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*Epiphyllum phyllanthus* (L.) Haw. (Cactaceae: Hylocereeae)**

Author(s): Odair J. G. Almeida Adelita A. S. Paoli Luiz A. Souza J. Hugo Cota-Sánchez

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# Seedling morphology and development in the epiphytic cactus *Epiphyllum phyllanthus* (L.) Haw. (Cactaceae: Hylocereeae)<sup>1</sup>

Odair J. G. Almeida<sup>2,3</sup>

Departamento de Botânica, IB, UNESP, Av. 24A, 1515, Rio Claro, SP, 13506-900, Brazil and Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK, S7N5E2, Canada

Adelita A. S. Paoli

Departamento de Botânica, IB, UNESP, Av. 24A, 1515, Rio Claro, SP, 13506-900, Brazil

Luiz A. Souza

Departamento de Biologia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, PR, 87020-900, Brazil

J. Hugo Cota-Sánchez

Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK, S7N5E2, Canada

ALMEIDA, O. J. G. (Departamento de Botânica, IB, UNESP, Av. 24A, 1515, Rio Claro, SP, 13506-900, Brazil and Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK, S7N5E2, Canada), A. A. S. PAOLI (Departamento de Botânica, IB, UNESP, Av. 24A, 1515, Rio Claro, SP, 13506-900, Brazil), L. A. SOUZA (Departamento de Biologia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, PR, 87020-900, Brazil) AND J. H. COTA-SÁNCHEZ (Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK, S7N5E2, Canada). Seedling morphology and development in the epiphytic cactus *Epiphyllum phyllanthus* (Cactaceae: Hylocereeae). *J. Torrey Bot. Soc.* 140: 196–214. 2013.—Seedling morphology is relevant in classification, taxonomy, and vegetation studies to understand plant life cycles, germination succession and requirements, and developmental progression. However, most morphological studies of seedlings lack analysis of organ anatomy, impeding the comprehension of series of development and establishment in a particular environment. Here, we have taken a traditional anatomical approach to examine the stages of seedling development in *Epiphyllum phyllanthus*, a holo-epiphytic cactus of tribe Hylocereeae. The goals were 1) to offer a comprehensive description of growth series in *E. phyllanthus* seedlings based on morphological and anatomical analysis and 2) to examine the initial growth phases in the life cycle of this species to identify organ development and understand their adaptive significance in relation to seedling establishment. Our results include descriptions of seed morphology, embryonic features, and seedling vascularization pattern in the root, hypocotyl, cotyledons, and epicotyl. The morphological and developmental patterns in *E. phyllanthus* seedlings have potential phylogenetic and ontogenetic implications in the Cactaceae. Characters such as the presence of mucilage on the seed coat, the lack of seed operculum, and large cotyledons in *E. phyllanthus* are comparable to basal cacti, but the root anatomy is more similar to columnar relatives. At the familial level, there is an apparent trend in decreasing number of phloem and xylem poles in the stele of primary root, correlated with degree of specialization and advanced phylogenetic position: tetrarch to septarch–octarch in basal lineages, tetrarch *Cereus*-type in columnar species, to the diarch vascular system in Rhipsalideae and some species with cylindrical/globose stem.

Key words: adventitious roots, anatomy, cotyledons, phylloclade, transition zone.

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<sup>3</sup> Author for correspondence, E-mail: odair1000@hotmail.com

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Cactaceae, a family within the Caryophyllales (Stevens 2001 onwards) comprising ca. 1,430 species and 100 genera (Hunt et al. 2006), is divided into four subfamilies: Pereskioideae, Opuntioideae, Cactoideae, and Maihuenioideae (Anderson 2001). Cacti are typically associated with dry desert environments; however, around 150 species (10%) exhibit the epiphytic habit and have evolved adaptations to develop in mesic environments of Neotropical forests and woodlands (Benzing 1990, Wallace and Gibson 2002). It is the upper strata of the forest canopy, though limited in water supply, which provides

epiphytes with more light compared to the lower and darker understory level. Epiphytic plants have the ability to cope with ecophysiological constraints, such as the scarcity of nutrients characteristic of tree canopies due to the smaller amount of suspended soil compared to the forest floor, the instability of the substrate, and water stress (Nieder et al. 2001). The ability to adjust to adverse parameters of forest canopies makes the epiphytic lifestyle a remarkable condition in plants. In addition, epiphytic cacti exhibit a suite of morphological traits indispensable to cope with desiccation, such as thick cuticle, flat stems, succulence, and a specialized adventitious root system (Benzing 1990). Furthermore, the roots of epiphytes grow on sites that dry rapidly, such as in pockets of soil debris or directly on trunks of the phorophyte, but have soil sheaths made of debris, mucilage, and root hairs, all of which prevent dehydration (Andrade and Nobel 1997).

It has been suggested that seedling morphology plays a relevant role in classification and taxonomy in addition to vegetation studies to understand plant life cycles, germination succession and requirements, and developmental progression (Duke 1965, Oliveira 1993); however, for the most part, morphological studies of seedlings lack analysis of organ anatomy, impeding the comprehension of the progression series of seedling development and establishment in a particular environment. The vast majority of studies dealing with seedlings of cacti have focused on their differential performance and establishment in conjunction with the role of nursing plants and biotic and abiotic factors on the survival and recruitment of offspring. As a result, the morphology and anatomy and initial developmental stages in plantlets of the Cactaceae remain largely unexplored. Both embryological and post-seminal studies in cacti are scarce, hindering the understanding of morphogenetic mechanisms and anatomical zonation during development, an issue that can be relevant in modern classifications because variation in apical dimensions and zone sizes have putative ontogenetic and phylogenetic implications (Mauseth 1978). In the late 19<sup>th</sup> century, a pioneer study (Ganong 1898) in the morphology and anatomy of seedlings in the Cactaceae made significant contributions to this field. Other enquiries encompassing seedling morphology in numer-

ous taxa of cacti emerged in the 20<sup>th</sup> and 21<sup>st</sup> centuries, for instance, *Pereskia* Mill., *Opuntia* Mill., *Echinopsis* Zucc. (Fraire 1910); *Mammillaria* Haw. and *Hylocereus*, among other genera (Buxbaum 1950); *Opuntia* (Wiggins and Focht 1967, Freeman 1969, Hamilton 1970); *Cereus* Mill. (Cota-Sánchez 1982, Almeida et al. 2009); *Cephalocereus fluminensis* (Miq.) Britton & Rose (Salles 1987); *Stenocereus queretaroensis* (F.A.C. Weber.) Buxb. (Loza-Cornejo et al. 2003); various species of the subfamily Cactoideae (Cota-Sánchez 2004), and *Lepismium* Pfeiff. and *Rhipsalis* Gaertn. (Secorun and Souza 2011).

