern Peru	MBG 1349-1960	SASK 180683
R. neves-armondii K. Schum. f. *megalantha* (Loefgr.) Barthlott & N.P. Taylor	Holo-epiphyte or lithophite	Brazil	MBG 7312-1939	SASK 180684
R. puniceo-discus G. Lindb.	Holo-epiphyte	Brazil	MGB 7314-1939	SASK 180685
R. teres (Vell.) Steud.	Holo-epiphyte or lithophite	Brazil	MGB 993-1995	SASK 180582
Schlumbergera russeliana (Hook.) Britton & Rose	Holo-epiphyte	Brazil	UofS 44-2006	SASK 180686
S. truncata (Haw.) Moran	Holo-epiphyte or lithophite	Brazil	MBG 1677-1989B	SASK 180687

TABLE 2. Floral attributes, nectary type, secreting structures, and nectar sugar concentration 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 (µL/flower)	%NCW (mean ± s.d.)	Putative Pollinator
Tribe Hylocereeae										
Disocactus ackermannii	4	Salverform Radial Red Scentless	11.57	7.03	4	Open chamber	Trichomes and stomata	>90	25.44 ± 3.29	Hummingbird
Epiphyllum guatemalense	2	Salverform Radial White-yellowish Sweet fragrance	21.12	*	1 night	Diffuse chamber	Trichomes and stomata	*	30.32 ± 2.13	Hawkmoth
E. oxypetalum	2	Salverform Zygomorphic, curved Strongly sweet Fragrant	32.65	21.51	1 night	Diffuse chamber	Trichomes and stomata	Ca. 20	22.36 ± 2.37	Hawkmoth
E. phyllanthus	2	Salverform Long tubular Radial Sweet fragrance	21.98	1.30	1 night	Open chamber	Trichomes and stomata	Ca. 5	16.64 ± 1.57	Hawkmoth
E. phyllanthus (ENN)	4^{Δ}	n/a	n/a	n/a	n/a	n/a	Unknown	< 1	77.00 ± 0.82	Ant
Epiphyllum X Fern la Borde	2	Salverform Slightly bilateral Purple-red Scentless	7.81	3.97	2	Open chamber	Trichomes and stomata	Ca. 25	22.67 ± 0.58	Bird/butterfly
Hylocereus setaceus	3	Salverform Radial White-cream Strongly sweet Fragrant	27.32	14.56	1 night	Diffuse chamber	Trichomes and stomata	Ca. 160	27.71 ± 0.76	Bat and Hawkmoth
H. setaceus (ENN)	3^{+}	n/a	n/a	n/a	n/a	n/a	Stomata (abaxial surface)	< 1	73.40 ± 1.14	Ant
H. undatus	3	Salverform Radial	28.91	26.25	1 night	Open chamber	Trichomes and	*	*	Bat and Hawkmoth

		White-cream Strong sweet Fragrant					stomata			
Selenicereus anthonyanus	6	Salverform Radial Pinkish-cream Strong sweet Fragrant	13.62	9.31	1 night	Open chamber	Trichomes and stomata	*	*	Hawkmoth
Weberocereus panamensis	2	Funnelform, Radial, Sweet light Fragrant	20.3	13.8	1 night	Open chamber	Trichomes and stomata	Ca. 20	18.22 ± 1.00	Hawkmoth
Tribe Rhipsalideae										
Hatiora gaertneri	5	Bowl-shape Radial Scarlet-red Scentless	3.50	4.67	9	Furrow	Stomata	Ca. 1.0	76.50 ± 2.07	Bee
Lepismium bolivianum	6	Bell-shape Radial Red-yellowish Scentless	1.51	1.74	2	Furrow	unknown	< 1.0	51.00 ± 7.33	Bee
L. cruciforme	20	Bell-shape Radial Cream-pinkish Scentless	0.92	1.07	2	Furrow	Stomata	0.6-0.8	59.40 ± 6.68	Bee
L. warmingianum	5	Bell-shape Radial White Scentless	1.76	1.39	2	Furrow	Stomata	*	*	Bee
Rhipsalis baccifera subsp. *baccifera*	6	Rotat, Radial Green-whitish Scentless	0.43	0.44	2	Annular	unknown	Ca. 1.0	50.00 ± 6.32	Bee
R. baccifera subsp. *horrida*	4	Rotate Radial Green-whitish Scentless	0.69	0.72	2	Annular	unknown	Ca 1.0	55.33 ± 3.05	Bee
R. cereuscula	9	Bell-shaped Radial White Scentless	1.36	1.41	2	Embedded Ring	Stomata	6.0-7.0	62.38 ± 4.47	Bee
R. floccosa	9	Rotate Radial Cream Scentless	0.71	1.73	2	Annular	Stomata	1.0-2.5	71.46 ± 2.72	Bee

R. grandiflora	23	Rotate Radial Cream-yellowish Scentless	1.11	1.63	2	Annular	Stomata	4.2-7.0	70.52 ± 4.45	Bee
R. mesembryanthemoides	4	Rotate Radial White Scentless	1.2	1.4	2	Annular	unknown	1.0-2.0	60.75 ± 5.24	Bee
R. micrantha f. *micrantha*	9	Rotate Radial White Scentless	0.76	0.91	2	Annular	unknown	Ca. 10.0	69.22 ± 1.39	Bee
R. neves-armondii f. megalantha	8	Rotate Radial White Weakly sweet Fragrant	2.53	1.34	3	Annular	Stomata	Ca. 0.6	72.43 ± 1.13	Bee
R. puniceo-discus	4	Rotate Radial Cream-pinkish Scentless	1.02	1.61	2	Annular	unknown	Ca. 1.0	72.00 ± 2.00	Bee
R. teres‡	8	Rotate Radial White Scentless	0.88	1.06	3	Annular	Stomata	Ca 1.0	70.63 ± 2.20	Bee
Schlumbergera russeliana	6	Tube-like Radial Pink Scentless	6.49	29.86	3	Furrow - Holder subtype	unknown	5.0-7.0	34.25 ± 2.43	Hummingbird
S. truncata	3	Tube-like Zygomorphic Pink-white Scentless	7.64	5.81	4	Furrow - Holder subtype	Stomata	8.0	37.0 ± 1.55	Hummingbird

*Measurements not taken. $^{\Delta}$Extranuptial nectar sampled from four different extranuptial nectaries (ENN) in two flowers in which the floral nectar was measured. $^{+}$Extranuptial nectar sampled from three different ENN in one flower for which the floral nectar was measured, in *H. setaceus*. ‡*Rhipsalis teres* information from Almeida et al. (2012).

TABLE 3. Description of nectary types and their taxonomic occurrence in members of tribes Hylocereeae and Rhipsalideae. Our descriptions follow Buxbaum (1953).

Tribe	Genus and/or Species	Nectary type	Nectary description	Picture/Drawing
Hylocereeae	*Disocactus ackermannii; Epiphyllum phyllanthus; Epiphyllum X Fern la Borde; Hylocereus undatus; Selenicereus; Weberocereus*	Open chamber	Nectary type with nectarial tissue surface extended beyond the floral tube, but the primary stamens are inserted at the same height of the floral tube wall	(Fig. 1A4, 1E4) / (Fig. 4 E-F)
	Epiphyllum guatemalensis; E. oxypetalum; Hylocereus setaceus	Diffuse chamber	Nectary type with nectarial surface extended beyond the floral tube, but the primary stamens are inserted at different heights of the floral tube.	(Fig. 1B4, 1D4) / (Fig. 4 G)
Rhipsalideae	*Hatiora gaertineri*; *Lepismium*	Furrow	A bowl or bell shape nectary in which the bases of the stamen filaments create undulations (furrows) in the nectariferous tissue	(Fig. 2A3-A6, B3, B4, C3, C4) / (Fig. 4 B-C)
	Rhipsalis	Annular	A 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) / Fig. 4 A
	Schlumbergera	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. 4 D

FIGURE 1. Floral morphology (photos) and nectary micromorphology (SEM) in selected Hylocereeae representatives. (A1-A8) *Disocactus ackermanii.* (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 face of scale. (D1-D7) *H. 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).

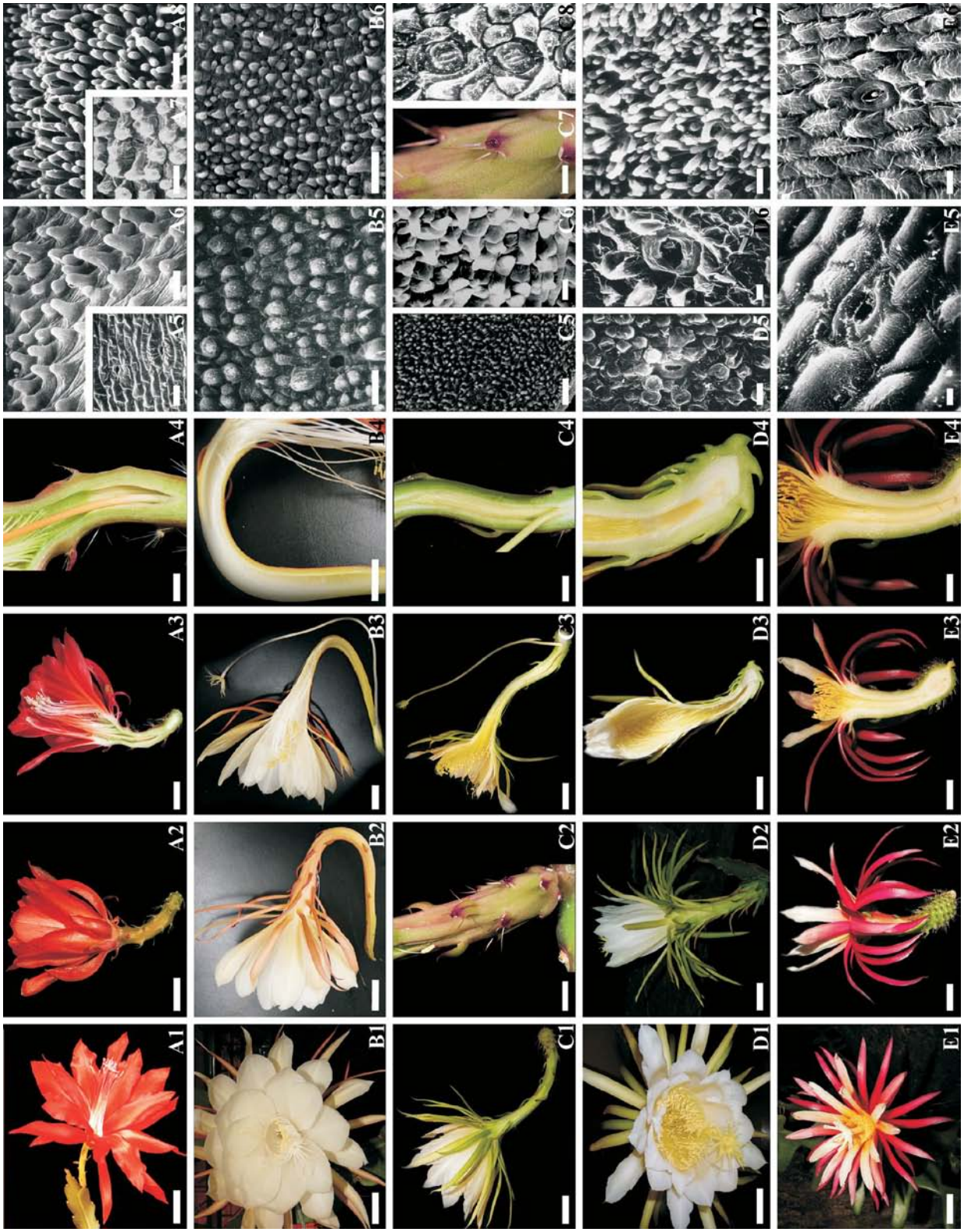

A1
A2
A3
A4
A5
A6
A7
A8
B1
B2
B3
B4
B5
B6
C1
C2
C3
C4
C5
C6
C7
C8
D1
D2
D3
D4
D5
D6
D7
D8
E1
E2
E3
E4
E5
E6

Figure 2. Floral morphology (photos) and micromorphology (SEM) of nectary in *Hatiora* and *Lepismium* (Rhipsalideae). (A1-A7) *Hatiora 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).

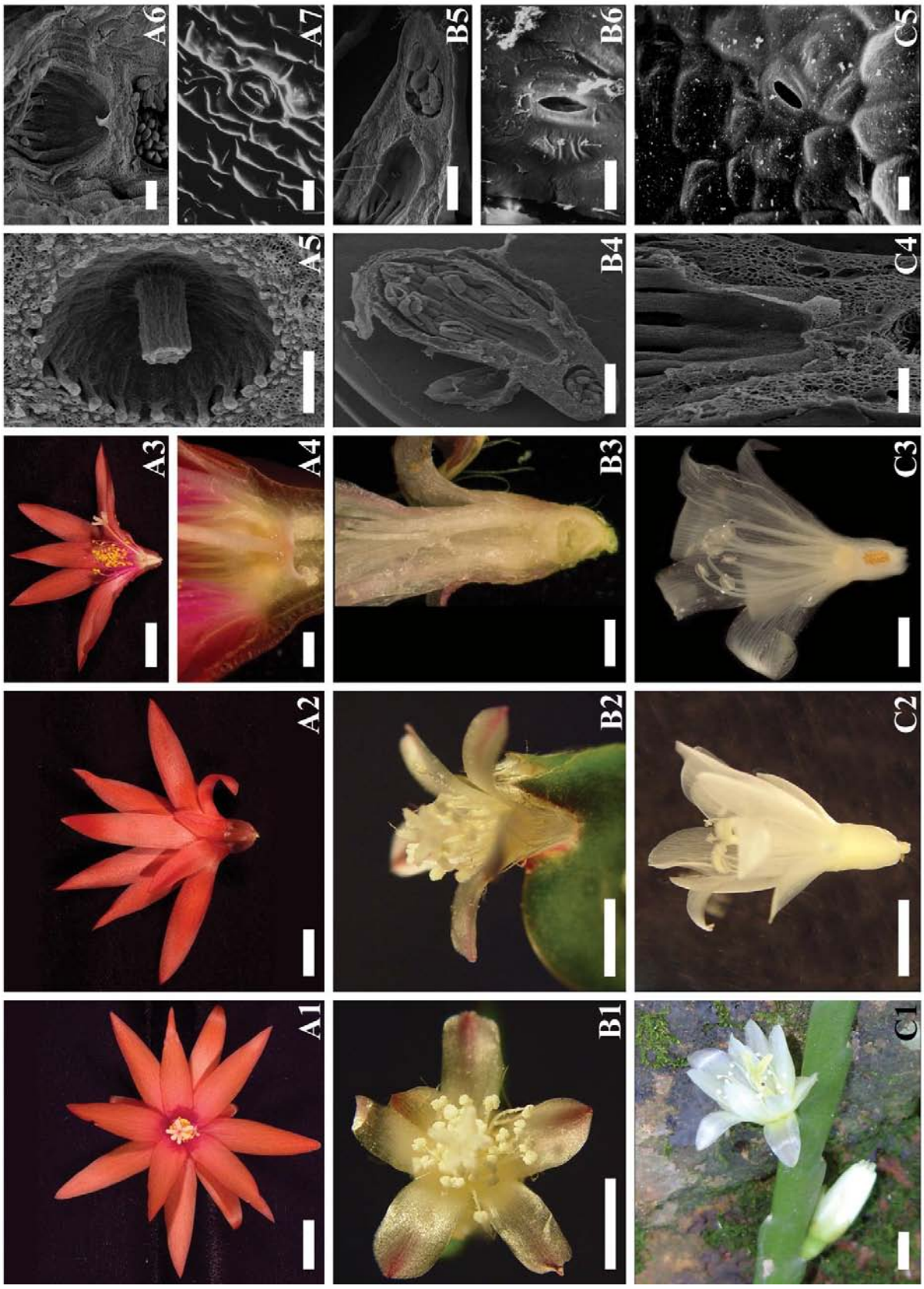

A6
A7
B5
B6
C5
A5
B4
C4
A3
A4
B3
C3
A2
B2
C2
A1
B1
C1

FIGURE 3. Floral morphology (photos) and micromorphology (SEM) of nectary in *Schlumbergera truncata* and selected species of *Rhipsalis* (Rhipsalideae). (A1-A7) *R. 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* fma. *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's epidermis. (D9) SEM view stomata on nectary epidermis. Scale bars: 2 mm (A1-A3, B4, C3), 500 µm (A4, A6, D7), 20 µm (A5, D8), 10 µm (A7, B8, C5, D9), 5 mm (B1, B2, C1, D4), 4 mm (B3), 300 µm (B5, B6, C4), 50 µm (B7), 10 mm (C2, D2, D3), 15 mm (D1), 1 mm (D6), 3 mm (D5).