Despite the scarcity of anatomical investigations in seedlings of numerous angiosperm lineages, there have been considerable advances in plant anatomy, a research area that has long been used as a valuable source of data in systematics. Some morpho-anatomical explorations have led to the establishment of hypotheses about evolutionary trends and polarization of character states in plants. Stuessy (2009) eloquently describes the early applications of morphological data in the 15<sup>th</sup> century to the real development of plant anatomy in the 19<sup>th</sup> century with the concomitant development of sophisticated techniques employed in recent decades. Clearly, early and recent contributions have added to the body of knowledge, evidence of the significance of morpho-anatomical characters, the conservative nature of which often suggests homology and common origin. Within this scope we have taken a traditional anatomical approach to examine the stages of seedling development in *Epiphyllum phyllanthus*. This species is a member of the Hylocereeae, a tribe including both facultative and obligate epiphytic cacti (Anderson 2001). However, *E. phyllanthus* is a true epiphytic (holoepiphytic) plant, spending its entire life cycle without contact with the forest floor in tropical Central and South America (Benzing 1990). It has light to intermediate green, branched, flattened stems marginally lobed or toothed, with conspicuous midribs, and areoles occasionally with hairs (Anderson 2001). The main goals of this investigation were 1) to offer a comprehensive description of growth series in *E. phyllanthus* seedlings based on morphological and anatomical analysis and 2) to examine the initial growth phases in the life cycle of this epiphytic species to identify organ development and understand their adaptive significance in

relation to seedling establishment in its natural environment. We also performed a literature survey in an attempt to characterize general trends in seedlings of the Cactaceae and present a general discussion embracing the taxonomic and phylogenetic implications of seedling morphology in the family.

**Materials and Methods.** **PLANT MATERIAL.** Seeds for germination studies were obtained from ripe fruits collected at the Parque do Ingá, a municipal conservation area in Maringá, Paraná, Brazil. A total of 30 fruits were collected from eight individuals separated at least 10 m from each other. The fruits were opened on a sieve and the seeds from each fruit harvested and maintained in separated batches, i.e., 30 fruits and 30 batches, and then the batches were rinsed with tap water to remove the pulp and mucilage. Voucher specimens (O.J.G. Almeida s/n and O.J.G. Almeida 001 and 002) were deposited at the herbarium of the Universidade Estadual de Maringá (Acc. No. HUEM 12,673) and the herbarium Rioclarense of the Universidade Estadual Paulista - UNESP/RC (Acc. No. HRCB 48,934 and 48,935, respectively). Additional seedlings already established around the mother plant were collected in the Parque do Ingá for morpho-anatomical comparative studies of artificially versus naturally propagated seedlings.

**SEED GERMINATION AND SEEDLING MORPHOLOGY.** For the investigation of seedling morphology, 200 seeds were evenly distributed in ten Petri dishes with two layers of water soaked filter paper and germinated in climatically controlled growing chambers at 25 °C. Seed germination started on the third day and continued for five more days. Seven to ten days following germination, the seeds were transferred to coconut fiber substrate to monitor developmental changes during the next 210 days. Throughout the first month observations were performed once a day and in the subsequent months were made once a week.

The general shape and external morphology of seedlings was investigated using fresh and preserved material fixed in formaldehyde-acetic acid-alcohol (FAA50) following Johansen (1940). The phylloclade surface area was analyzed using a Zeiss DSM940A scanning electron microscope (SEM) with samples fixed

in Karnovsky solution (Karnovsky 1965). In addition, fixed seedlings in FAA50 were clarified in Petri dishes to observe the vasculature network system in the cotyledonary node. Clarification was performed in three stages: 1) immersion in 20% sodium hydroxide for 12 hours and then rinsed with distilled water 2×, 2) immersion in 10% sodium hypochlorite for two hours and rinsed with distilled water 2×, and 3) staining for six hours with a few drops of 1% safranin in Petri dishes containing distilled water (Foster 1949, modified). Visual inspections were conducted and photographs taken immediately after staining in order to prevent color fading. The illustrations of seed and post-seminal development were made based on line drawings and microscopic photographs obtained with a digital camera.

**SEEDLING ANATOMY.** Several healthy seedlings exhibiting sequential developmental stages (from three to 210 days old, in addition to the seedlings collected near the mother plant) were fixed in FAA50 and later transferred and preserved in 70% ethanol for the anatomical study. The samples were then dehydrated in a graded ethanol series from 70% to 100% ethanol, embedded in 2-hydroxyethyl methacrylate Leica historesin and sectioned (cross and longitudinal, 8 to 12 µm thickness) with a rotary microtome. Tissue sections were stained with 0.05% toluidine blue pH 4.6 (O'Brien et al. 1965) and mounted on slides with Entellan synthetic resin. The presence of organic compounds in different organs/parts of the seedlings was determined with micro-chemical tests in combination with different dyes/chemicals performed on manually prepared sections of fresh and fixed tissue. Lipids were identified with Sudan IV, starches with Lugol's solution, phenolic compounds with a combination of ferric chloride and sodium carbonate (Johansen 1940), lignified walls with phloroglucinol with hydrochloric acid (Sass 1951), polysaccharides and pectins with ruthenium red (Jensen 1962), and proteins with mercuric bromophenol blue (Mazia et al. 1953). The anatomical illustrations were made using photographs taken with the image capturing system of a Leica stereo- and optical microscope using the Leica IM50 program, v. 5. All photos in morphological and anatomic plates included here were edited using Adobe Photoshop CS3 software. In view of the lack of a seedling terminology for the

Cactaceae, the morphological descriptions provided here are based on terms proposed by different authors. That is, cotyledon morphology and venation pattern is according to Hickey (1979) and description of epicuticular waxes follows Barthlott et al. (1998). Taxonomic authorities for scientific names are according to the Tropicos (Tropicos.org) database of the Missouri Botanical Garden.

**Results.** Following, we report major morpho-anatomical structures characterizing the early stages of seedling development in *Epiphyllum phyllanthus*, from seed germination to seven months and at an older stage. For completeness of the developmental series, our account includes a brief description of seed morphology, embryonic features prior to and after germination, and traits present in later stages of growth. We also highlight the seedling's vascularization pattern in the root, hypocotyl, cotyledons and epicotyl (phylloclade or flattened photosynthetic stem).

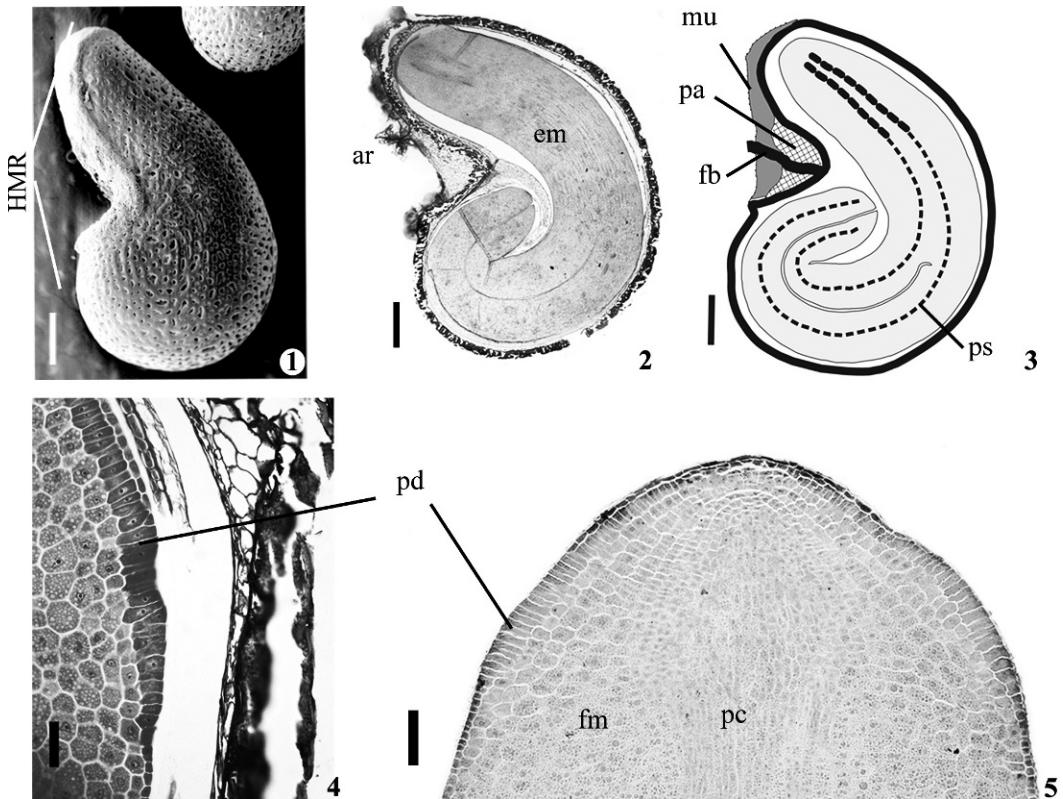
**SEED MORPHOLOGY AND POST-SEMINAL DEVELOPMENT.** The seeds of *Epiphyllum phyllanthus* are black, shiny, ovoid in shape with ornamented seed coat, and an aril on the hilum micropylar region (HMR) (Fig. 1). Seeds vary from 3.70 to 4.90 mm in length (average 4.12 mm) and 2.20 to 2.80 mm in width (mean 2.51 mm). The embryo is typically curved and well developed (Fig. 2), with lipids and proteins as storage material. The embryo's ground meristem is homogeneous, the procambial strands are immersed in the embryonic axis, and each cotyledon has one procambial strand (Fig. 3). Near the radicular portion of the embryo and the collet region, the protoderm cells have different shape and size (the anticlinal walls are more elongated) compared to other protoderm cells (Fig. 4, 5). These cells are responsible for the formation of trichomes in the primary root and collet region, as indicated below.

Two hours after contact with water, the seeds develop mucilage on the HMR, and 24 hours later this slimy substance covers the entire seed coat surface. The first signal of germination is the protrusion of the radicle and a portion of the embryonic axis (Fig. 6) the third day following seed imbibition. No operculum is present in *E. phyllanthus* seeds, which show epigeal germination of a seedling

on which small trichomes begin to develop from the protodermal cells with elongated anticlinal walls on the radicle, mainly from collet region (Fig. 4, 5) from the second to sixth day. The seedlings are phanerocotyledonary (exposed cotyledons), but the seed coat covers the cotyledons until the fifth day (Fig. 7, 8), when a tuft of long unicellular trichomes is evident on the radicular area (Fig. 7, 8). At this stage, primary root development is incipient; the cotyledons are small, greenish, sessile and asymmetric, and from the sixth day onwards, once the seed coat separates from the seedling, the white hypocotyl starts accumulating chlorophyll and turns green (Fig. 9). The taproot is less than 2 mm; it ceases growth with simultaneous development of the adventitious roots at the base of the collet region and hypocotyl (Fig. 10), thereby linking the epicotyl with this vital absorptive organ and the substrate.

By the end of the second week following germination, the seedlings have two large and expanded green cotyledons of unequal size (the largest measuring 8.0–12.0 mm in length and the smallest 5.0–7.0 mm, with both cotyledons averaging 2.5 mm in width (Table 1). The cotyledons are plain-convex and oblong-ovoid in shape, with entire, smooth margin, obtuse apex, and glabrous epidermis. The hypocotyl is also glabrous and greenish (Fig. 11). New primordia of adventitious roots emerge from the hypocotyl (Fig. 10, Table 1). The two first weeks seem to be critical in the establishment of *E. phyllanthus* seedlings as there is a high incidence of mortality (over 50%), indicating the vulnerability of the early stages of development. Survival experiments in viviparous offspring of *E. phyllanthus* indicate that transplanting one-week-old seedlings is critical for establishment; high mortality occurred in three treatments used: 69% on the phorophyte surface with substrate accumulated on crevices, 58.6% on the ground (local substrate), and 44.8% under controlled conditions in potting soil (Cota-Sánchez and Abreu, 2007), supporting the vulnerability of early developmental stages and selection of substrate, nutrient and water availability.

No significant morphological changes were observed up to the 180<sup>th</sup> day compared to the fast-growing early stages (Table 1). After six months the epicotyl begins to develop between the cotyledons, and by the seventh month it becomes a flat dark green phylloclade with



FIGS. 1–5. Seed and embryo of *Epiphyllum phyllanthus*. FIG. 1. SEM view of mature seed. FIG. 2–5. Longitudinal sections. FIG. 2. Longitudinal section of mature seed and embryo. FIG. 3. Diagram of mature seed and embryo in longitudinal section. FIG. 4. Detail of the radicular apex. FIG. 5. Radicular apex of the embryo. (ar-aril; em-embryo; fb-vestigial funicular vascular bundle; fm-ground meristem; HMR-hilum micropylar region; mu-mucilage; pa-parenchymatous cells of hilum cup; pc-procambium; pd-protoderm ps-procambium strand). Scale bars = 500  $\mu$ m (Fig. 1–3), 50  $\mu$ m (Fig. 4, 5).

crenate margin and circular base; areoles form on areas between the notches of the phylloclade margin that initially bear multicellular trichomes. In seven-month-old seedlings the cotyledons are fleshy, shiny green, and the hypocotyl has cylindrical shape (Fig. 12). Same-age seedlings collected near the mother plant (Fig. 13) exhibit comparable morphological features to those of seedlings grown in

the greenhouse (Fig. 12), except for the larger phylloclade in the former.

**SEEDLING ANATOMY.** The tissues of *E. phyllanthus* seedlings have primary growth up to the sixth month after germination, and those closer to seven months old reveal a more complex internal histological organization as a consequence of the development of secondary

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FIGS. 6–13. Post-seminal development in *Epiphyllum phyllanthus*. FIG. 6. Seed: first day of germination. FIG. 7–9. Seedling development during the third, fourth, and sixth day post-germination, respectively. FIG. 10. Base of the hypocotyl with adventitious roots in an eight-day-old seedling. FIG. 11. Fourteen-day-old seedling. FIG. 12. Seven-month-old seedling. FIG. 13. Seedling collected near mother plant. (ar-adventitious root; cl-collet; co-cotyledon; HMR-hilum micropylar region; hp-hypocotyl; hra-hypocotyl-radicle axis; phr - part of phorophyte and adventitious root; pl-phylloclade; pr-primary root; ra-radicle; sc-seed coat; tr-trichome). Scale bars = 2 mm (Fig. 6–8, 10, 11), 3 mm (Fig. 9), 10 mm (Fig. 12), 20 mm (Fig. 13).