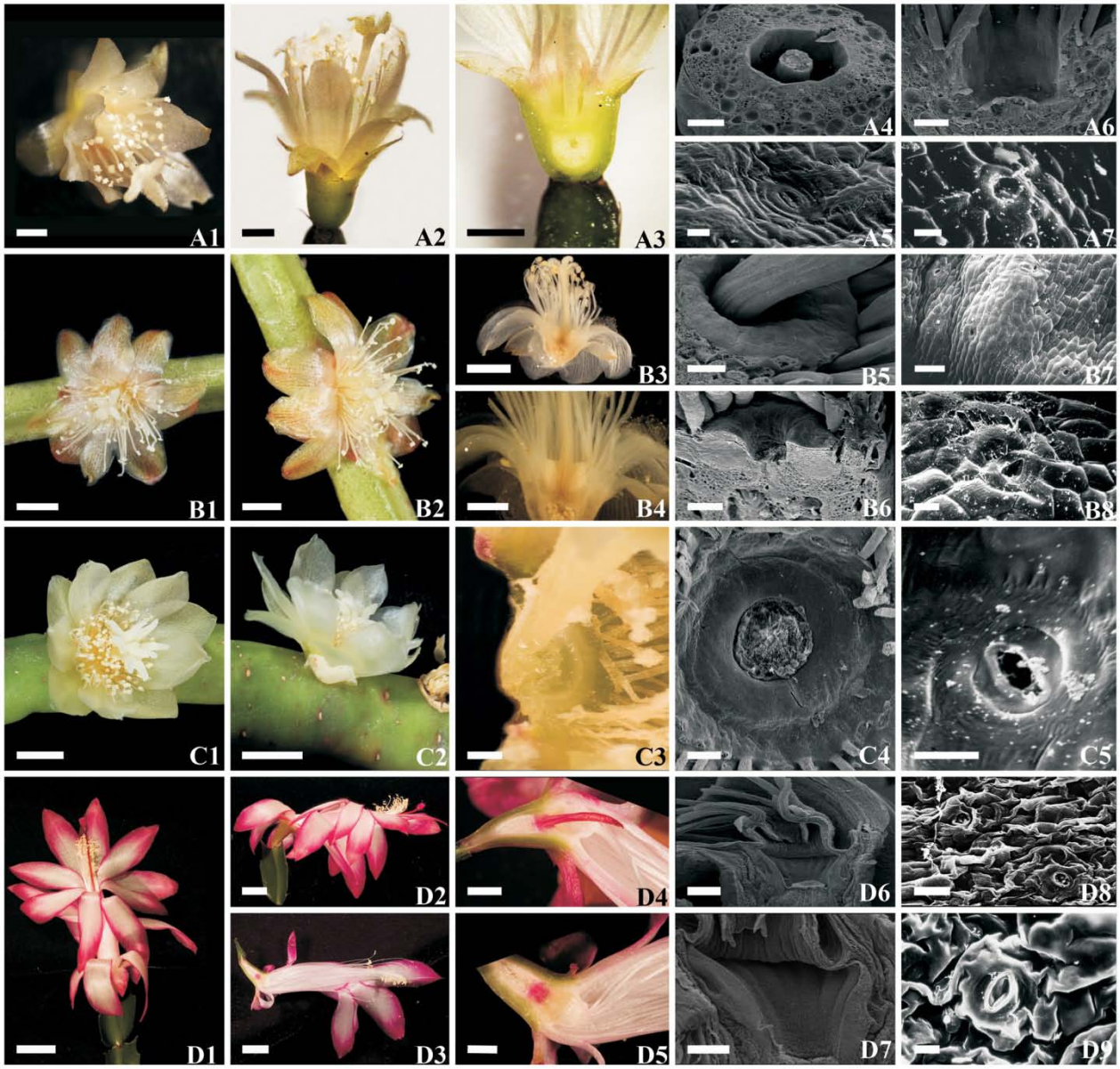

A1
A2
A3
A4
A5
A6
A7
B1
B2
B3
B4
B5
B6
B7
B8
C1
C2
C3
C4
C5
D1
D2
D3
D4
D5
D6
D7
D8
D9

FIGURE 4. Line drawings of Rhipsalideae (A-D) and Hylocereeae (E-G) flowers and nectaries in longitudinal section. A. Annular nectary in *Rhipsalis grandiflora*. B and C. Furrow nectary in *Lepismium warmingianum* and *Hatiora gaertneri*, respectively. D. Furrow nectary, holder subtype in *Schlumbergera truncata*. E. Open chamber nectary in *Selenicereus anthonyanus.* F. *Epiphyllum phyllanthus* - detail of uppermost part of floral tube where the primary stamens form the open chamber nectary– . G. *Hylocereus setaceus* – detail of the uppermost part of floral tube with primary stamens inserted at different levels forming the diffuse chamber nectary. an: annular nectary; ch: chamber nectary; fu: furrow nectary; fuh: furrow nectary/holder subtype; ps: primary stamens; ss: secondary stamens. Bars: 2 mm (A, B), 5mm (C), 1 cm (D-G).

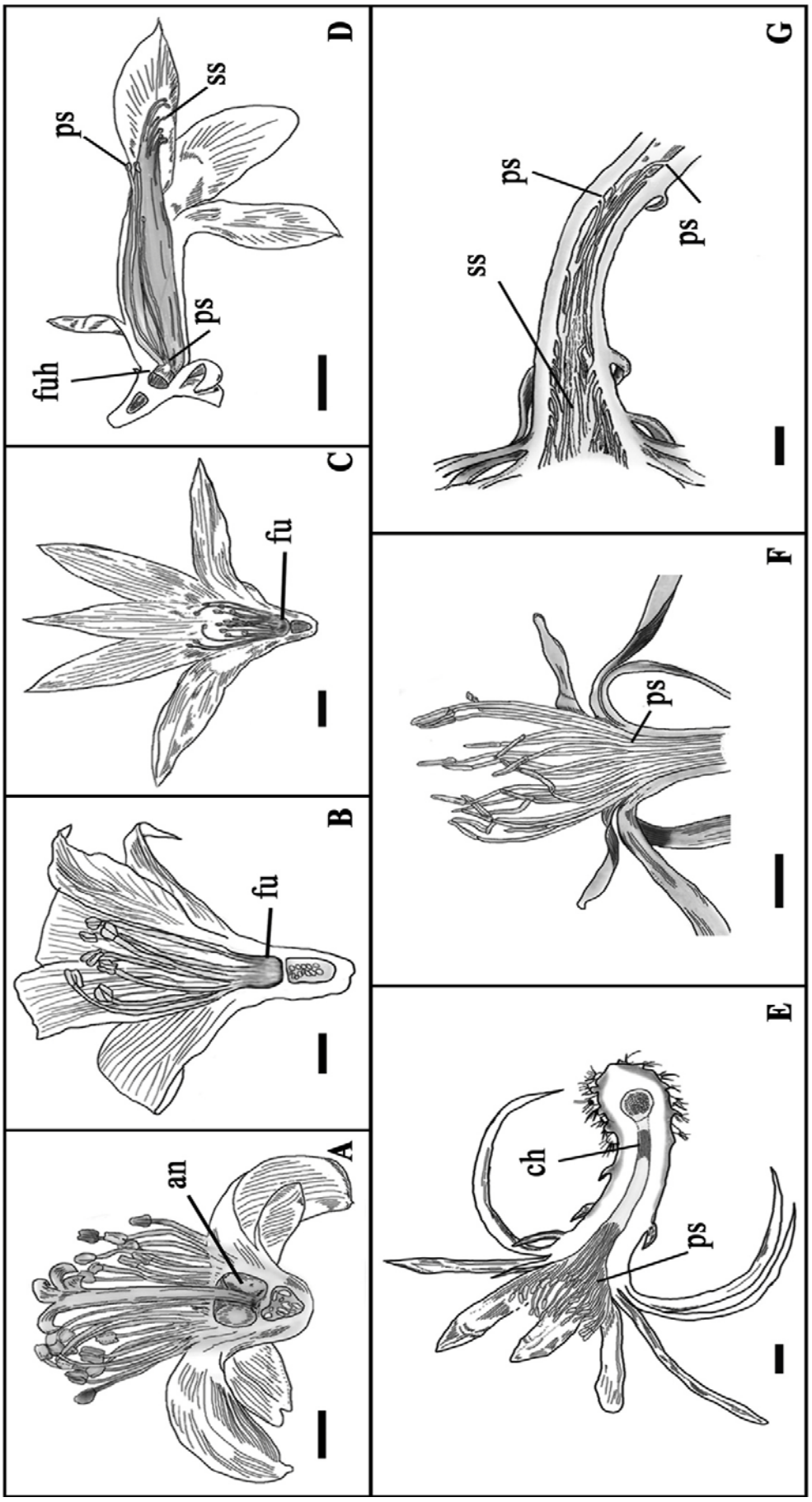
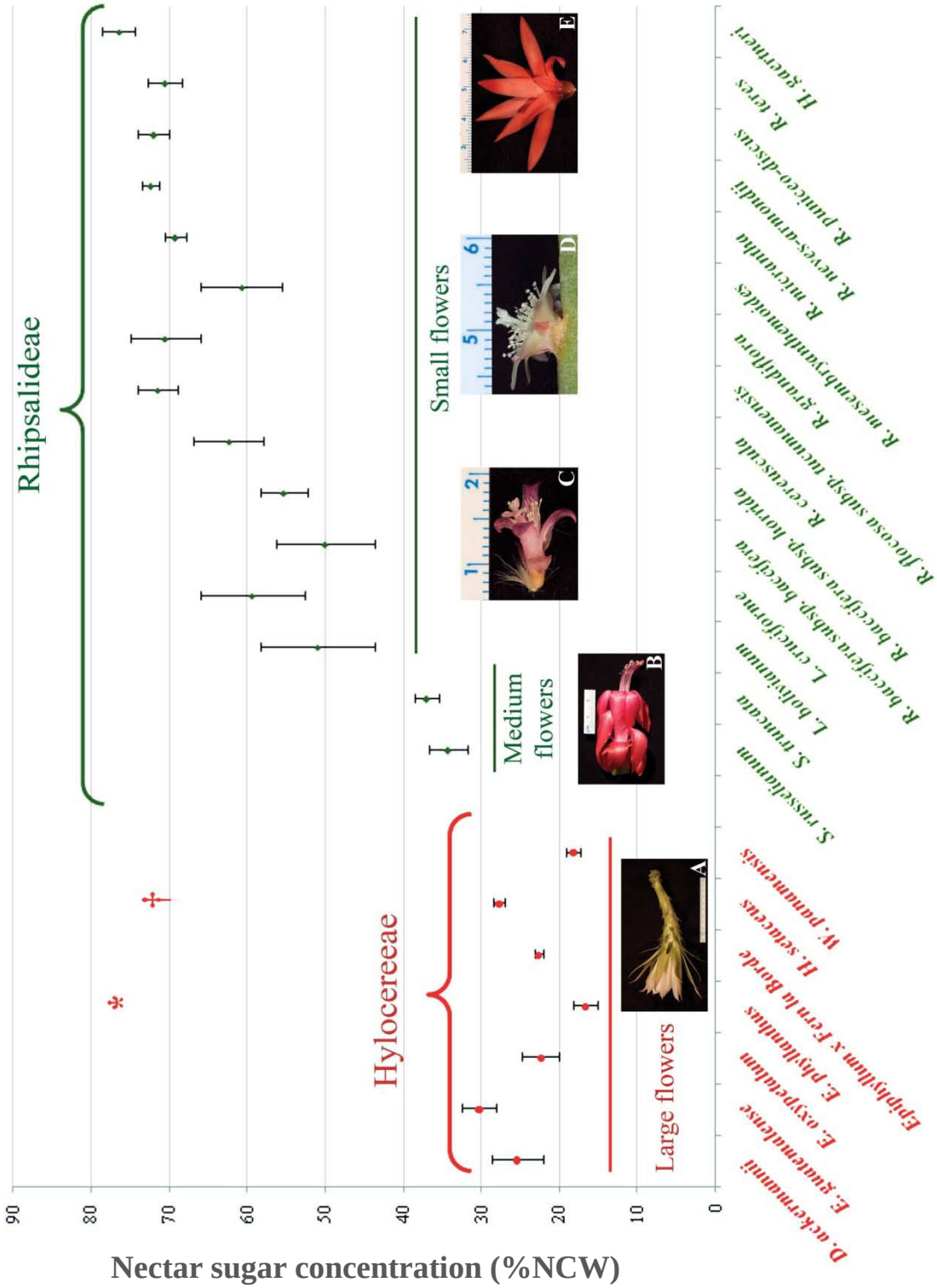

A
an
B
fu
C
fu
D
ps
ss
fuh
ps
E
ch
ps
F
ps
G
ss
ps
ps

FIGURE 5. Mean values (Average ± SD) 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 scale of (*) *Epiphyllum phyllanthus* (77.00) and (†) *Hylocereus setaceus* (73.40). (A) *Weberocereus panamensis* – scale: 10 cm. (B) *Schlumbergera russelianum* – scale: 2.5 cm. (C) *Lepismium cruciforme* – scale: 1.5cm. (D) *Rhipsalis floccosa* subsp. *tucumanensis* – scale: 1.5cm. (E) *Hatiora gaertneri* – scale: 5.5cm.


Rhipsalideae
Hylocereeae
Small flowers
Medium flowers
Large flowers
A
B
C
D
E
D. ackermannii
E. guatemalense
E. oxypetalum
E. phyllanthus
Epiphyllum x. Fern la Borde
H. scleracens
W. pammenensis
S. russelianum
S. truncata
L. boliviannum
L. cruciforme
R. baccifera subsp. baccifera
R. baccifera subsp. horrida
R. cereuscula
R. floccosa subsp. hucumanensis
R. grandiflora
R. mesembryanthemoides
R. micrantha
R. neves-armondii
R. puniceo-disca
R. teres
H. guatemalensis
Nectar sugar concentration (%NCW)
0
10
20
30
40
50
60
70
80
90

CAPÍTULO 2

Fruit development in three cacti epiphytic species: contributions to understanding its structure, and implications in fruit nomenclature

Odair José Garcia de Almeida[1, 2], Hugo Cota-Sánchez[2], Arthur R. Davis[2], Adelita Aparecida Sartori Paoli[1].

(1) Departamento de Botânica, IB, UNESP, Rio Claro, SP (13506-900) Brasil

(2) Department of Biology, University of Saskatchewan, Saskatoon, SK (S7N5E2) Canada

(3) Departamento de Biologia, UEM, Maringá, PR (87020-900) Brasil

A ser submetido ao periódico Systematic Botany

ABSTRACT **-** In the Cactaceae, the most species have inferior ovary in their flowers, and it produces a very special and interesting type of fruit in which receptacular tissue is involved. The fruit of the cacti is perhaps one of the most interesting and complex formations in which the most different floral organs can participate. In general the fruit is described in the literature as a conspicuous, globose, to oblong, indehiscent *berry* with numerous seeds embedded in a fleshy pulp. However, we also find somewhat inconsistent classification of fruit, that is, there is an unevenness of names for fruit descriptions among different authors. Thus, this work aims, through a traditional morpho- and anatomical approach with optical and scanning electron microscopy to study the structure and development of fruit after anthesis, using three species of epiphytic cacti *Hylocereus undatus*, *Lepismium warmingianum* and *Rhipsalis cereuscula.* The three species show similar fruit development that supports to consider the cactus fruit *cactidium* type with accessory structures of pericarpelar origin.