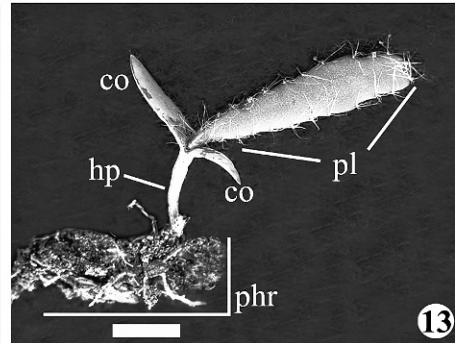
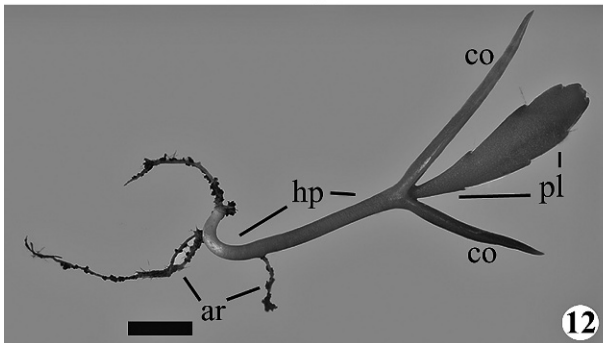
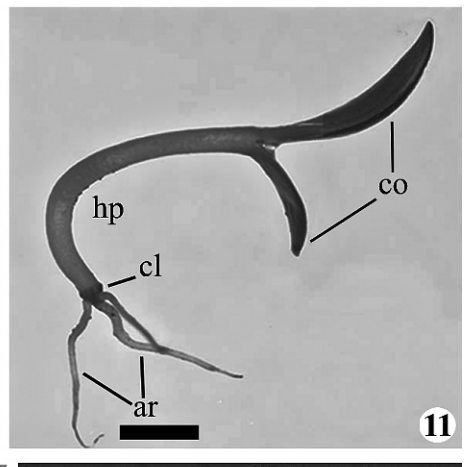
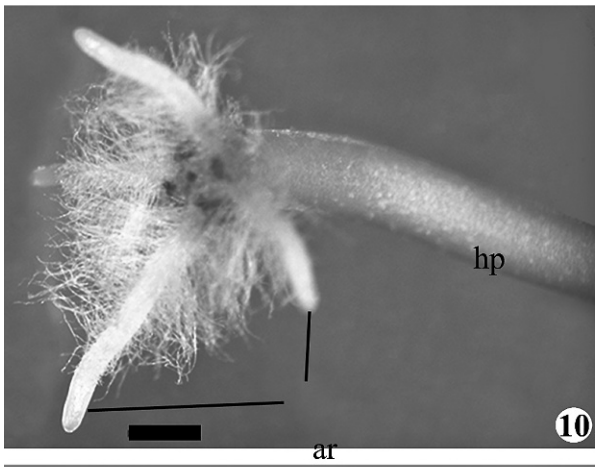
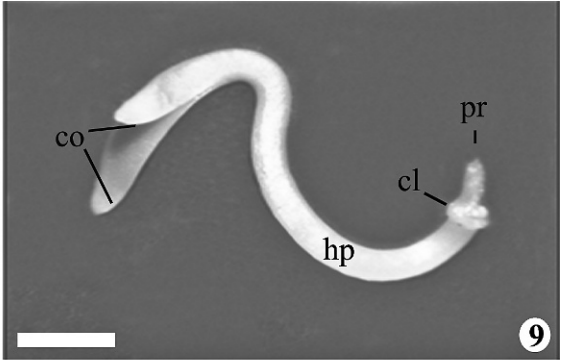
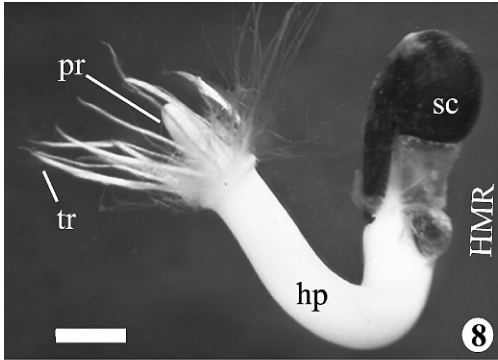
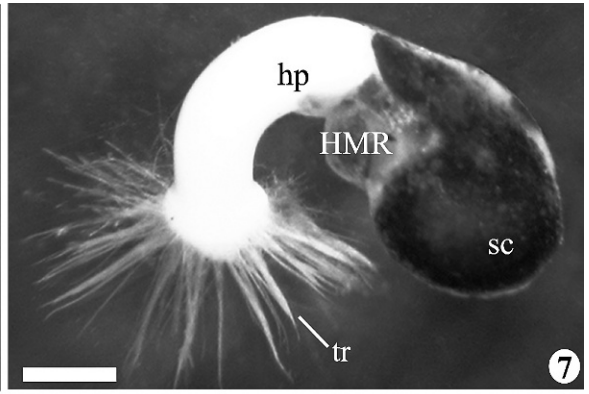
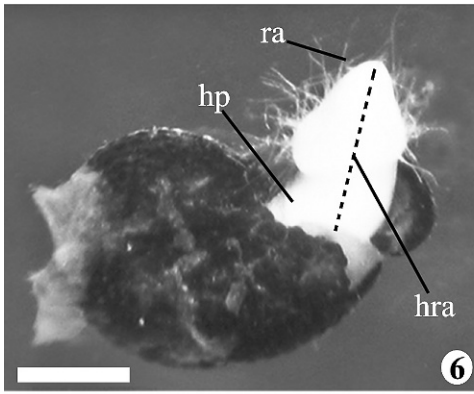


Table 1. Morphological development and chronological appearance of structures in seedlings of *Epiphyllum phyllanthus*. Asterisk(\*) = structure present but measurements not taken.

Age after germination (days)	Length of hypocotyl (mm)	Length of cotyledons (mm)		Width of cotyledons (mm)		Length of adventitious root (mm)	Phylloclade (epicotyl) (mm)	
		Larger	Smaller	Larger	Smaller		Length	Width
3	6.0	-	-	-	-	-	-	-
6	7.0	7.0	4.0	*	*	*	-	-
14	16.0	8.0–12.0	5.0–7.0	2.5	2.5	5.0–10.0	-	-
30	16.0–23.0	10.0–15.0	6.0–11.0	3.0	3.0	5.0–10.0	-	-
120	16.0–23.0	10.0–15.0	6.0–11.0	3.0	3.0	10.0–20.0	-	-
210	28.0	20.0	12.0	6.0	6.0	>20.0	17.0–25.0	5.0

tissues and lateral meristems (phellogen and cambium).

**PRIMARY ROOT AND ADVENTITIOUS ROOTS.** As indicated, the primary root in *E. phyllanthus* seedlings quickly atrophies, but when functional, this structure has uniseriate epidermis with pluricellular trichomes and cortical parenchyma with large cells of various shapes, with size decreasing towards the root's stele region. The endodermis of the primary root lacks Casparian strips; the central cylinder has uniseriate pericycle, four strands of primary phloem, and xylem with tracheary elements around the pith (Figs. 14, 20).