Key-workds – *Cactidium*, fruit typology, Hylocereeae, inferior ovary, ontogeny, Rhipsalideae.

INTRODUCTION

In the classical definition of Gaertner (1788) the fruit is interpreted as the mature ovary. Nevertheless, some group of plants may have their fruits developed from the flower as a complex unity, besides the carpels (ovary), other floral parts may be involved in the fruit formation, such as receptacle, sepals, petals, stamens, peduncle and bracts (Roth 1977). So that, other authors have showed different definitions for the fruit, such as the flower in the state of seed maturation (Knoll 1939; Leins and Erbar 2010). Thus, in this work fruit will be considered the result of the ovary developed and matured, which may have other parts of the flower, or even of the inflorescence, as adopted in Souza (2006). According to Spjut (1994) the fruits derived from an inferior ovary theoretically have extraovarian tissues that surround the pericarp, the origin of which may be receptacular or appendicular (from fused perianth parts).

In the Cactaceae, the most species have inferior ovary in their flowers, and it produces a very special and interesting type of fruit in which receptacular tissue is involved. The fruit of the cacti is perhaps one of the most interesting and complex formations in which the most different floral organs can participate (Roth 1977). The cactus fruit exhibits a multitude of shapes which is, in general, dictated by the peculiar shape of the gynoecium. The areoles of the receptacle remain active until the flowering period has passed. The areoles of the pericarpel zone of the receptacle sometimes cease activity after flowering, but they may also remain active when the fruit is mature, for instance, in *Pereskia* Mill. and *Opuntia* Mill. (Buxbaum 1955). The term pericarpel sensu Buxbaum (1953) refers to that receptacle tissue which surrounds the ovary.

During the 1960's Boke (1963, 1964, 1966, 1968) clarified the origin of the ovary and the receptacular tissues involved in the Cactaceae flowers through the ontogeny in flowers from *Pereskia* as reviewed in Roth (1977), but since then little attention has been given to

this subject. For instance, Rosa and Sousa (2003) have studied fruit development in *Pereskia aculeata* Mill., Fuentes-Peres et al. (2009) the flower in *Opuntia* spp., and Almeida et al. (2010) the flower of *Epiphyllum phyllanthus* (L.) Haw. On the other hand, quite a few investigations have focused on embryology (Strittmatter et al. 2002), the gynoecium development and auxins role in masculinization in the dioecious cactus *Opuntia stenopetala* Engelm. (Orozco-Arroyo et al. 2012), seed development (Engleman 1960) and seed morphology (Barthlott and Hunt 2000; Arias and Terrazas 2004) to name a few. Recently Loza-Cornejo et al. (2012) have reported the morphological aspects of fruit, seed, and germination in globose cacti; however, there still is very little information about fruit development in the Cactaceae.

In general the fruit descriptions in the literature are according to Barthlott and Hunt (1993) in which the authors described: "the cactus fruit is usually a conspicuous, globose, to oblong, indehiscent *berry* with numerous seeds embedded in a fleshy pulp. Colors can be green to white, yellow, all shades of red and bright blue to almost black". However, as we search a little deeper into the literature we find somewhat inconsistent classification of fruit, that is, there is an unevenness of names for fruit descriptions among the authors. For instance, most authors have reported *berry* as said before by Barthlott and Hunt (1993), but we can still find other types, such as *acrosarcum* form Spjut (1994), *flesh capsule* (Souza and Lorenzi 2005), *melonidium* (Barroso et al. 1999), *cactidium* (Hertel 1959; Rosa and Souza 2003; Souza 2006). Conversely, two authors have brought out good concepts for the cactus fruit, the first one is in Buxbaum (1955), who has classified the fruits as an *accessory fruit*, and the second one is about Mauseth (2006) who has defined that the events after the pollination and fertilization happen just as in an apple fruit, that is, the *true fruit* develops inside the base of the long-shoot, which itself develops as a *false fruit*, and the boundary between inner true fruit and outer false fruit is not readily apparent. Nevertheless, works dealing with the

ontogeny of this reproductive organ and the nomenclatural issues in the Cactaceae are still lacking.

The most studies in the Cactaceae have focused onto the fruit of prickly pears (*Opuntia* spp.) and little has been investigated about the fruit composition and aspects of fruit cultivation of species of cacti (Esquivel 2004; Loza-Cornejo et al. 2012). Many species of cacti have been used as food, in addition to *Opuntia* spp., for instance, *Stenocereus* (Berger) Riccob. spp., *Pachycereus* (Berger) Riccob. spp., *Ferocactus* (Berger) Britton & Rose spp., *Mammillaria* Haw., *Peniocereus greggii* (Engelm.) Britton & Rose, *Pereskia aculeata* Mill., *P. guamacho* Weber and *Cereus repandus* Haw. are some examples of cacti eaten in the New World. In fact, almost all fleshy fruits of cacti are edible; including several epiphytic species, in which some of them are widely cultivated, such as *Hylocereus undatus* Haw. Britton & Rose and *H. triangularis* (L.) Britton & Rose that produce the dragon fruit which is cultivated in regions of the New Word, Europe and Asia; and *Hylocereus setaceus* (Salm-Dyck) Ralf Bauer and *Epiphyllum anguliger* (Lem.) G. Don which are cultivated and consumed for food in South America (Anderson 2001). With this in mind, this work aims to study the structure and development of fruit after anthesis, using three species of epiphytic cacti *Hylocereus undatus*, *Lepismium warmingianum* (Schum.) Barthlott and Hunt and *Rhipsalis cereuscula* Haw., as models, addressing the following question: Can the fruit anatomy help to support a general classification of the fruit for the family? In addition, contribute with basic knowledge about the development of this structure in a histological view, which are useful for any further systematic and/or evolutionary study that need morpho- and anatomical groundwork. Since, according to Loza-Cornejo et al. (2012) some morphological characteristics of fruits may be used to support further systematic studies of Cactoideae's genera.

MATERIAL AND METHODS

Materials Studied and Species Information **-** Flowers in post-anthesis and fruits at several stages of development were collected at the Ingá Park - a municipal conservation unity – in Maringá, Paraná, Brazil and at the Pavuna Ecological Park in Botucatu, São Paulo, Brazil. Voucher materials were deposited at the Herbário da Universidade Estadual de Maringá (HUEM) and at the Herbário Rioclarense (HRCB), according to Table 1.

Hylocereus undatus, included in the subfamily Hylocereeae, is a species of the Queens of the Night, also known as Pitahaya or Dragon Fruit; it is a climbing and secondary hemi-epiphytic (e.i., starting living with its roots in the soil, but in the maturity it lost the link with the ground, becoming completely epiphytic); produces massive numbers of three-winged, sinuate, horny and dark-green stem; its distribution is uncertain because of its history, perhaps is a native species of the Caribbean coast; it is widespread in the New World tropics and in the southeastern Asia (Anderson 2001; Hunt et al. 2006).

Lepismium cereuscula, included in the subfamily Rhipsalideae, is an epiphytic shrub, much branched. It has pendent, dark green and slender stem segments; it occurs in Brazil, eastern Paraguay, and northeastern Argentina (Anderson 2001; Hunt et al. 2006).

Rhipsalis cereuscula, included in the Rhipsalideae, is a shrubby to bushy epiphytic plant, much branched with dimorphic and cylindrical stem segments; it occurs in Bolivia, Paraguay, Uruguay, southern Brazil, and Argentina (Anderson 2001; Hunt et al. 2006).

TABLE 1. Information about the specimens.

Species	**Site**	**Collectors /Numbers**
Rhipsalis cereuscula Haw.	Inga Park, Maringá/PR - Brazil	O.J.G. Almeida / 006
Lepismium warmingianum (Schum.) Barth.	Inga Park, Maringá/PR - Brazil	O.J.G. Almeida /007

L. warmingianum (Schum.) Barth.	Pavuna Ecological Park, Botucatu/SP - Brazil	L. B. Santos & O.J.G. Almeida / 452
L. warmingianum (Schum.) Barth.	Inga Park, Maringá/PR - Brazil	O.J.G. Almeida / 009
Hylocereus undatus (Haw.) Britton & Rose	Inga Park, Maringá/PR - Brazil	O.J.G. Almeida / 016

Morpho- and Anatomical Analysis **-** The morphological analysis was performed in fresh and fixed material evaluated under Leica stereoscope microscope. The botanical material was fixed in formalin acetic alcohol (FAA 50) from seven to ten days and later transferred into 70% alcohol, following the protocol of Johansen (1940). For the anatomical study, it was used hydroxyethyl methacrylate Leica HistoResin (according to the fabricant instructions) or in Paraplast wax using a modified version of the protocol in Davis et al. (1988). For HistoResin work, the material was dehydrated in ethyl alcohol series, embedded in Leica HistoResin, sectioned (cross and longitudinally) at 8 to 12 µm thicknesses with a rotary microtome and then, the sections were stained with toluidine blue O at 0.05%, pH 4.6 (O'Brien et al. 1965). For the wax work, the material was dehydrated in an increasing ethanol-*n*-butanol series (Jensen 1962), and then embedded in Paraplast wax in an incubator (60 °C). Plant material sectioning (cross and longitudinally) was conducted with a rotary microtome at 7-12 µm width with sections being heat mounted on glass slides, stained with 0.05% toluidine blue O in 20 mmol/L sodium benzoate buffer, pH4 (O'Brien and McCully 1981), and then submerged in xylene to remove wax from tissue. The slides were then covered with glass cover slips and sealed with Permount or Entellan synthetic resins. The optical microscopy images were obtained by image capturing under a Leica ICC50 photomicroscope system and Leica Application Suite program, version 1.8.1.

In order to identify certain substances, microchemical tests were performed on manually prepared sections of fresh and fixed material. Sudan IV was used to identify lipids, Lugol's solution to identify starch, and ferric chloride with sodium carbonate to identify phenolic substances (Johansen 1940), Phloroglucinol with hydrochloric acid to identify lignified walls (Sass 1951), Ruthenium red to identify polysaccharides and pectins (Jensen 1962), mercuric bromphenol blue to identify protein (Mazia et al. 1953).

SEM Analysis **-** For the SEM micromorphological analysis the material investigated 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 48h, dehydrated in a graded acetone series to 100%, critical-point dried with liquid CO_2 (Polaron Instruments E3000), and then affixed on aluminum stubs following Almeida et al. (2012). After gold coating (Edwards Sputter Coater S150B), sections of different portions of the filaments were examined with a Philips SEM 505 at 29 kV, and micrographs were taken using Polaroid 665 positive/negative film and the Animator DV (image capture) program.

The images were edited and plates were labeled and assembled using the programs Adobe Photoshop CS3 and Corel Photo-Paint X3, version 13.

RESULTS

In *H. undatus* the development starting with a pericarpel/ovary structure of about five centimeter long and 4 cm diameter in the post-anthesis flower (Fig. 1A) that may become a mature fruit of about 15 cm long and 10 cm diameter, and it is pinkish in color with a white pulp (Fig. 1B-E); whereas in the *L. warmingianum* and the *R. cereuscula* species the pericarpel/ovary structure in post-anthesis flowers have less than a 0,5 cm (Fig. 1F, H) and the mature fruits in these species have less than two cm in the former and less than one cm in

the latter (Fig. 1G, I). *Lepismium warmingianum* has a dark purple color mature fruit (Fig. 1G), and *R. cereuscula* has a translucent mature one (Fig. 1I).

Ontogeny: In order to provide a better understanding of the development of the fruit, in this study we are considering pericarp the whole unit of pericarpelar and carpelar origin, located between the external epidermis (exocarp) and the inner epidermis (endocarp).

Fruit of Hylocereus undatus - The flowers of *H. undatus* in post-anthesis stage (Fig. 1A) are characterized as having a young pericarp (pericarpel/ovary tissues in origin) (Fig. 2A) made from one layer of epidermis (exocarp), which contains stomata and is covered by cuticle, one-two layers of collenchymatic hypodermis (Fig. 2B, C, E). There is relatively compact parenchyma tissue composed of about 50-60 layers of large, isodiametric cells, many mucilage secretory cavities and vascular small bundles (the pericarpel region, in the flower); following the compacted parenchyma there is one line of large vascular bundle that surrounded another parenchyma region compounded for a non-compacted tissue with more than 60 layers of small cells, lot of intercellular space, several small and tiny vascular bundles (Fig. 2A, D, F), (this innermost region was the ovary in the flower) and the “ring” of large vascular bundles (Fig. 2 D, F) is the boundary between these two merged regions. So that, the structure which forms the pericarp is compounded by: external epidermis (exocarp), compacted parenchyma, a ring of large vascular bundles, a non-compacted parenchyma (mesocarp), and internal epidermis (endocarp).

The cells of the young pericarp start to elongate anticlinally (i.e., perpendicular to the surface), but the number of layers remains more or less the same, and as the fruit grows the cells of the epidermis and the hypodermis undergo anticlinal cellular divisions (in an arrangement similar to that showed in the Figures 2A, C and D), to equalize with the development of the pericarpel cells; at the same time the cells of the ovary itself start to

increase in size, decreasing the intercellular spaces (Fig. 2G). In the placental and the funiculus regions the cells increase their size, start to accumulate starch, and undergo cellular division (Fig. 2H). During the ripening process of the fruit, both the pericarp and the placenta have their cells increased in size; and the funiculus have their parenchyma cells increased in number (by cell division) and in size (by cell enlargement). Close to the final stage of maturation of the pericarp, the cells of the innermost region collapse; and in the mean time this region also is compressed for the layers of the external region, and placenta/funiculus developed tissues. (Fig. 2I-L). At the final stage of maturation of the fruit, the pericarp still retains large parenchyma cells with very thin cellular walls. Many cells have collapsed and the mucilage has filled the intercellular spaces (Fig. 2K, L). The funiculus cells are also enlarged with very thin cellular walls and the cells that surrounds the seed has accumulated large amounts of mucilage (Fig. 2L).

Fruits of Lepismium warmingianum and of Rhipsalis cereuscula - In the small fruits of the *L. warmingianum* and *R. cereuscula* analyzed, they have general structure relatively similar to that one seen in *Hylocereus undatus* fruit, however, in much smaller proportion.

The flower in anthesis of both have a single layer epidermis made by small and rectangular cells, covered with a cuticle, and the pericarpel region has about nine-ten layers of large, different sized and more or less isodiametric parenchyma cells; in this region there are huge mucilage secretory cavities (that are even more remarkable in *L. warmingianum*) which surround the inner tissues, followed by vascular bundles, and 10-14 layers of parenchyma cells that gradually decrease in size (the ovary tissue).

Similar to the fruit development in *Hylocereus undatus*, in *L. warmingianum* and *R. cereuscula* the number of layers remains relatively the same in both the pericarpel and the ovary wall regions, which compound the pericarp itself (Fig. 3A-D; 4A-D). Nevertheless, the

size of all structures is smaller than that one of *H. undatus.* During the fruit development of these two species, the cells have increased their size, initially in the external regions of pericarp (pericarpel), after that in the innermost region of the pericarp (ovary); at the mean time, the epidermis and the subepidermal layer have undergone periclinal enlargement and some cellular division to equalize the cellular growth in the pericarpel and ovary regions (pericarp) (Fig. 3C, D, G, H, K, I; 4C, D, G, H, K, L). The external shape of the pericarpel in *R. cereuscula* does not undergo change until the end of the fruit maturation process (Fig. 4A, B, E, F, I, J) and the increase in size of the fruit is a result of the enlargement of parenchyma cells of pericarp and the mucilage secretory cavities as a whole. In *Lepismium warmingianum* the enlargement of the mucilage secretory cavities was remarkably greater, resulting in a fruit shape different from that shape of the pericarpel (in post-anthesis stage), that is, the pericarpel shape of *L. warmingianum* had four-five angles (ribs) (Fig. 3A, B, E, F); however during the fruit development the shape has been changed to a spherical form (Fig. I, J). In both species, the cells become much larger during maturation, as we said before, with very thin cellular walls, with some of them having collapsed and all the intercellular space being replaced with mucilage; the internal layers of the fruit had their cells compressed by the growth of secretory cavities in the external region of the pericarp, as well as, by the growth and development of the seeds (Fig. 3L; 4K, L). As in *H. undatus,* in *L. warmingianum* and *R. cereuscula* the parenchyma cells that surround the seed accumulate large amount of mucilage (Fig. 4K, L).

DISCUSSION

The period of ripening of the fruit mainly concerns changes in cell wall structure and in cell contents; it may also affect the structure of the surface and the formation of intercellular spaces (Roth 1977; Souza 2006). As seen in our results there are not many cell divisions in the fruit wall during its development after anthesis. In the species studied here

there were evident cell divisions in the funiculus and placenta regions, essentially in the large fruit of *H. undatus*. Certainly, the cell division takes place during flower development (in pre-anthesis events). Cell enlargement is the main event responsible for the fruit growth, followed by the increase in mucilage production and storage in the secretory cavities, which break down releasing mucilage contents into the intercellular spaces during final stages of the fruit maturation. In fact, during the cell enlargement process the volume of the vacuoles increases, which is correlated with a large uptake of water, that can amount up to 90% of the size in fresh fruits (Nitsch 1953); the capacity to produce fleshy fruit with water abundance brings positive consequences to the cactus species, such as mutualistic interactions with birds. For instance, Guaraldo *et al.* (in press) have confirmed a specialized case of seed dispersal system involving species from *Rhipsalis* and *Euphonia* (small birds) in Neotropical forests in Brazil. Another factor that may be related to the succulence in the cactus fruit is the rare phenomenon of vivipary recorded in several epiphytic cacti species both from Hylocereeae and Rhipsalideae (Conde 1975; Lombardi 1993; Cota-Sánchez 2004; Cota-Sánchez and Abreu 2007). In addition, as pointed out in Cota-Sánchez and Abreu (2007), the characteristics of the fruit in several viviparous cacti such as humidity, fresh and mucilaginous pericarp, and pulp may provide a gradual series of opportunities for seedling establishment, these features were also observed in the development of the fruit in *Epiphyllum phyllanthus* (Almeida 2009), which is also a potential viviparous species. The fruit anatomy may facilitate the germination of seeds inside the fruit because it allows gas exchange between internal and external of the fruit, and further, the light passing through the tiny portion of the translucent fruit wall (Simão et al. 2010).

Buxbaum (1955) was one of the pioneers in the study of the fruit morphology in the cactus family, and according to him all forms of fruits may be generalized by the peculiar shape of the gynoecium. Here, we found this kind of pattern in *Hylocereus undatus* and

Rhipsalis cereuscula, and the same was found in *E. phyllanthus* by Almeida (2009), in which it's possible to see somewhat of a similarity between the external pericarpel shapes of the post-anthesis flower and to the mature fruit. In contrast of the mature fruit of the *Lepismium warmingianum* which has a different shape when compared to the external pericarpel shape of the post-anthesis flower, i.e., the pericarpel has about four to five ribs, when seen in transversal section; whereas the mature fruit is round with no ribs. The shape and color of the fruit in *Lepismium* Pfeiff. are useful in taxonomic treatments (Barthlott and Taylor 1995; Anderson 2001; Hunt et al. 2006), and so the development as showed here can be useful as well.

The fruit typology in Cactaceae is an interesting subject; since in the literature it is possible to find a couple of different names for the same structure. Although many authors considered it such a *berry*, we agree with Souza (2006) which consider berry a large term and keep this definition to simple and fresh fruit from superior ovary. Concerning to Cactaceae, Hertel (1959) classified its fruits as "*pomaceo*" type, originated from inferior ovary, and Cactidium subtype fleshy, tri-pluricarpelar, unilocular, involved in tissue from stem origin, colorless, greenish, orange or red. Spjut (1994) described the cactus fruit as *acrosarcum*, a simple indehiscent fruit characterized by an undifferentiated pericarp that is surrounded by an accrescent fleshy exocarp derived from perianth or receptacle; the author has yet considered synonym to the term *cactidium* from Hertel (1959). Other authors have different names for that structure, for instance, Barroso et al. (1999) included the fruit of Cactaceae as *melonidium*: fruit originated of superior or inferior ovary, with central space without delimitation of loculus or, if divided in loculus, these are more or less large and have no visible increase of placentary tissue with tissue that fills the fruit cavity, they are mostly of yellow color; Souza (2006) is in agreement to Hertel (1959)'s classification. On the other hand, Mauseth (2006) and Anderson (2001) have showed interesting description of the cactus

fruit with more information from a structural point of view, they have reported the fruit structure in Cactaceae as a true fruit developed inside the base of the long-shoot, which itself develops as a false fruit; just as in an apple fruit, and the boundary between inner true fruit and outer false fruit is not readily apparent. The results showed here for *Hylocereus undatus, Lepismium warmingianum* and *Rhipsalis cereuscula,* in addition to results of the fruit development in *Epiphyllum phyllanthus* described by Almeida (2009) corroborate Mauseth and Anderson descriptions. However, in order to contribute for a consistent definition for the fruit in the Cactaceae, we suggest considering it as *cactidium* type with accessory structures of pericarpelar origin. It is worth to mention that cactus family is the unique group of seed plants that have areoles in its reproductive structure (pericarpel), which may produce scales-leaves, bristles, spines or ever other fruits, as in *Opuntia*; and according to Taylor (1997) the areoles represent the hallmark of Cactaceae.

ACKNOWLEDGEMENTS

We thank A.P. Lonardoni and D. Falconer for comments on early drafts of the manuscript; and G. Liu and M. Mierau for technical assistance. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil, Ph.D. scholarship (141861/2009-6) to OJGA, grants (300495/2010-2 and 474068/2009-9) to AASP; the Emerging Leaders in the Americas Program (ELAP-Canada) for providing an exchange grant to OJGA; the National Geographic Society (Grant No. 7382-02), and the University of Saskatchewan Tri-Council Bridge (Grant No. 411051) to JHCS.

LITERATURE CITED

Almeida, O. J. A. 2009. *Morfoanatomia dos órgãos reprodutivos e da plântula de Epiphyllum phyllanthus (L.) Haw. (Cactaceae)*. M.S. thesis. Rio Claro, São Paulo: Universidade Estadual Paulista.

Almeida, O. J. G., A. A. S. Paoli and J. H. Cota-Sánchez. 2012. A macro- and micromorphological survey of floral and extrafloral nectaries in the epiphytic cactus *Rhipsalis teres* (Cactaceae: Rhipsalideae). *Flora* 207:119-125. doi:10.1016/j.flora.2011.11.004

Almeida, O. J. G., A. A. S. Paoli and L. A. Souza. 2010. Flower morpho-anatomy in *Epiphyllum phyllanthus* (Cactaceae). *Revista Mexicana de Biodiversidad* 81:65-80.

Anderson, E. F. 2001. *The cactus family*. Portalnad: Timber press.

Arias, S. and T. Terrazas. 2004. Seed morphology and variation in the genus *Pachycereus* (Cactaceae). *Journal of Plant Research* 117: 277-289. doi: 10.1007/s10265-004-0156-4

Barroso, G. M., M. P. Morin, A. L. Peixoto and C. L. F. Ichaso. 1999. *Frutos e sementes. Morfologia aplicada à sistemática de dicotiledôneas*. Viçosa: Editora UFV.

Barthlott, W. and D. Hunt. 1993. Cactaceae. Pp. 161-196 in *The families and genera of vascular plants* vol. 2, eds. K. Kubitzki, J. G. Rohwer and V. Bittrich. Berlin: Springer Verlag.

Barthlott, W. and D. Hunt. 2000. Seed-diversity in the Cactaceae subfam. Cactoideae. *Succulent Plant Research* 5:1-173.

Barthlott, W. and N. P. Taylor. 1995. Notes towards a monograph of Rhipsalideae (Cactaceae). *Bradleya* 13: 43-79.

Boke, N. 1963. Anatomy and development of the flower and fruit of *Pereskia piptitache. American Journal of Botany* 50:843-858.

Boke, N. 1964. The cactus gynoecium: a new interpretation. *American Journal of Botany* 51:598-610.

Boke, N. 1966. Ontogeny and structure of the flower and fruit of *Pereskia aculeata. American Journal of Botany* 53:534-542.

Boke, N. 1968. Structure and development of the flower and fruit of *Pereskia diaz-romeroana. American Journal of Botany* 55:1254-1260.

Buxbaum, 1953. *Morphology of cacti*. Section 2. The flower. Pasadena: Abbey Garden Press.

Buxbaum, 1955. *Morphology of cacti*. Section 3. Fruits and Seeds. Pasadena: Abbey Garden Press.

Conde, F. L. 1975. Vivipary in *Epiphyllum*. *Cactus Succulent Journal (U.S.)* 47: 38–39

Cota-Sánchez, J. H. and D. D. Abreu. 2007. Vivipary and offspring survival in the epiphytic cactus *Epiphyllum phyllanthus* (Cactaceae). *Journal of Experimental Botany* 58: 3865-3873. doi:10.1093/jxb/erm232

Cota-Sánchez, J.H. 2004. Vivipary in the Cactaceae: its taxonomic occurrence and biological significance. *Flora* 199: 481–490

Davis, A. R., R. L. Peterson, and R. W. Shuel. 1988. Vasculature and ultrastructure of the floral and stipular nectaries of *Vicia faba* (leguminosae). *Canadian Journal of Botany* 66:1435-1448.

Engleman, E. 1960. Ovule and seed development in certain cacti. *American Journal of Botany* 47:460-467.

Esquivel, P. 2004. Los frutos de las cactáceas y su potencial como materia prima. *Agronomía Mesoamericana* 15:215-219.

Fuentes-Perez, M., T. Terrazas and S. Arias. 2009. Anatomía floral de cinco species de *Opuntia* (Opuntioideae, Cactaceae) de México. *Polibotânica* 27:87-100.

Gaertner, J. 1788. *De fructibus et seminibus plantarum* vol. 1. Stuttgart: Academiae Carolinae.

Guaraldo, A. C., B. O. Boeni, and M. A.Pizo. In press. Specialized seed dispersal in epiphytic cacti and convergence with mistletoes. *Biotropica.*

Hertel, R. J. G. 1959. Contribuições para a fitologia teórica. II. Alguns conceitos na carpologia. *Humanitas* 4: 1-43.

Hunt, D., N. Taylor, and G. Charles. 2006. *The new cactus lexicon*. Milborne Ports: Dh books.

Jensen, W.A. 1962. *Botanical histochemistry: principle and practice*. San Francisco: WH Freeman.

Johansen, D.A. 1940. *Plant microtechnique*. New York: McGraw Hill Books.

Knoll, F. 1939. *Über den Begriff "Frucht.'' Der Biologe* 8: 154-160

Leins, P and C. Erbar. 2010. *Flower and fruit. Morphology, ontogeny, phylogeny, function and ecology*. Stuttgart: Schweizerbart Science Publishers.

Lombardi, J. A. 1993. Viviparity in *Rhipsalis pilocarpa* Löfgren (Cactaceae). *Ciência e Cultura* 45: 407.

Loza-Cornejo, S., T. Terrazas, and L. Lópes-Mata. 2012. Fruits, seeds and germination in five species of globose Cacteae (Cactaceae). *Interciencia* 37:197-203.

Mauseth, J. D. 2006. Structure-Function relationships in highly modified shoots of Cactaceae. *Annals of Botany* 98:901-926. doi:10.1093/aob/mcl133

Mazia, D., P.A. Brewer and M. Alfert. 1953. The cytochemical staining and measurement of protein with mercuric bromphenol blue. *Biological Bulletin* 104: 57-67.

Nitsch, J. P. 1953. The physiology of fruit growth. *Annual Review of Plant Physiology* 4:199-236.

O'Brien, T. P. and M. E. McCully. 1981. *The Study of Plant Structure: Principles and Selected Methods*. Melbourne: Termarcarphi.

O'Brien, T.P., N. Feder and M.E. Maccully. 1965. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59: 368-373.

Orozco-Arroyo, G., S. Vásques-Santana, A. Camacho, J. G. Dubrovsky and F. Cruz-García. 2012. Inception of maleness: auxin contribution to flower masculinization in the dioecious cactus *Opuntia stenopetala*. *Planta* 236: 225-236. doi: 10.1007/s00425-012-1602-5.

Rosa, S. M. and L. A. Souza. 2003. Morfo-anatomia do fruto (hipanto, pericarpo e semente) em desenvolvimento de *Pereskia aculeata* Miller (Cactaceae). *Acta Scientiarum, Biological Sciences* 25:415-428.

Roth, I. 1977. Fruit of Angiosperms. Pp. 1-675 in *Encyclopedia of plant anatomy*, ed K. Linsbauer. Berlin: Gebrüder Borntraeger.

Sass, J. E. 1951. *Botanical microtechnique*. Ed. 2 Iowa Ames, IA: State University Press.

Simão, E., A. T. Nakamura and M. Takaki. 2010. The germination of seeds of *Epiphyllum phyllanthus* (L.) Haw. (Cactaceae) is controlled by phytochrome and by nonphytochrome related process. *Biota Neotropical* 10: 115-119.

Souza, L. A. 2006. Fruto. Pp. 11-123 in *Anatomia do fruto e da semente*, org. L. A. Souza. Ponta Grossa: Editora UEPG.

Souza, V. C. and H. Lorenzi. 2005. *Botânica sistemática: guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APGII.* Nova Odessa: Instituto Plantarum.

Spjut. R. W. 1994. A systematic treatment of fruit types. *Memories of the New York Botanical Garden* 70:1-182.

Strittmatter, L. I., V. Negrón-Ortiz and R. J. Hickey. 2002. Subdioecy in *Consolea spinosissima* (Cactaceae): breeding system and embryological studies. *American Journal of Botany* 89:1373-1387.

Taylor, N. P. 1997. Brazilian cacti. Pp. 199-202 in *Cactus and Succulent Plants – Status survey and conservation action plan*, comp. S. Oldfield. Cambridge: IUCN/SSC Cactus and Succelent Specialist Group.

FIG. 1. Fruit morphology of *Hylocereus undatus* (A-E), *Lepismium warmingianum* (F-G) and *Rhipsalis cereuscula* (H-I). A. Post-anthesis young fruit. B. Immature fruit. C. Mature fruit. D. Mature fruit in longitudinal section (LS). E. Detail of the fruit (LS). F. Post-anthesis flower and young fruits. G. Young and mature fruits. H. Post-anthesis flower and immature fruit. I. Mature fruit. Bars – 4 cm (A, B), 5 cm (C), 3cm (D), 1 cm (E, G), 5 mm (F, H, I).