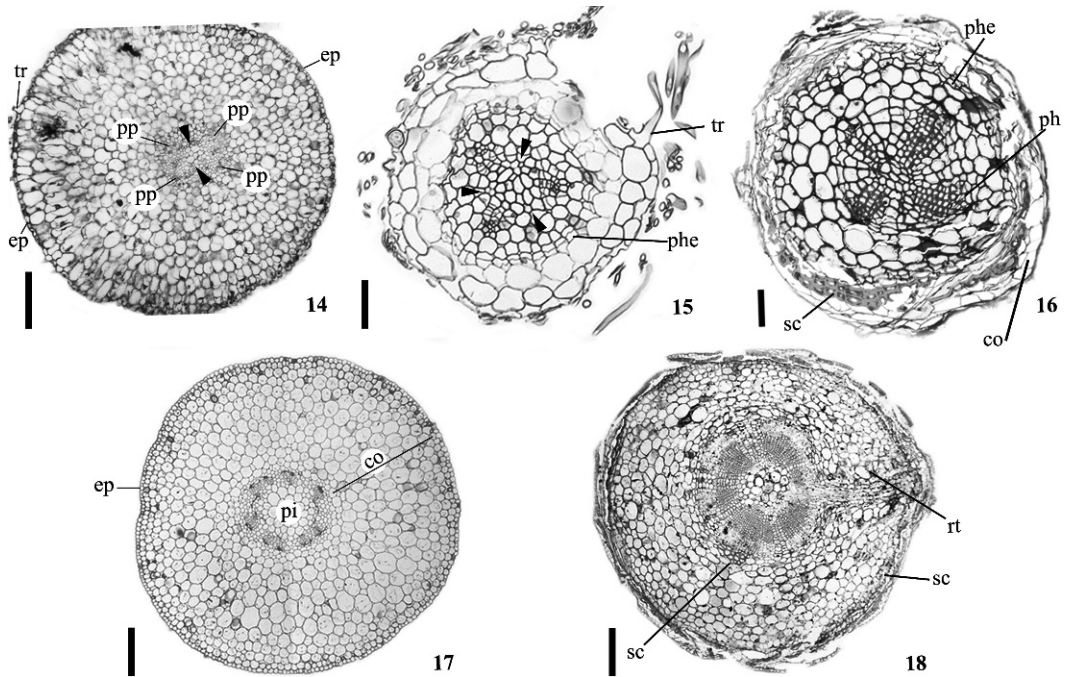
The epidermis of the adventitious roots is also uniseriate, composed of thin-walled cells and unicellular trichomes (Fig. 15), unlike the cortex, made of large exodermis cells and elongate parenchyma cells. No typical anatomical structures corresponding to an endodermis were identified at this stage. Provascular cells form the central cylinder, which has reduced pith and primary xylem alternating with primary phloem (Fig. 15). The number of protoxylematic poles varies along the adventitious roots. Specifically, the root is triarch (Fig. 15) in the apex or distal region and tetrarch in the middle and basal portions (Fig. 16), near the stem. The adventitious roots in the secondary tissue develop phellogen (from pericycle) and phellem, the latter with characteristic clusters of sclerenchyma cells (Fig. 16). There are abundant parenchyma rays in the vascular secondary tissues.

**TRANSITION ZONE, COLLET AND HYPOCOTYL.** The transition region (located between the root and the hypocotyl, including the collet) of *E. phyllanthus* seedlings exhibits a reorganization of the tracheary elements and strands of phloem (Fig. 19). This leads to a gradual change from exarch to endarch condition from

the collet region to the upper third portion of the hypocotyl (Fig. 20–22). The collet and the hypocotyl show structural differences in the epidermis, hypoderm, and vascular system. The epidermis of the collet has abundant, long unicellular trichomes, which assist in fixing the seedling to the substrate. The cortical parenchyma consists of isodiametric cells, and the stele has four vascular strands of phloem and tracheary elements of primary xylem circularly arranged around the pith parenchyma, a similar pattern also seen in the primary root (Fig. 20). The hypocotyl, in turn, displays a relatively uniform organization throughout, differing basically in the arrangement of the vascular system. The epidermis is glabrous, uniseriate (Fig. 17) with thin cuticle and parallelocytic stomata, one to two layers of collenchymatous hypodermic cells, and the vascular system distributed throughout the hypocotyl axis. The hypocotyl's basal part bears six vascular strands of phloem (Fig. 21), and tracheary elements of primary xylem surround the pith (Fig. 21, 22). The vascular system consists of two poles of protoxylem and eight or nine phloem strands distributed rather uniformly throughout the lower half of the hypocotyl (Fig. 20–22), but this pattern changes in the upper half of the hypocotyl, where the vascular system is clearly organized in two cotyledonary and two epicotyledonary traces (Fig. 23). Each cotyledonary trace has protoxylem/metaxylem elements and two phloem strands flowing in the direction of the cotyledon (Fig. 24–26), whereas each epicotyledonary trace is composed of one phloem strand with several tracheary elements running into the epicotyl (phylloclade) (Fig. 23).

The epidermic phellogen forms the periderm as a consequence of secondary growth, and at first, this new layer is present only at the base of the hypocotyl (Fig. 18). The cork, or phellem, has sclerenchyma arranged in continuous bands





FIGS. 14–18. View of the root and hypocotyl of seedlings of *Epiphyllum phyllanthus* in cross section. FIG. 14. Taproot (primary root) in a three-day-old seedling (arrow-head indicates protoxylem pole). FIG. 15. Primary growth of adventitious root (arrow-head indicates protoxylem pole). FIG. 16. Initial secondary growth adventitious root (seedling collected near mother plant). FIG. 17. Hypocotyl in primary growth, median region in a six-month-old seedling. FIG. 18. Hypocotyl in secondary growth (seedling collected near mother plant). (co-cortex; ep-epidermis; ph-phloem; phe-phellogen; pi-pith; pp-primary phloem; rt-adventitious root trace; sc-sclerenchyma; tr-trichome). Scale bars = 50  $\mu$ m (Fig. 14), 100  $\mu$ m (Fig. 15), 200  $\mu$ m (Fig. 16, 17), 250  $\mu$ m (Fig. 18).

around the hypocotyl, a different organization to that seen in the root (characterized by discontinuous clusters of sclerenchyma cells - see Fig. 16). The cortical parenchyma has secretory cavities and clusters of sclerenchyma around the stele, near the phloem (Fig. 18). The fascicular and interfascicular cambium of the central cylinder produces secondary tissues (xylem and phloem); the pith retains its parenchymatous nature along with the parenchyma cells of the xylem, which contain starch granules.

**COTYLEDONS.** Two fairly large cotyledons are typical in 30-day-old and older *E. phyllanthus* seedlings. The secondary veins of these leafy structures flow freely, curving towards the margin, forming a venation pattern known as camptodromous-cladodromous (Fig. 27, 28). The cotyledons have uniseriate and cutinized epidermis composed of irregular cells (cuboid to rectangular) with sinuous anticlinal walls, parallelocytic stomata (Fig. 29, 31), and

a layer of continuous crusts of epicuticular waxes. Chlorophyllous parenchyma with cells of diverse sizes makes up the mesophyll. The vasculature is organized in a main central midrib with two collateral vascular bundles (Fig. 29, 30). Similar morphological patterns were observed in seedlings up to 210 days old, except that as the seedlings aged, increased numbers and larger mucilage secretory cavities were present near the epidermis.