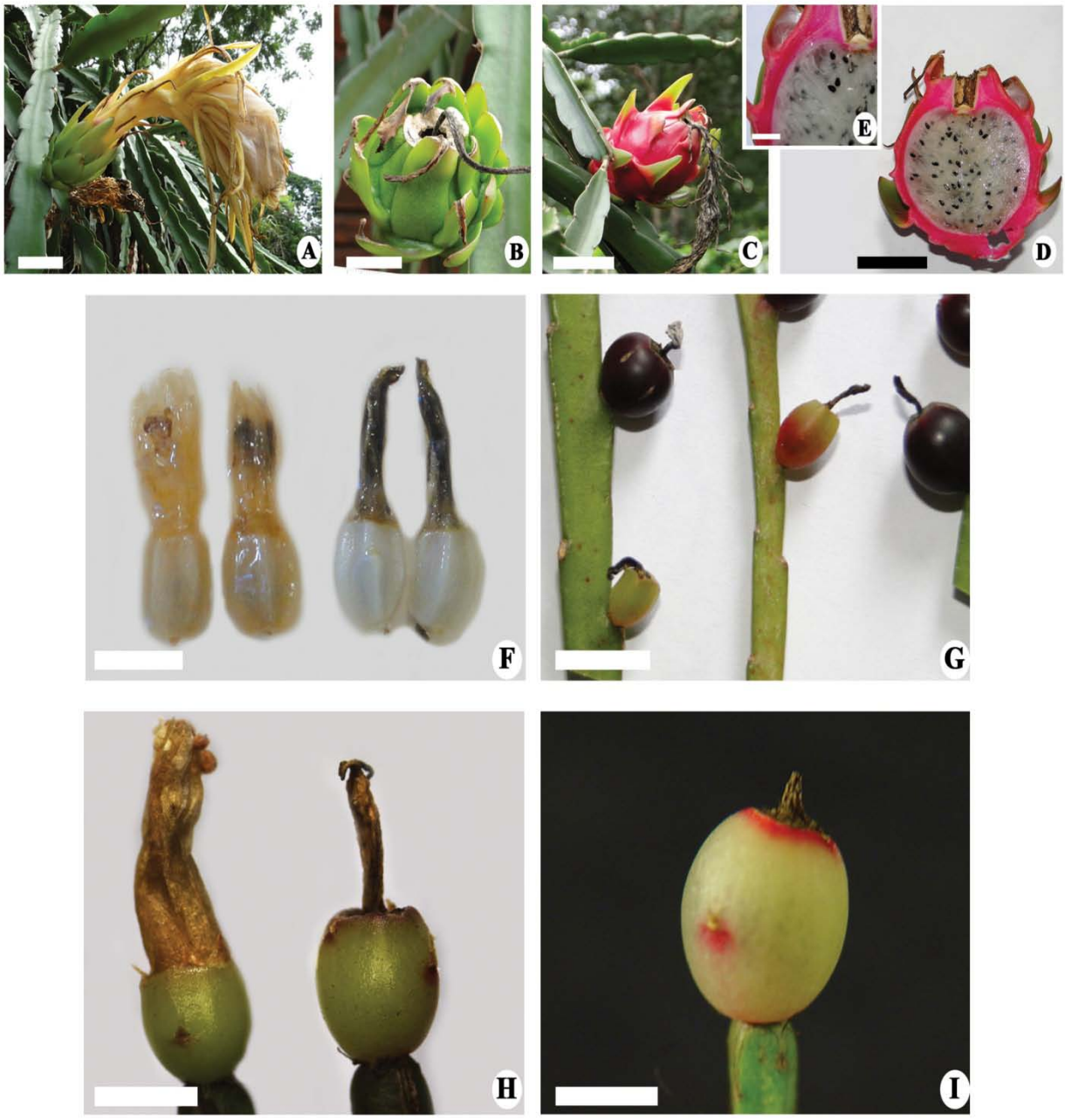

A
B
C
E
D
F
G
H
I

FIG. 2. Anatomy of the fruit of *Hylocereus undatus* in cross (CS) and longitudinal (LS) sections. A-C. Young fruit (post-anthesis) - LS. B. Detail of the outermost parenchyma layers of the pericarp. C. Detail of the epidermis and hypodermis. D-G. Young fruit (post-anthesis) - CS. E. Detail of the outermost region - young pericarp. F. Detail of the large vascular bundle in between the outer and inner parts of the pericarp. G-H. CS of fruit in development. G. Innermost part of the pericarp and funiculus. H. Detail of the funiculus cells and vascular bundle. I-L. Mature fruit in LS. J. Detail of the outer part. K. Detail of the middle part. L. Detail of the inner part of the pericarp. cu – cuticle, ex – exocarp, fu – funiculus, hp – hypodermis, ov – ovary in origin, pc – pericarp, pe – pericarpel in origin, sl – scale-leaf, vb – vascular bundle. Bars – 2mm (A, D, I), 100 µm (B), 50 µm (C), 400 µm (E, G, F), 200 µm (H, J-L).

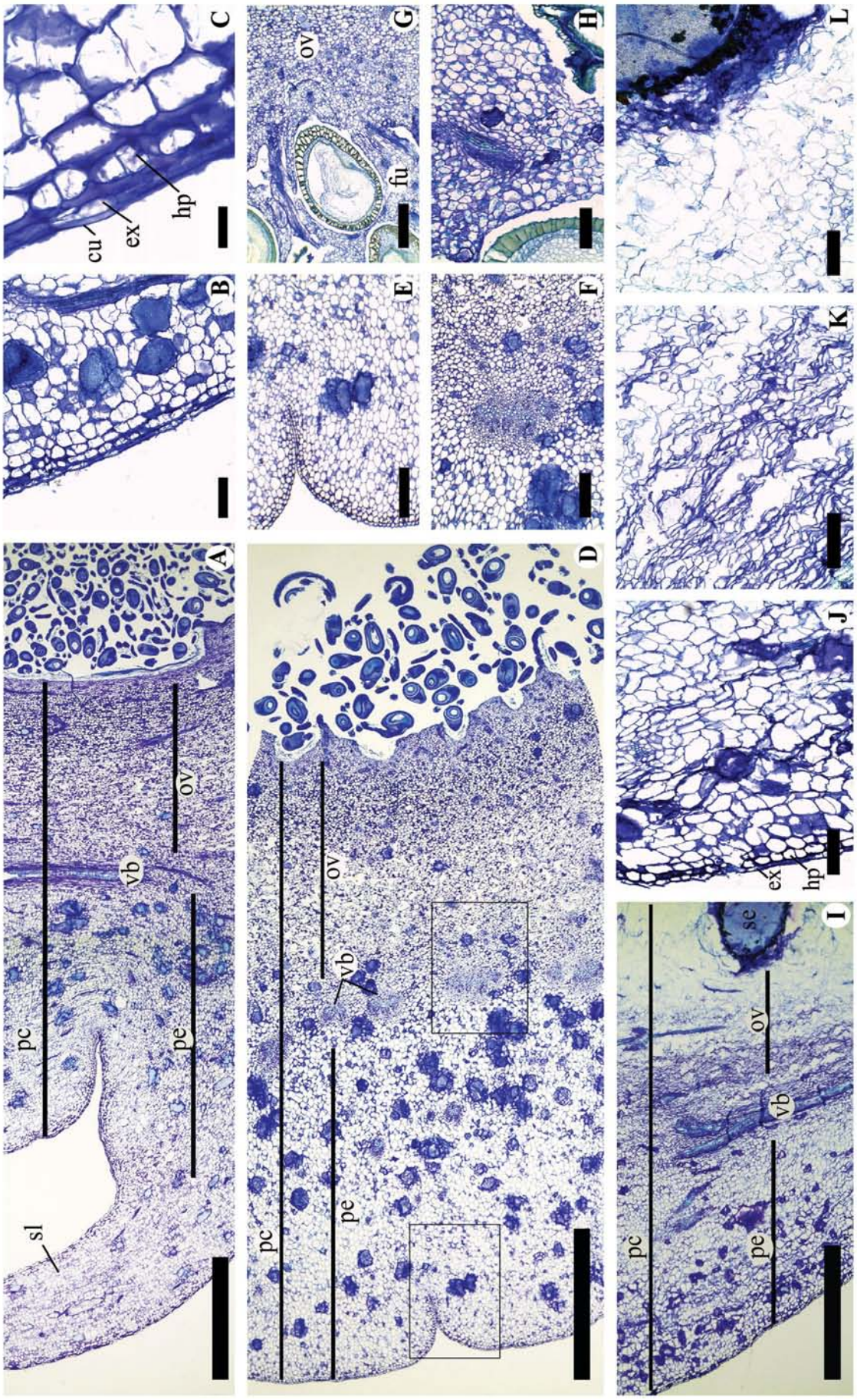

A
sl
pc
vb
ov
pe
B
C
cu
ex
hp
D
pc
ov
vb
pe
E
F
G
ov
fu
H
I
pc
se
ov
vb
pe
J
ex
hp
K
L

FIG. 3. Anatomy and SEM of the fruit of *Lepismium warmingianum* in cross (CS) longitudinal (LS) sections. A. Post-anthesis young fruit (LS). B. Post-anthesis young fruit (CS). C. Detail of the pericarp (pericarpel+ovary). D. SEM of the pericarp and ovules. E. Young fruit (LS). F. Young fruit (CS). G. Detail of the pericarp. H. SEM part of the young fruit. I. Mature fruit (CS). J. Mature fruit (CS). K. Detail of the pericarp. L. SEM of the pericarp and seed of mature fruit. ex – exocarp, ov – ovary in origin, pc – pericarp, pe – pericarpel in origin. Bars – 2 mm (A, E, I, J), 1 mm (B, F), 200 µm (C), 300 µm (G, K), 250 µm (D), 500 µm (H, L).

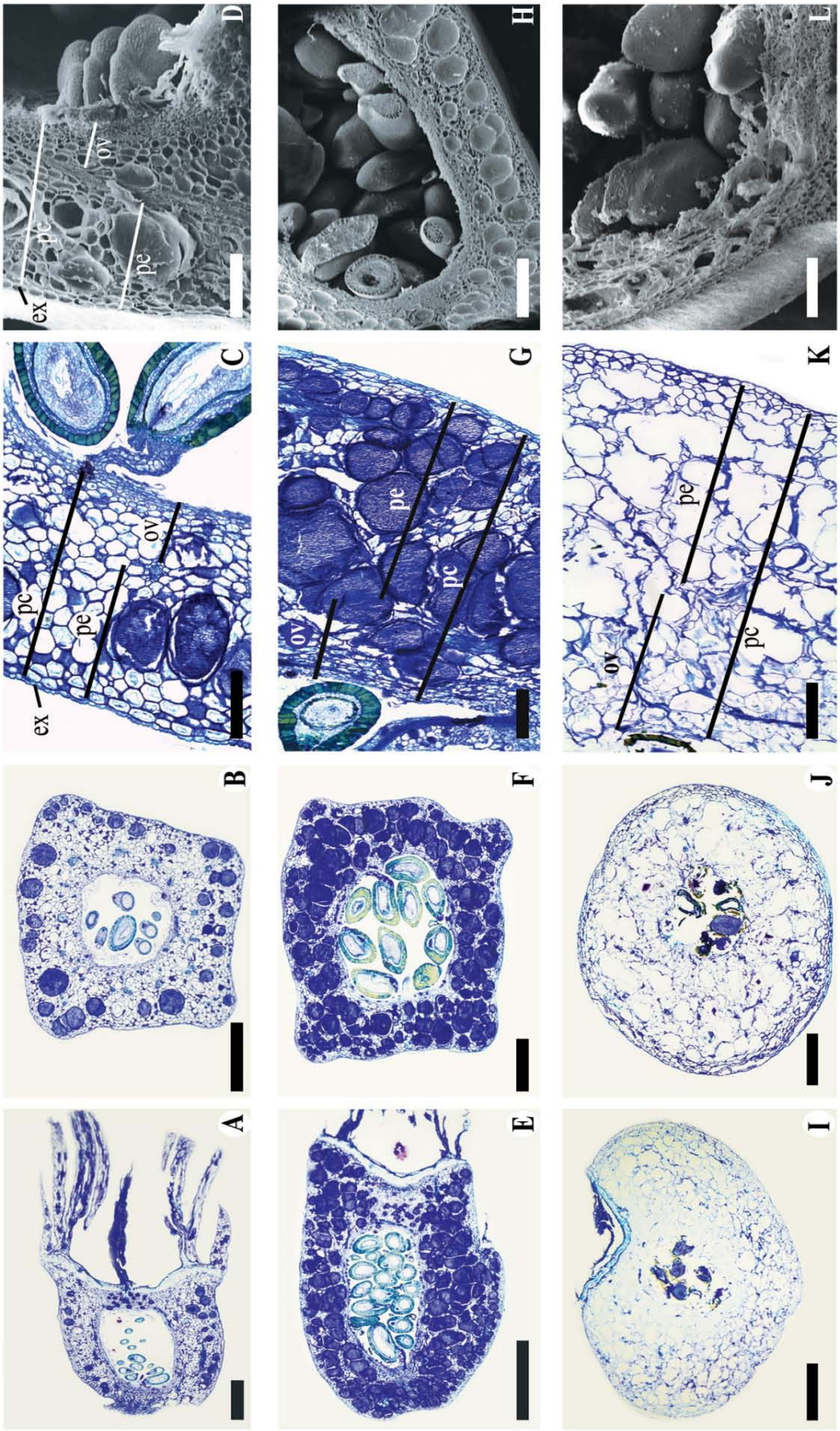
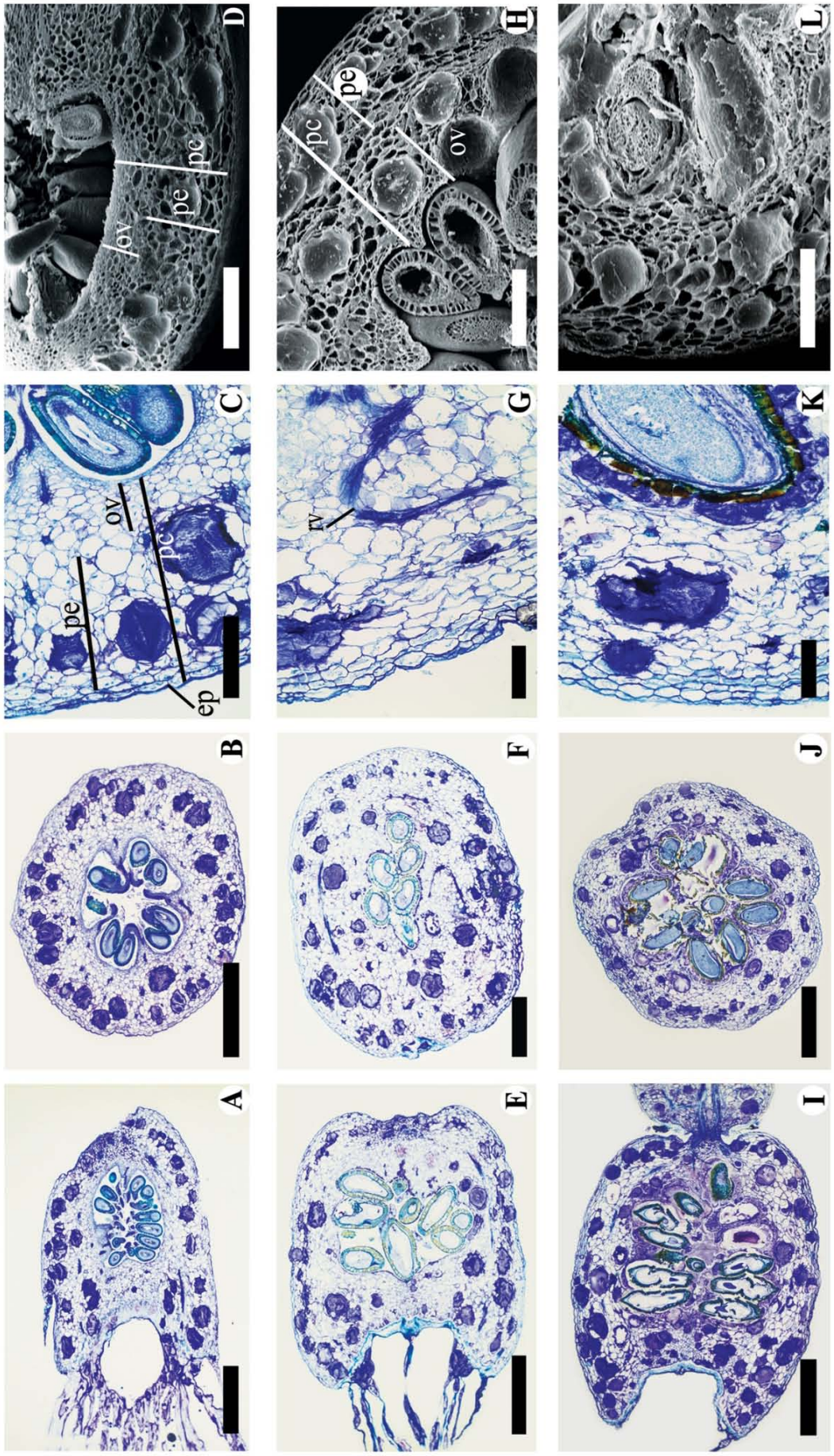

A
B
C
D
E
F
G
H
I
J
K
L
ex
pe
ov
pc

FIG. 4. Anatomy and SEM of the fruit of *Rhipsalis cereuscula* in cross (CS) longitudinal (LS) sections. A. Post-anthesis young fruit (LS). B. Post-anthesis young fruit (CS). C. Detail of the pericarp. D. SEM of the pericarp and ovules. E. Young fruit (LS). F. Young fruit (CS). G. Detail of the pericarp. H. SEM part of the young fruit. I. Mature fruit (CS). J. Mature fruit (CS). K. Detail of the pericarp. L. SEM of the pericarp and seed of mature fruit. ex – exocarp, ov – ovary in origin, pc – pericarp, pe – pericarpel in origin, rv – recurrent vascular bundle. Bars – 1 mm (A, B, F), 300 µm (C),1,5 mm (E, I, J), 200 µm (G, K), 400 µm (H), 500 µm (D, L).


D
ov
pe
pc
H
pc
pe
ov
L
C
ov
pc
pe
ep
G
tv
K
B
F
J
A
E
I

CAPÍTULO 3

Estrutura e desenvolvimento seminal de *Hylocereus undatus* (Hylocereeae), *Lepismium warmingianum* e *Rhipsalis cereuscula* (Rhipsalideae) com contribuição à taxonomia

Odair José Garcia de Almeida[1,2], Hugo Cota-Sánchez[2], Arthur R. Davis[2], Adelita Aparecida Sartori Paoli[1].

(1) Departamento de Botânica, IB, UNESP, Rio Claro, SP, 13506-900, Brasil.

(2) Department of Biology, University of Saskatchewan, Saskatoon, SK, S7N5E2, Canada

A ser submetido para publicação no periódico Plant Systematics and Evolution

RESUMO

Cactaceae é composta por 124 gêneros e 1438 espécies exclusivas das Américas, exceto *Rhipsalis baccifera* que ocorre naturalmente na África e Ásia. O conhecimento da semente é importante para entender as relações dentro da família devido à grande diversidade em tamanho, forma, coloração e padrão da testa. Aliado a isso, esses caracteres são considerados estáveis, pois não são modificados por fatores ambientais. Esse estudo visou elucidar a estrutura e o desenvolvimento de sementes de três espécies de cactáceas epífitas, *Hylocereus undatus* (Hylocereeae), *Lepismium warmingianum* e *Rhipsalis cereuscula* (Rhipsalideae), desde a flor em pós-antese, com o uso de microscopia óptica e eletrônica de varredura. Os resultados revelaram características com potenciais taxonômicos como: o tipo e a morfologia do óvulo, o tamanho e a forma da semente (incluindo região hilo-micropilar e presença/ausência de quilha), e a morfologia do embrião. Esses caracteres podem ser usados como modelo em futuros estudos com um número maior de táxons, a fim de encontrar atributos comuns a determinados grupos na família Cactaceae.

Palavras-chave: Anatomia - Cactaceae – Cactos epífitos – Morfologia – Óvulo – Semente

INTRODUÇÃO

O potencial taxonômico dos caracteres seminais foi notado há mais de 150 anos, mas foi com o advento da MEV, a partir da década de 1960, que a observação, interpretação e ilustração de uma variedade de microestruturas, antes invisíveis ao microscópio óptico, foram facilitadas aos pesquisadores (Barthlott & Hunt 2000). Desde então surgiram trabalhos como o pioneiro estudo feito por Buxbaum (1955) que contém uma descrição geral da semente para toda a família; o artigo de Engleman (1960) sobre o desenvolvimento das espécies *Astrophytum myriostigma* Lem., *Thelocactus bicolor* Britton & Rose e *Toumeya papyracantha* Britton & Rose; o de Barthlott & Hunt (2000), no qual os autores analisaram a morfologia externa da semente em MEV de mais de 100 gêneros da subfamília Cactoideae; o de Rosa & Souza (2003) que descreveram o desenvolvimento da semente de uma espécie do gênero basal *Pereskia* Mill.; o de Arias & Terrazas (2004) que investigaram as variações morfológicas da semente em *Pachycereus* (Berger) Britton & Rose *spp.* e, o de Cota-Sánchez & Bomfim-Patrício (2010) que analisaram a morfologia externa das sementes em MEV, além de outras características para encontrar padrões de variações intraespecíficas em *Rhipsalis baccifera* (Muell.) Stearn., dentre outros estudos.

Cactaceae é composta por 124 gêneros e 1438 espécies (Hunt et al. 2006) que estão frequentemente relacionadas a ambientes áridos. Entretanto, cerca de 130 espécies ocorrem em florestas neotropicais e tropicais como cactáceas epífitas (Wallace & Gibson 2002). Essas espécies são exclusivas nas Américas, com exceção da epífita *R. baccifera* que ocorre na África e Asia (Barthlott & Hunt 1993). Está dividida em quatro subfamílias: Pereskioideae, Maihuenioideae, Opuntioideae e Cactoideae *sensu* Anderson (2001), sendo Cactoideae a mais diversa, apresentando maior número de espécies e maior variação de formas e hábitos (desde espécies terrestres, escandentes, rupícolas e epífitas). Compreende nove tribos, nas quais duas possuem, exclusivamente, espécies epífitas ou litófitas: Hylocereeae, característica da

América Central com poucas espécies que se estendem até a América do Sul; e Rhipsalideae característica da América do Sul, onde a maioria das espécies ocorre na Mata Atlântica Brasileira (Barthlott & Hunt 1993).

Sabe-se que o conhecimento da semente é importante para entender as relações dentro da família, devido a grande diversidade em tamanho, forma, coloração e padrão do tegumento externo (testa) (Barthlott & Hunt 2000). Além disso, esses caracteres são considerados estáveis, pois não apresentam modificações por fatores ambientais (Anderson 2001). Assim, este estudo teve como objetivo descrever e comparar o desenvolvimento estrutural da semente, desde a flor em pós-antese, em três espécies de cactáceas epífitas, *Hylocereus undatus* (Haw.) Britton & Rose (Hylocereeae), *Lepismium warmingianum* (Schum.) Barth. e *Rhipsalis cereuscula* Haw. (Rhipsalideae), a fim de levantar caracteres morfoanatômicos e de desenvolvimento que possam ser úteis em novas classificações; além de contribuir como modelo para estudos futuros envolvendo a morfologia e filogenia deste grupo peculiar de plantas.

MATERIAL E MÉTODOS

Informações sobre as espécies

Sementes de *Rhipsalis cereuscula*, *Lepismium warmingianum* e *Hylocereus undatus*, provenientes de frutos em vários estágios de desenvolvimento foram coletadas entre os anos de 2009 a 2011 no Parque do Ingá (Unidade Municipal de Conservação) em Maringá, PR e no Parque Ecológico da Pavuna em Botucatu, SP, Brasil. Materiais herborizados foram depositados no Herbário da Universidade Estadual de Maringá (HUEM) e no Herbário Rioclarense HRCB, na Universidade Estadual Paulista (UNESP) conforme a Tabela 1.

Hylocereus undatus, é uma espécie lianescente e posteriormente epífita secundária (nos primeiros estágios de vida mantém o contato das raízes com o solo, mas na maturidade

torna-se completamente epífita), está incluída em Hylocereeae. Produz grande número de segmentos caulinares verde-escuro, de formato triangular com espinhos nas aréolas. Por ser uma espécie largamente cultivada nas Américas e no sudeste Asiático, sua origem é incerta, mas acredita-se que seja nativa da Costa Caribenha (Anderson 2001; Hunt 2006).

Lepisimum warmingianum pertence à Rhipsalideae, é um arbusto epífito pendente de segmentos caulinares achatados. Ocorre no Brasil, leste Paraguaio e nordeste Argentino (Anderson 2001; Hunt 2006).

Rhipsalis cereuscula pertence à Rhipsalideae, é um arbusto epífito que se ramifica muito, e apresenta segmentos caulinares cilíndricos. A espécie é natural da Bolívia, Paraguai, Uruguai e Sul do Brasil (Anderson 2001; Hunt 2006).

Tabela 1. Coleta e registro em Herbário das espécies.

Espécie	**Local de coleta**	**Coletor /Número**
Rhipsalis cereuscula Haw.	Parque do Ingá, Maringá/PR - Brasil	O.J.G. Almeida / 006
Lepismium warmingianum (Schum.) Barth.	Parque do Ingá, Maringá/PR - Brasil	O.J.G. Almeida /007
L. warmingianum (Schum.) Barth.	Parque Ecológico da Pavuna, Botucatu/SP - Brasil	L. B. Santos & O.J.G. Almeida / 452
L. warmingianum (Schum.) Barth.	Parque do Ingá, Maringá/PR - Brasil	O.J.G. Almeida / 009
Hylocereus undatus (Haw.) Britton & Rose	Parque do Ingá, Maringá/PR - Brasil	O.J.G. Almeida / 016

Análise em microscopia óptica e em Microscopia Eletrônica de Varredura

Para a análise morfológica das sementes, as amostras foram fixadas em FAA50 por sete dias e depois transferidas para etanol 70% (Johansen 1940), sendo analisadas em estereomicroscópio Leica. As ilustrações foram realizadas por câmera digital Sony Cyber-Shot modelo DSX-HX1.

Para a análise em Microscopia Eletrônica de Varredura (MEV), o material fixado e conservado em etanol 70% foi cuidadosamente dissecado e submetido à desidratação gradual em série etílica. Devido à grande quantidade de mucilagem e capacidade em reter água, dos tecidos, foi realizada uma série adicional de desidratação em etanol/acetona: *i*) etanol/acetona (3:1) = 30 min; *ii*) etanol/acetona (1:1) = 30 min; *iii*) etanol/acetona (1:3) = 30 min; *iv*) acetona (100%) = 30 min; e *v*) acetona (100%) = 30 min. Imediatamente após a desidratação, as amostras foram submetidas ao ponto crítico de secagem in CO_2 líquido (Polaron Instruments E3000), fixadas em suportes de alumínio, metalizadas em ouro (Edwards Sputter Coater S150B) e, examinadas em MEV (Philips SEM 505). As imagens de MEV foram adquiridas por revelação de filmes positivo/negativo Polaroid 665 e/ou através do programa Animator DV (captura de imagens).

Para o estudo anatômico, sementes em vários estágios de desenvolvimento foram embebidas em hidroxietil metacrilato (Leica historesin) (conforme instruções do fabricante) e/ou em parafina (Paraplast wax) usando uma versão modificada do protocolo utilizado por Davis et al. (1988). As amostras, previamente, fixadas em FAA50, foram desidratadas em série etílica, embebidas em historresina, conforme instruções do fabricante, seccionadas transversal e longitudinalmente em micrótomo de rotação (8 – 12 µm). As secções foram coradas em azul de Toluidina com pH de 4,6 (O'Brien et al. 1965) e montadas em lâmina e lamínula com resina sintética Entellan. Em parafina, o material fixado em FAA50 foi desidratado em série crescente de etanol/n-butanol (Jensen 1962), depois embebido em Paraplast dentro de estufa à 60º C por pelo menos 10 dias, sendo realizadas duas trocas de

Paraplast para remoção de qualquer resíduo de n-butanol. O material foi seccionado com 7-12 µm de espessura em micrótomo de rotação, montado em lâmina aquecida e corado com azul de Toluidina (O'Brien & McCully 1981). Após remoção da parafina, utilizando xilol, as lâminas foram cobertas com lamínulas usando resina sintética Permount ou Entellan. Testes microquímicos foram realizados para identificar substâncias em material fresco ou fixado seccionado manualmente. Utilizou-se Lugol para detecção de amido; cloreto férrico, acrescido de carbonato de cálcio, para verificar a ocorrência de substâncias fenólicas; Sudan IV para identificar lipídeos (Johansen 1940); vermelho de Rutênio para identificar polissacarídeos e pectinas (Jensen 1962) e mercúrio de azul bromofenol para identificar proteínas (Mazia et al. 1953).

As imagens referentes ao estudo anatômico foram obtidas em sistema de captura de imagens Leica ICC50. As pranchas foram editadas utilizando o programa Photoshop CS3. A nomenclatura utilizada para descrever e classificar a semente foi baseada em Corner (1976), Martin (1946) e Barthlott & Hunt (2000).

RESULTADOS

As espécies analisadas apresentam óvulos bitegumentados, com micrópila delimitada pelo tegumento interno (Figs. 1A-C; 2A-C, J, K; 3A, G-I). Tanto o tegumento externo como o interno apresentam três camadas de células de paredes delgadas. O tamanho do funículo e o tipo de óvulo variam entre as espécies: *Hylocereus undatus* apresenta óvulo circinótropo com funículo longo (Figs. 1A,B), em *Lepismium warmingianum* o óvulo é anátropo (Figs. 2A, B) e, *Rhipsalis cereuscula* possui óvulo circinótropo, com funículo bem reduzido (Fig. 1J).

Depois da fecundação o zigoto inicia processo de divisão celular, e o endosperma em formação é do tipo nuclear, caracterizado por apresentar núcleos livres que se dividem antes da formação da parede celular (Fig. 1B). O tegumento externo (testa) inicia o processo de

diferenciação inicialmente na camada mais externa, ou seja, na exotesta, na qual as células epidérmicas aumentam em tamanho e iniciam processo de produção e acúmulo de compostos fenólicos (Figs. 1D-F; 2B, E, K, N), exceto na região hilo-micropilar (RHM) (Figs. 1E; 3G, I). As demais camadas da testa (meso- e endotesta), bem como as camadas mais externas do tegmen (exo- e mesotegmen) permanecem parenquimáticas com paredes delgadas que se alongam periclinalmente. O endotegmen inicia processo de diferenciação, com deposição de suberina na parede das células, porém estas não aumentam tanto em tamanho quanto às células da exotesta (Figs. 1D; 2F).

Durante o desenvolvimento do embrião, as espécies compartilham as fases de embrião globular (Figs. 1D, E; 2D) e cordiforme (Fig. 1G) e, juntamente com o desenvolvimento do embrião, o endosperma nuclear (Figs. 1E, D; 2F) tem seus núcleos livres proliferados e celularizados (Figs. 1G; 2L, M). O tecido nucelar (Figs. 1C; 2C) permanece após a fertilização (Figs. 1D,E; 2F,N); e tanto o nucelo quanto o endosperma nutrem o embrião durante a sua formação. Ao final do desenvolvimento, o embrião ocupa praticamente todo interior da semente (Figs. 1I; 2G, O; 3E, M) com substância de reserva de natureza lipo-protéica. Em *H. undatus* o embrião é maior, curvado e possui dois grandes cotilédones (Figs. 1I, 3D, F), enquanto que os das sementes de *R. cereuscula* e *L. warmingianum* são menores, retos com dois pequenos cotilédones (Figs. 2G, O; 3M). Em todas as espécies o embrião apresenta protoderme formada por células cuboides (exceto no ápice radicular onde as células são alongadas no sentido anticlinal); meristema fundamental; e cordões procambiais no eixo hipocótilo-radícula, que se bifurcam e seguem um para cada cotilédone (Fig. 1I-K; 2G, O).

A semente madura varia em tamanho, sendo que em *H. undatus* têm entre 2,3-2,6 mm de comprimento (Figs. 1I, 3B) e as de *R. cereuscula* e *L. warmingianum* tem comprimento entre 1,0 e 1,2 mm (Figs. 2G, O; 3J, Q). A superfície externa da semente de *H. undatus* é lisa e a RHM é discreta (Fig. 3B), mas a semente possui uma quilha que se destaca na região

dorsal próximo à RHM (Figs. 3B, C). Nas espécies de Rhipsalideae a superfície é levemente sinuosa, com células da exotesta apresentando contornos evidentes, são maiores na largura e possuem formas variadas (Figs. 2I; 3J, K, Q, R); *L. warmingianum* possui uma proeminente quilha na região dorsal (Fig. 3J), enquanto *R. cereuscula* não apresenta quilha (Fig. 3A). Em todas as sementes a exotesta é constituída de grandes células lignificadas, (Figs. 1D, I, J; 2E-N; 3A, D, E, G-I, N, S). As demais camadas da testa (meso- e endotesta) juntamente às camadas mais externas do tegmen (exo- e mesotegmen) sofrem compressão devido ao crescimento do embrião. O endotegmen suberifica-se e forma uma bolsa que envolve o embrião (Figs. 1D, K; 2F; 3I, L). Permanecem 1-2 camadas de endosperma e de perisperma (Figs. 1J, K; 2P; 3M). A RHM forma um único complexo, que varia de forma e tamanho, sendo maior e curvada na semente de *H. undatus* (Figs. 3B, J). A diferenciação da RHM difere do restante do tegumento da semente, em consequência dessa região ser delimitada apenas pelo tegmen. Nesse local as células são menores (Figs. 1D-F; E, J, K, N), e a esclerificação não ocorre nas camadas mais externas (como acontece na testa), ou seja, as camadas superficiais de células na RHM permanecem parenquimáticas (Figs. 1D-F; 2C, K, N). A diferenciação da semente ocorre antes do amadurecimento do fruto.