**EPICOTYL (PHYLLOCLADE).** Polymorphic epidermal cells, parallelocytic stomata, and a layer of epicuticular waxes are distinctive in the epicotyl surface of *E. phyllanthus* seedlings (Fig. 32–34). Multicellular trichomes are present in the areoles (Fig. 32, 35, 36), which are located in the border of the phylloclade; their vasculature consists of traces originating from the central cylinder (Fig. 35–36). Cross sections of the phylloclade revealed a cutinized uniseriate epidermis with rectangular cells and the lack of hypodermis. The cortical

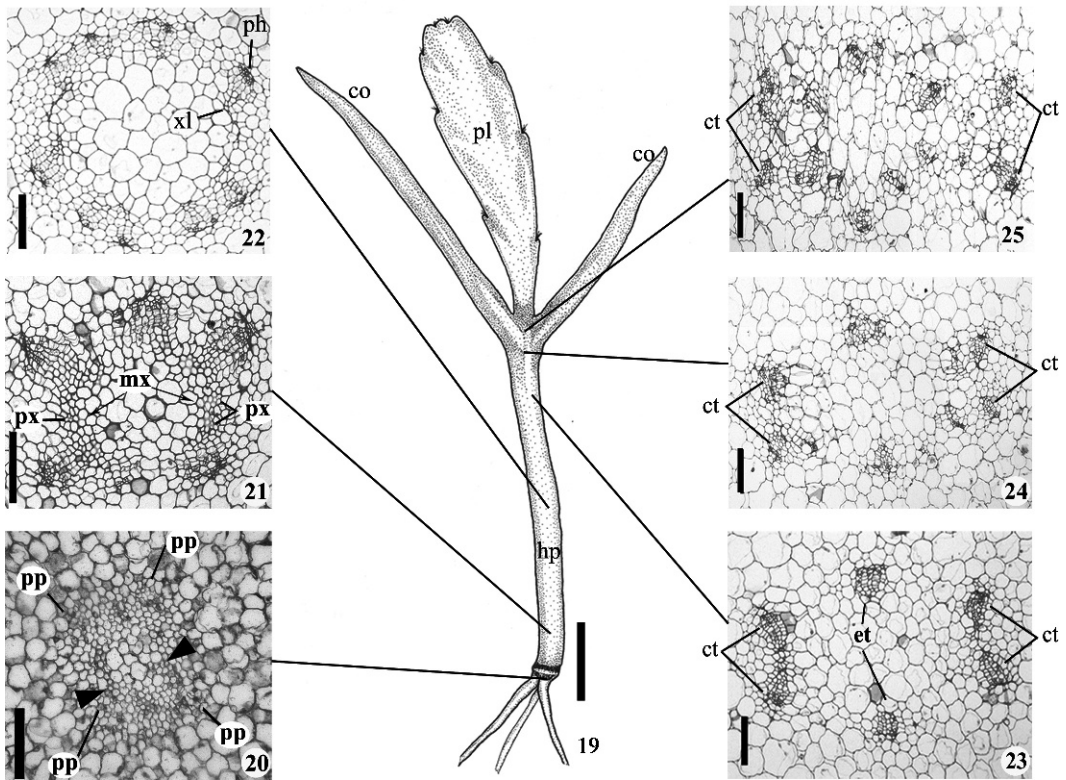


FIG. 19–25. Plate showing changes in the vascular system in *Epiphyllum phyllanthus* six-to seven-month-old seedlings, except for Fig. 20 showing structures of a one-week-old seedling. FIG. 19. Six-month-old seedling (general view). FIG. 20. Cross section of primary root which atrophies after one week (arrow-head indicates protoxylem pole). FIG. 21–22. Cross section of hypocotyl (basal and median regions). FIG. 23. Cross section of hypocotyl (upper region). FIG. 24–25. Cross section of cotyledonary node. (co-cotyledon; ct-cotyledonary trace; et-epicotyledonary trace; hp-hypocotyl; mx-metaxylem; ph-phloem; pl-phylloclade; pp-primary phloem; px-protoxylem; xl-xylem). Scale bars = 5 mm (Fig. 19), 100  $\mu$ m (Figs. 20–25).

parenchyma of the phylloclade is chlorophyllous with large polymorphic cells (Fig. 37) and mucilage-secreting cells scattered in the sub-epidermal layer. The central cylinder of the cortex has seven to eight collateral vascular bundles arranged around the small parenchyma pith. As in the hypocotyl, secondary growth (periderm from epidermal phellogen and secondary vascular tissues from cambium) is evident in the young phylloclade. Seedlings older than seven months also have an assembly of sclerenchyma close to the phloem, similar to that seen in the hypocotyl.

**Discussion.** SEED MORPHOLOGY AND GERMINATION. Ten types of seeds have been described in dicots based on embryo morphology, relative amount of endosperm, and position of the embryo in relation to endosperm (Baskin and Baskin 2001). Cactaceae seeds

are grouped within the peripheral category (large seeds with large embryo) (Martin 1946). The seeds of *E. phyllanthus* are well placed within this category because the embryo has large cotyledons (Fig. 2, 3) with comparatively large amount of protein and lipids as storage material and reduced perisperm and endosperm (O.J.G. Almeida et al. unpublished data). Conversely, in numerous members of the Cactaceae, the enlarged hypocotyl of the embryos store food reserves, which compensates in part for the characteristic small cotyledons and minute endosperm (Goebel 1905), e.g., *Cereus*, *Ferocactus*, *Mammillaria*, among others (Table 2). The characteristically large cotyledons of *E. phyllanthus* seedlings have also been reported in viviparous offspring of this species (Cota-Sánchez and Abreu 2007). Few reports of seedlings with large and expanded cotyledons exist in cactus