DISCUSSÃO

O óvulo pode conter características de importância taxonômica, pelo menos, para a categoria de gênero dentre os representantes de cactos epífitos, principalmente, em relação ao tamanho do funículo: como foi verificado no óvulo anátropo em *Lepismium* Pfeiff., circinótropo em *Rhipsalis* Gaerth. com funículos reduzidos e circinótropo com um longo funículo em *Hylocereus* (Berger) Britton & Rose. *Epiphyllum phyllanthus* (L.) Haw., outra espécie epífita de Hylocereeae, também apresenta óvulos circinótropos de longo funículo (Almeida et al. 2010). Apesar dos óvulos apresentarem algumas características aparentemente

distintas entre gêneros, também apresentam características comuns aos integrantes de Cactaceae: são bitegumentados com micrópila delimitada apenas pelo tegumento interno, como descrito por Corner (1976), Bouman (1984) e Werker (1997).

As principais características da semente nas espécies analisadas são comuns para Cactaceae, especialmente quanto à morfologia. Segundo, Barthlott & Hunt (2000) sete tipos principais de sementes foram descritos para Cactaceae, levando-se em conta o formato, a saber: *Cereus*, *Rhipsalis*, *Arequipa*, *Notocactus*, *Thrixanthocereus*, *Astrophtum* e *Blossfeldia*, derivadas do tipo ancestral *Pereskia*, conforme discutido por Cota-Sánchez & Bomfim-Patrício (2010). Nesse caso as sementes de *L. warmingianum* e *R. cereuscula* podem ser classificadas como tipo *Rhipsalis* (“*narrowly mussel-type*”), de tamanho pequeno (0,9-1,1mm) e RHM que forma complexo único; as de *H. undatus* como tipo *Rhipsalis*, de tamanho grande e RHM em complexo único, curvado e maior que nas sementes de *L. warmingianum* e *R. cereuscula*, chamado pelos autores de “*curved-band variant*”. Os demais gêneros de Hylocereeae, *Epiphyllum* Haw. (Barthlott & Hunt 2000; Almeida 2009); *Selenicereus* (Berger) Britton & Rose, *Disocactus* Lind., *Weberocereus* Britton & Rose (Barthlott & Hunt 2000) também possuem sementes grandes e com RHM que formam um complexo único e curvado.

De acordo com Corner (1976), sementes de Cactaceae são consideradas exotestais por apresentarem a exotesta como a principal camada mecânica da testa, com células grandes e de paredes lignificadas. As espécies analisadas nesse estudo estão de acordo com as observações do autor para a família.

A presença de perisperma nas sementes de Cactaceae é descrita na literatura por Corner (1976). Entretanto, do ponto de vista do desenvolvimento, o nucelo persistente (perisperma), juntamente com o endosperma nutrem o embrião durante seu desenvolvimento, sendo quase totalmente consumidos durante esse processo. Na semente madura restam apenas

resquícios celulares tanto de perisperma como de endosperma e as substâncias de reserva encontram-se no próprio embrião, que é de natureza proteica. Padrão similar de desenvolvimento foi encontrado na semente de *Epiphyllum phyllanthus* (Almeida et al. in press).

Segundo Johri et al. (1992) em subfamílias basais (Pereskeioideae e Opuntioideae) foi observado perisperma bem desenvolvido localizado na curvatura da semente, entre o hipocótilo e os cotilédones, ao passo que em Cactoideae, subfamília considerada derivada, o perisperma desapareceu e a função de reserva passou para o hipocótilo. De fato, Rosa & Souza (2003) encontraram uma faixa de perisperma composta por uma a cinco camadas de células com reserva oleaginosa e amilácea e ainda, resíduos do endosperma na semente madura de *Pereskia aculeata* Mill., apoiando essa idéia.

A morfologia do embrião na semente madura difere nas espécies estudadas, e isso pode ser uma característica útil para distinguir as tribos Hylocereeae e Rhipsalideae, uma vez que, *Rhipsalis* e *Lepismium* apresentaram cotilédones reduzidos, enquanto que *Hylocereus* apresentou embrião com cotilédones relativamente grandes. Em *Epiphyllum phyllanthus* (Almeida 2009), também é registrada a presença da semente de embrião com grandes cotilédones similares aos observados em *Hylocereus undatus*. De acordo com a classificação de Martin (1946), o embrião é periférico em Cactaceae; tanto nas espécies estudadas aqui como em *E. phyllanthus* (Almeida et al. in press), o embrião se enquadra nessa categoria, ocupando grande parte do interior da semente, e com grande quantidade de substâncias lipoprotéicas como reserva.

A quilha (ou crista) encontrada na região dorsal próximo da RHM da semente de *Hylocereus undatus* pode representar uma característica comum ao gênero, pois as espécies *H. lemairei* (Hook.) Britton & Rose, *H. minutiflorus* Britton & Rose e *H. trigonus*(Haw.) Saff. (Barthlott & Hunt 2000) também compartilham essa estrutura, ao passo que outras

espécies epífitas de Hylocereeae não apresentam, como por exemplo, *E. phyllanthus* (Barthlott & Hunt 2000; Almeida 2009); *Selenicereus spp.*, *Disocactus spp. Weberocereus spp.* (Barthlott & Hunt 2000). Para Rhipsalideae, é prematura qualquer especulação quanto à presença ou ausência de quilha na semente, devido ao número limitado de dados em relação à quantidade de gêneros, espécies e subespécies existentes.

Assim os caracteres observados como o tipo de óvulo, morfologia, o tamanho e a forma da semente (incluindo a RHM e a presença ausência de quilha), e morfologia do embrião, podem ser usados como modelo em futuros estudos com um número maior de táxons, a fim de encontrar atributos comuns a determinados grupos dentro da família. Para um sistema filogenético ser formulado é preciso estudos pormenorizados de todas as partes da planta (Buxbaum 1955). À luz das modernas filogenias moleculares para a família (Nyfeler 2002; Hernández-Hernández et al. 2011; Bárcenas et al. 2011), e para grupos de espécies epífitas (Calvente et al. 2011, Korotkova et al. 2011), dados estruturais morfoanatômicos são ferramentas valiosas para entender melhor o curso da evolução e filogenia.

AGRADECIMENTOS

Os autores são gratos à A. Lonardoni pela revisão crítica do manuscrito; à G. Liu por auxílio técnico junto ao MEV; ao Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, por auxílio financeiro (474068/2009-9) e bolsa produtividade em pesquisa (300495/2010-2) para AASP, bolsa de doutorado (141861/2009-6) para OJGA; ao Governo do Canadá (CBIE/DFAT) por auxílio financeiro, através do programa ELAP para OJGA; “National Geographic Society” (7382-02) e Universidade de Saskatchewan “Tri-Council Bridge” (411051) por auxílios financeiros à JHCS.

REFERÊNCIAS

Almeida O J G (2009) Morfoanatomia dos órgãos reprodutivos e da plântula de *Epiphyllum phyllanthus* (L.) Haw. (Cactaceae). Dissertação, Universidade Estadual Paulista Julio de Mesquita Filho

Almeida OJG, Paoli AAS, Souza LA (2010) Flower morpho-anatomy in *Epiphyllum phyllanhtus* (Cactaceae). Rev Mex Biodivers 81:65-80

Almeida OJG, Paoli AAS, Souza LA, Cota-Sánchez, JH (in press) Seedling morphology and development in the epiphytic cactus *Epiphyllum phyllanthus* (L.) Haw. (Cactaceae:Hylocereeae). J Torrey Bot Soc

Anderson EF (2001) The cactus family. Timber Press, Portland, OR

Arias S, Terrazas T (2004) Seed morphology and variation in the genus *Pachycereus* (Cactaceae). J Plant Res 117: 277-89. doi: 10.1007/s10265-004-0156-4

Bárcenas RT, Yesson C, Hawkins JA (2011) Molecular systematics of the Cactaceae. Cladistics 27:470-489

Barthlott W Hunt D (2000) Seed-diversity in the Cactaceae. Succul Plant Res 5:1-173

Barthlott W, Hunt DR (1993) Cactaceae. In: Kubitzki KR, Bittrich JG, (ed) The Families and genera of vascular plants, 3rd edn. Springer, Berlin, pp 161-197.

Bouman F (1984) The ovule. In: Johri BM (ed.) Embryology of Angiosperm, 3rd edn. Springer-Verlag, Berlin, pp 123-155

Buxbaum F (1955) Morphology of cacti. Section III. Fruits and seeds. Abbey Garden Press, Pasadena

Calvente A, Zappi DC, Foirest F, Lonmann (2011) Molecular phylogeny of tribe Rhipsalideae (Cactaceae) and taxonomic implications for *Schlymbergera* and *Hatiora*. Molec Phylog Evol 58:456-468. doi:10.1016/j.ympev.2011.01.001

Corner EJH (1976) The seeds of dicotyledons. Cambridge University Press, Cambridge

Cota-Sánchez H, Bomfim-Patrício C (2010) Seed morphlogy, polyploidy and the evolutionary history of the epiphitic cactus *Rhipsalis baccifera* (Cactaceae). Polibotánica 29:107-129

Cota-Sánchez JH, Abreu DD (2007) Vivipary and offspring survival in the epiphytic cactus *Epiphyllum phyllanthus* (Cactaceae). J Exp Bot 58:3865-3873. doi:10.1093/jxb/erm232

Davis AR, Pylatuik JD, Paradis JC, Low NH (1988) Nectar-carbohydrate production and composition vary in relation to nectary anatomy and location within individual flowers of several species of Brassicaceae. Planta 205: 305-318. doi.org/10.1007/s004250050325

Engleman EM (1960). Ovule and seed development in certain cacti. Am J Bot 47:460-467

Hernández-Hernández T, Hernández HM, De-Nova JA, Puente R, Eguiarte LE, Magallón S (2011) Phylogenetic relationships and evolution of growth form in Cactaceae (Caryophyllales, Eudicotyledoneae). Am J Bot 98:44-61

Hunt D (2006) The new cactus lexicon. Dhbooks, London

Jensen WA (1962) Botanical histochemistry: principle and practice. WH Freeman, San Francisco

Johansen DA (1940) Plant microtechnique. McGraw Hill Books, New York

Johri BM, Ambegaokar KB, Srivastava PS (1992) Comparative embriology of Angiosperms. Vol. 1. Springer-Verlag, Berlin.

Korotkova N, Borsch T, Quandt D, Taylor NP, Müller KF, Barthlott W (2011) What does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic Rhipsalideae (Cactaceae). Am J Bot 98:1549-1572. doi: 10.3732/ajb.1000502

Martin, AC (1946) The comparative internal morphology of seeds. Am Midl Nat 36:513-660.

Mazia D, Brewer PA, Alfert M (1953) The cytochemical staining and measurement of protein with mercuric bromphenol blue. Biol Bull 104:57-67

Nyffeler R (2002) Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trn* K/*mat* K and *trn* L-*trn* sequences. Am J Bot 58:456-468

O'Brien TP, McCully, ME (1981) The Study of Plant Structure: Principles and Selected Methods. Termarcarphi, Melbourne

O'Brien TP, Feder N, Maccully ME (1965) Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59:368-373

Rosa SM., Souza LA (2003) Morfo-anatomia do fruto (hipanto, pericarpo e semente) em desenvolvimento de *Pereskia aculeata* Miller (Cactaceae). *Acta Sci Biol Sci* 25:415-428.

Wallace RS, Gibson AC (2002) Evolution and systematics. In: Nobel PS (ed) Cacti biology and uses, University of California Press, Berkeley, pp 1-21

Werker E (1997) Seed anatomy. Gebrüder Borntrager, Berlin

Fig. 1 Anatomia da semente de *Hylocereus undatus* em seções longitudinais (SL) e transversais (ST). A. ST de parte do ovário pós-antese. B. Óvulos em SL e ST de flor pós-antese. C. Detalhe da região micropilar do óvulo (pós-antese). D. Semente jovem, embrião globular. E. Detalhe da semente jovem, embrião globular e parte da RHM. F. Semente jovem RHM e funículo, G. Semente jovem, embrião mais ou menos cordiforme. H. Detalhe de parte da semente madura em fruto próximo do amadurecimento. I. Semente madura. J-I. Detalhes da região ventral na semente madura. (ce: camada de células esclerenquimáticas, cm: camada de células mucilaginosas, cp: camada de células parenquimáticas, eg: embrião globular, em: embrião, en: endosperma, ep: embrião, formato cordiforme, et: endotégmen, ex: exotesta, fu: funículo, fv: feixe vascular, nu: nucelo, mi: micrópila, mu: mucilagem, pe: perisperma, po: polpa, ov: ovário, RHM: região hilo-micropilar, se: saco embrionário, si: sinérgide, te: tegumento externo, ti: tegumento interno, ts: tegumento seminal, zi: zigoto). Barras: 1mm (A, I), 200µm (B, D, H), 50 µm (C, F, K), 300 µm (E, J), 100 µm (G).

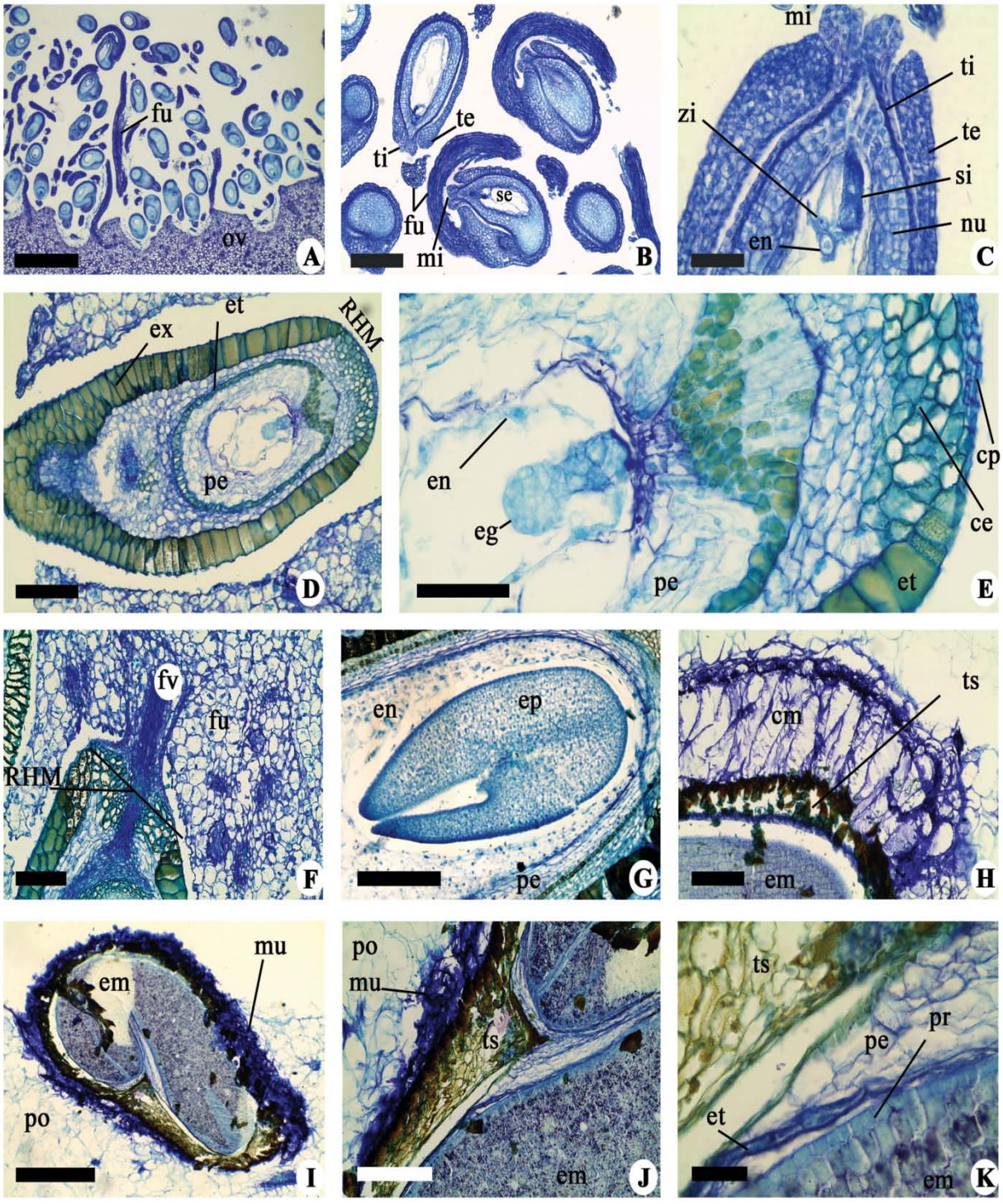

fu
ov
A
te
ti
se
fu
mi
B
mi
ti
zi
te
si
nu
en
C
et
ex
RHM
pe
D
en
eg
cp
ce
pe
et
E
fv
fu
RHM
F
en
ep
pe
G
ts
cm
em
H
mu
em
po
I
po
mu
ts
em
J
ts
pr
pe
et
em
K