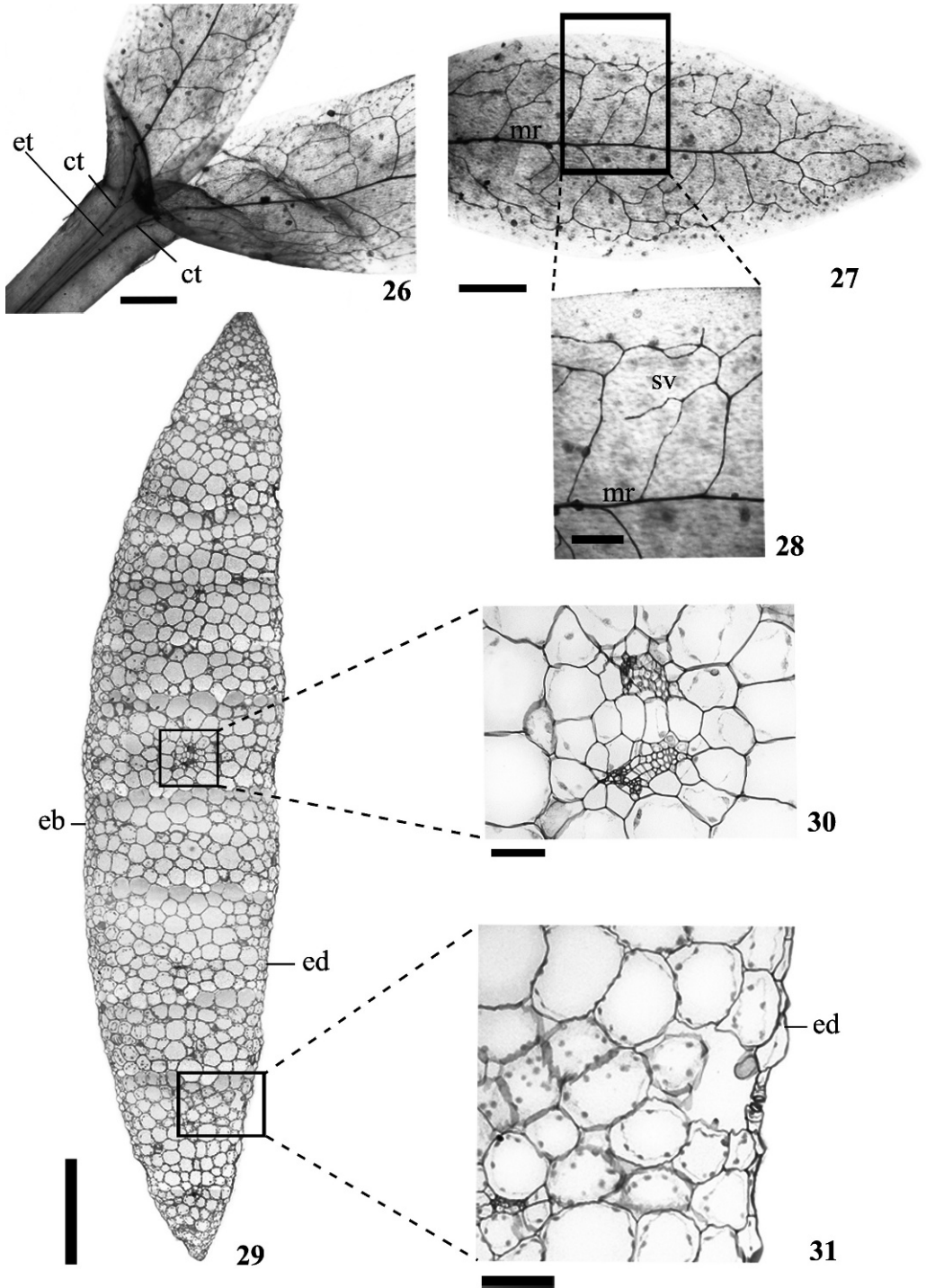


FIG. 26–31. Portions of clarified seedling and cotyledon of *Epiphyllum phyllanthus*. FIG. 26. Cotyledonary ramification in the cotyledonary node. FIGS. 27–28. Details of clarified cotyledon. FIG. 29–31. Cross section views. FIG. 29. Cotyledon. FIG. 30. Detail of the midrib with two vascular bundles. FIG. 31. Detail of the epidermis (adaxial surface). (eb-epidermis of abaxial face; ed-epidermis of adaxial face; ct-cotyledonary trace; et-epicotyledonary trace; mr-midrib; sv-secondary veins). Scale bars = 1 mm (Fig. 26, 27), 500  $\mu$ m (Fig. 28), 600  $\mu$ m (Fig. 29), 50  $\mu$ m (Fig. 30), 100  $\mu$ m (Fig. 31).

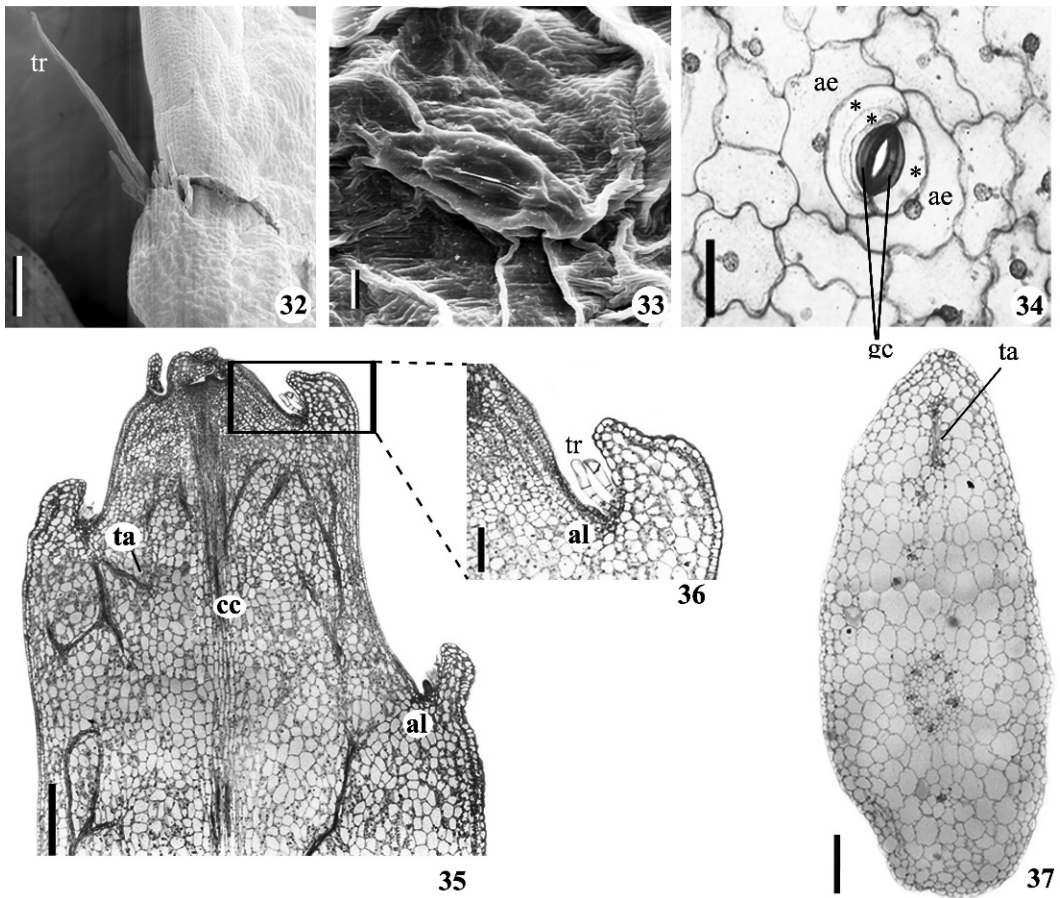


FIG. 32–37. Phylloclade (epicotyl) of *Epiphyllum phyllanthus*. FIG. 32. SEM view of the phylloclade's crena. FIG. 33–34. Epidermis in frontal view. FIG. 33. SEM-wax deposition on stomata. FIG. 34. Parallelocytic stomatal complex and adjacent epidermal cells (\* = subsidiary cell). FIG. 35. Phylloclade apex in longitudinal section. FIG. 36. Detail of the areole with trichomes in longitudinal view. FIG. 37. Base of the phylloclade in cross section. (ae-adjacent epidermal cell; al-areole; cc-central cylinder; gc-guard cell; ta-trace of the areole; tr-trichome). Scale bar = 200  $\mu$ m (Fig. 32, 37), 5  $\mu$ m (Fig. 33), 30  $\mu$ m (Fig. 34), 300  $\mu$ m (Fig. 35), 100  $\mu$ m (Fig. 36).

literature, and most species investigated are terrestrial (Table 2), except for *Hylocereus* (Buxbaum 1950, Bravo-Hollis 1978), a closely related species to *Epiphyllum* (within Hylocereeae), and *Peniocereus* (Bravo-Hollis 1978).