Fig. 2 Anatomia de sementes de *Lepismium warmingianum* e *Rhipsalis cereuscula* em seções longitudinais (SL), transversais (ST) e paradérmico (SP). A-I. *Lepismium warmingianum*. A. Cavidade seminal de fruto jovem (pós-antese) em SL, com sementes jovens em SL e ST, notar semente jovem proveniente de óvulo anátropo. B. Semente jovem (pós-antese) proveniente de óvulo anátropo, notar RHM. C. Semente jovem em ST. D. Detalhe da cavidade embrionária em SL, com embrião em estágio globular. E. Semente em desenvolvimento SL. F. Detalhe da cavidade embriológica (SL). G. Semente madura em SL. H. Detalhe da testa (SL) de semente desenvolvida. I. Detalhe da exotesta em SP. J-P. *Rhipsalis cereuscula*. J. Cavidade seminal de fruto em pós-antese em ST com sementes jovens em SL. K. Semente jovem (pós-antese) em ST. L. Detalhe da cavidade embriológica em SL, com embrião em estágio de coração. M. Parte da cavidade seminal de fruto em desenvolvimento (ST). N. Parte de semente jovem em SL. O. Semente desenvolvida em SL. P. Detalhe da região da testa de semente desenvolvida em SL. (cv: cavidade embrionária, ce: camada de células esclerenquimáticas, cp: camada de células parenquimáticas, eg: embrião globular, em: embrião, en: endosperma, ec: embrião, forma de coração, et: endotégmen, ex: exotesta, fu: funículo, fv: feixe vascular, hi: hipocótilo, nu: nucelo, mi: micrópila, mu: mucilagem, pe: perisperma, ov: ovário, RHM: região hilo-micropilar, su: suspensor, te: tegumento externo, ts: tegumento seminal). Barras: Barras: 400µm (A), 200 µm (B, G, M), 50 µm (C, D, F, H, I, K, L, P), 250 µm (E), 250 µm (J), 300 µm (N, O).

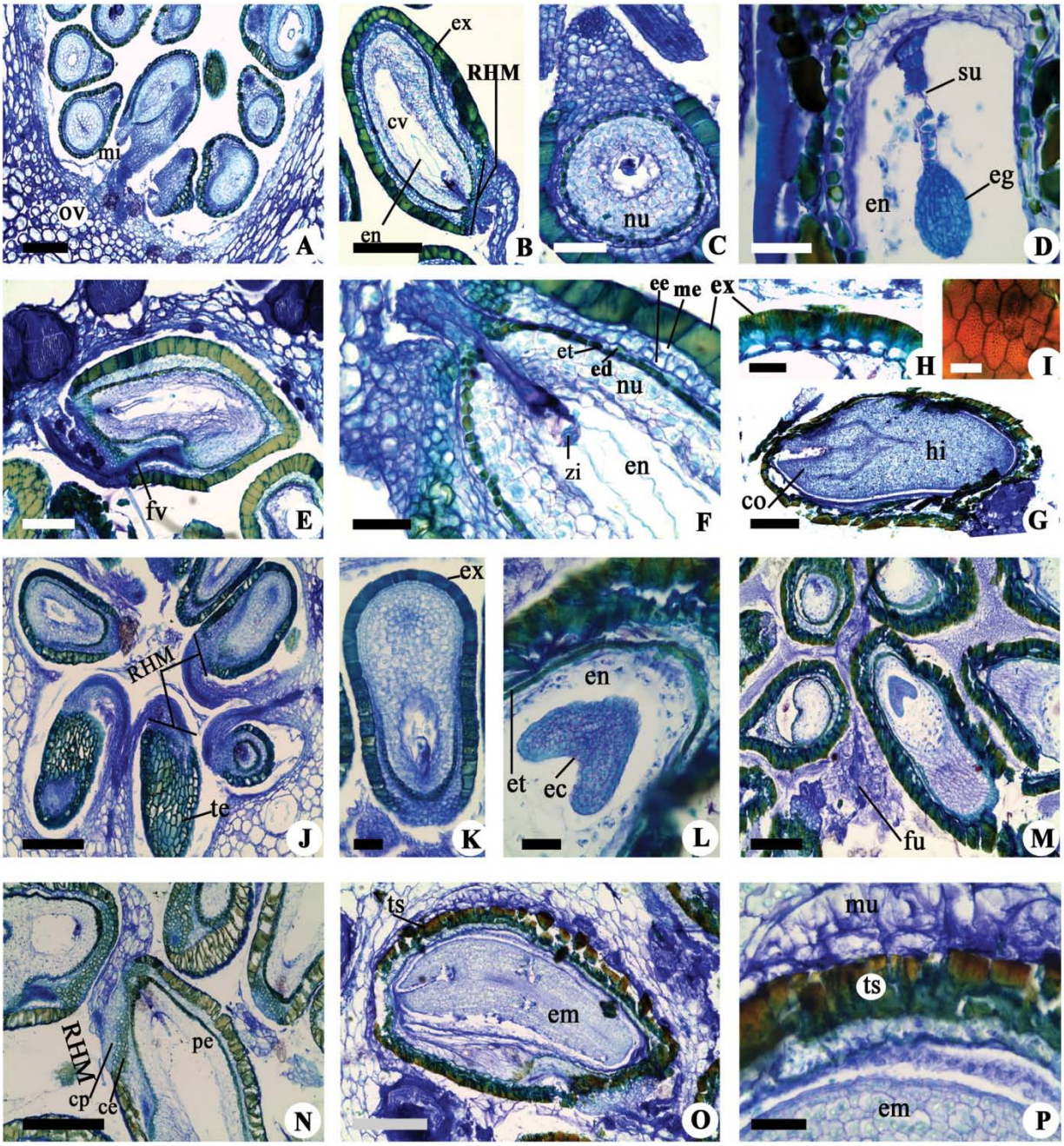
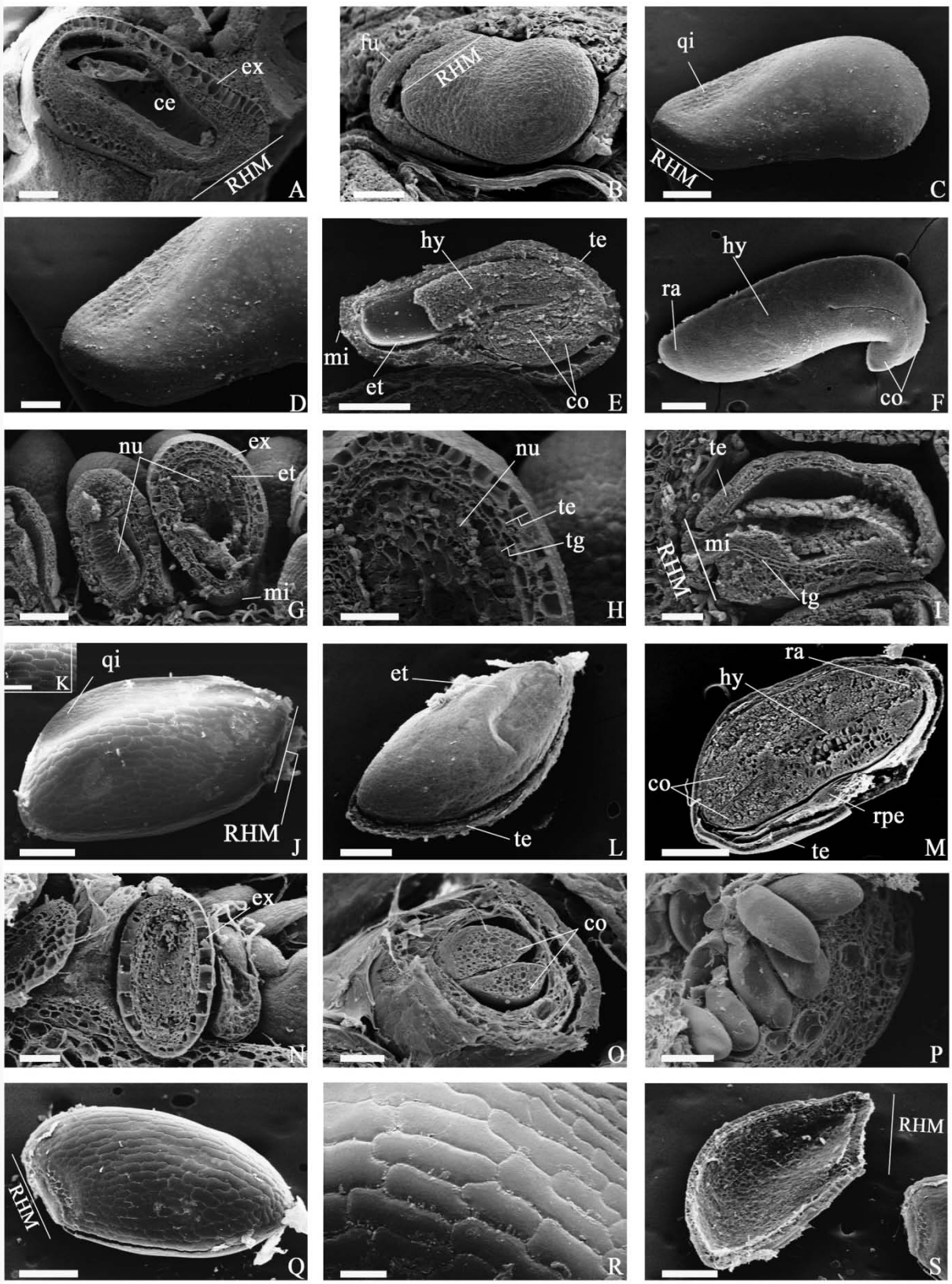

mi
ov
A
ex
RHM
cv
en
B
nu
C
su
eg
en
D
fv
E
ee
me
ex
et
ed
nu
zi
en
F
H
I
hi
co
G
RHM
te
J
ex
K
en
et
ec
L
fu
M
RHM
cp
ce
pe
N
ts
em
O
mu
ts
em
P

Fig. 3 MEV da semente de cactáceas epífitas. **A-F.** *Hylocereus undatus*. **A.** Semente jovem em seção longitudinal. B. Semente jovem. C**.** Semente madura. **D.** Detalhe da RHM e de parte da quilha. **E.** Semente em seção longitudinal. **F.** Embrião em vista lateral. **G-M.** *Lepismium warmingianum*. **G.** Semente jovem proveniente de óvulo anátropo em seção longitudinal. **H.** Detalhe de parte dos tegumentos e do nucelo. **I.** Semente jovem. **J.** Semente madura, vista lateral **K.** Detalhe das células da exotesta. **L.** Semente em seção longitudinal, com embrião envolto pela camada de células do endotegmen. **M.** Semente em seção longitudinal, com embrião reto com pequenos cotilédones. N-S. *Rhipsalis* cereuscula. N. Semente jovem proveniente em seção transversal. M. Semente próxima do amadurecimento em seção transversal. P. Parte do fruto com sementes provenientes de óvulos circinótropos. Q. Semente madura em vista lateral. R. Detalhe da testa. S. Semente em seção longitudinal, vista interna da cavidade sem o embrião. (ce: cavidade embrionária, co: cotilédones, et: endotegmen, ex: exotesta, fu: funículo, hy: hipocótilo, mi: micrópila, nu: nucelo, qi: quilha, ra: radícula, RHM: região hilo-micropilar, rpe: restos de perisperma e endosperma, te: testa, tg: tegmen. Barra: 250µm (A,D,F,L,M,S), 200µm (B,J,Q), 500µm (C,E,H,P), 100µm (G,K,N,O), 50µm (H,I,R)


ex
ce
RHM
A
fu
RHM
B
qi
RHM
C
D
hy
te
mi
et
co
E
hy
ra
co
F
nu
ex
et
mi
G
nu
te
tg
H
te
RHM
mi
tg
I
K
qi
RHM
J
et
te
L
ra
hy
co
rpe
te
M
ex
N
co
O
P
RHM
Q
R
RHM
S

CONSIDERAÇÕES FINAIS

O conhecimento estrutural dos órgãos reprodutivos das espécies epífitas de Hylocereeae e Rhipsalideae, além da importância morfoanatômica para estudos taxonômicos, são ferramentas importantes para investigações sobre a biologia reprodutiva de Cactaceae.

A morfologia floral, a micromorfologia dos nectários, o modo de secreção juntamente com a concentração de açúcares no néctar são úteis para a sistemática, pois existe uma separação evidente entre Hylocereeae e Rhipsalideae e, entre os gêneros *Lepismium*, *Hatiora*, *Rhipsalis* e *Schlumbergera.* As flores das espécies de Hylocereeae são maiores, em sua maioria, apresentam antese noturna, possuem longo tubo floral (hipanto) com nectário do tipo câmara, onde o néctar (com baixa concentração de açúcares) é secretado tanto por tricomas como por estômatos, para animais noturnos (mariposas e morcegos). Em Rhipsalideae as flores, são menores, apresentam antese diurna, são brancas em sua maioria, possuem tubo floral bastante reduzido, nectário do tipo anelar (em *Rhipsalis*) ou "furrow" (em *Hatiora* e *Lepismium*) e néctar muito concentrado secretado por estômatos; com exceção de *Schlumbergera* que apresenta flores coloridas com tubo floral longo nectário do tipo "furrow", subtipo "holder" com néctar bastante diluído. Diferentemente de Hylocereeae o néctar é, em geral, para animais diurnos (borboletas, abelhas e pássaros).

Os frutos de *Hylocereus undatus*, *Lepismium warmingianum* e *Rhipsalis cereuscula*, são originados de ovário ínfero intimamente envolvido pelo pericarpelo (tecido de origem caulinar), sendo que essa estrutura, ovário/pericarpelo forma o pericarpo. O desenvolvimento do fruto justifica a sua classificação como cactídio, com uma parte acessória pericarpelar ou axial, para Cactaceae.

As características estruturais e o desenvolvimento das sementes de *H. undatus*, *L. warmingianum* e *R. cereuscula*, apresentam valor taxonômico, como o tipo de óvulo e a

morfologia, o tamanho e a forma da semente (incluindo a RHM e a presença/ ausência de quilha), e a morfologia do embrião. Esses caracteres podem ser usados como modelo em futuros estudos com número maior de táxons, a fim de encontrar atributos comuns a determinados grupos dentro da família.

Os resultados apresentados demonstram a importância do conhecimento da morfologia e da anatomia de órgãos reprodutivos para entendimento da classificação e evolução das espécies de Hylocereeae e Rhipsalideae. Entretanto, são necessários mais estudos para o conhecimento da evolução das estruturas florais em cactos epífitos e em Cactaceae como um todo.