One peculiar attribute of *E. phyllanthus* seeds is that shortly after being in contact with water, mucilage starts covering the seed coat, beginning in the HMR. Within 24 hours this slimy substance envelops the entire seed coat surface. The production of seed mucilage, known as myxospermy, is an adaptation in at least 100 families of angiosperms (Western 2012). Several functions have been attributed to mucilaginous substances on the seed testa surface, including enhancing contact area with

substrate to assist in germination (Mott 1974) and increasing germination rate by keeping water around the seed, a relevant advantage in dry environments (Labouriau 1973, Yang et al. 2012). However, mucilage on the seed coat has also been proposed as a main factor preventing germination (Witztum et al. 1969) since mucilage deposition on the seed surface absorbs water forming a shield that deprives the embryo of water and oxygen. Thus, seeds covered with mucilage undergo an anoxic or hypoxic process after imbibition (Witztum et al. 1969, Joly and Crawford 1983). In fact, mucilage deposition in *Jacaratia spinosa* (Aubl.) A. DC. seeds inhibits germination (Paoli et al. 1987). Ecological roles include

Table 2. Literature compilation of attributes of seeds and seedlings of the Cactaceae during germination. Question mark (?) indicates unknown data. Species are separated in subfamilies and tribes *sensu* Anderson (2001). Clade abbreviations follow Bárcenas et al. (2011). Enlarged cotyledons: > 5 mm; reduced cotyledons: < 5 mm.

	Taxa	Seed mucilage during germination	Seed operculum	Cotyledons	Source(s)
Cactoideae-Cacteae- "Mammilloid"	<i>Manmillaria</i> Haw., <i>Coryphantha</i> (Engelm.) Lem.	?	?	Reduced	Fraine (1910); J.H. Cota-Sánchez (pers. obs.)
Cactoideae-Cacteae- Clade C	<i>Ferocactus</i> Britton & Rose, <i>F. latispinus</i> (Haw.) Britton & Rose	?	?	Reduced	Buxbaum (1950), Cota-Sánchez (1984)
Cactoideae-HLP Clade- Hylocereae	<i>Disocactus ackermannii</i> (Haw.) Ralf. Bauer [= <i>Phyllocactus ackermannii</i> (Haw.) Salm-Dyck], <i>Epiphyllum anguliger</i> (Lem.) G. Don. (= <i>P. anguliger</i> Lem.), <i>E. hookeri</i> Haw. (= <i>P. hookeri</i> Salm-Dyck) <i>E. phyllanthus</i> (L.) Haw. [= <i>P. phyllanthus</i> (L.) Link].	?	?	Reduced	Ganong (1898); Fraine (1910)
Cactoideae-ACHLP Clade-Copiapoa	<i>Hylocereus</i> (A. Berger) Britton & Rose <i>Copiapoa</i> Britton & Rose	Yes	Absent	Enlarged	This study, Ganong (1898).
Cactoideae-BCT Clade-Cereae	<i>Cereus</i> Mill.	?	?	Enlarged Reduced	Buxbaum (1950), Bravo-Hollis (1978) Buxbaum (1950)
Cactoideae-BCT Clade-Trichocereae	<i>Echinopsis</i> Zucc., <i>Harrisia pomianensis</i> (F.A.C. Weber ex K. Schum.) Britton & Rose [= <i>Eriocereus bomplandii</i> (Parm. Ex Pfeiff.) Riccob.]	No	Present	Reduced	Fraine (1910), Cota-Sánchez (1982), Almeida et al. (2009)
Cactoideae-RNBCT Clade-Rhipsalideae	<i>Hatiora</i> Britton & Rose <i>Lepismium</i> Pfeiff., <i>Rhipsalis</i> Gaertn., <i>Schlumbergera truncata</i> (Haw.) Moran	?	?	Reduced	Fraine (1910), Buxbaum (1950)
Cactoideae-HLP Clade-Echinocereae	<i>Cephalocereus</i> Pfeiff. [= <i>Pilocereus albispinus</i> (Salm-Dick) Rumpler], <i>Echinocereus</i> Engelm., <i>Pachycereus</i> (A. Berger) Britton & Rose (= <i>Marginatocereus</i> Backeb.), <i>Stenocereus queretaroensis</i> (F.A.C. Weber) Buxb.	No, No, No	Present, Present, Present	Reduced Reduced, Reduced	Bona et al. (1997) Fraine (1910), Buxbaum (1950), Secorun and Souza (2011), O.J.G. Almeida and J.H. Cota-Sánchez (pers. obs.) Buxbaum (1950), Fraine (1910), Salles (1987), Loza-Cornejo et al. (2003)
Opuntioideae	<i>Peniocereus</i> (A. Berger) Britton & Rose [= <i>Nyctocereus oaxacensis</i> Britton & Rose; = <i>Wilcoxia viperina</i> (F.A.C. Weber) Britton & Rose]	?	?	Enlarged	Bravo-Hollis (1978)
Pereskioideae	<i>Opuntia</i> Mill.  <i>Pereskia</i> Mill.	Yes	Absent	Enlarged	Fraine (1910), Buxbaum (1950), Wiggins and Focht (1969), Hamilton (1970), Freeman (1969)
		?	Absent	Enlarged	Fraine (1910), Buxbaum (1950)

Table 3. Literature collection of stele organization in the primary root in members of Cactaceae. This study<sup>1</sup>, Secorun and Souza (2011)<sup>2</sup>, Almeida et al. (2009)<sup>3</sup>, Freeman (1969)<sup>4</sup>, Fraine (1910)<sup>5</sup>

Arranged according to Bárcenas et al. (2011) phylogeny	Diarch root (typical)	Tetrarch root - <i>Cereus</i> type (4 phloem and 2 xylem poles)	Tetrarch root (typical)	Septarch-octarch
Cactoideae - Cactaceae - "Mammilloid" Clade	<sup>5</sup> <i>Mammillaria donatii</i> Berge ex K. Schum., <sup>5</sup> <i>M. eriacantha</i> Link & Otto ex Pfeiff., <sup>5</sup> <i>M. hexacantha</i> Salm-Dyck, <sup>5</sup> <i>M. meiacantha</i> Engelm., <sup>5</sup> <i>M. multiceps</i> Salm-Dyck, <sup>5</sup> <i>M. pentacantha</i> Pfeiff., <sup>5</sup> <i>M. polyedra</i> Mart., <sup>5</sup> <i>M. rhodantha</i> Link & Otto, <sup>5</sup> <i>M. spinosissima</i> Lem., <sup>5</sup> <i>Echinocactus bicolor</i> Galeotti, <sup>5</sup> <i>E. ottonis</i> Link & Otto	<sup>5</sup> <i>Mammillaria missouriensis</i> Sweet		
Cactoideae - Cactaceae Cactoideae - HLP Clade - Hylocereae Cactoideae - HLP Clade - Echinocereae Cactoideae - BCT Clade - Cereae	<sup>5</sup> <i>Echinocereus cinctescens</i> Lem., <sup>5</sup> <i>E. ehrenbergii</i> (Pfeiff.) Rumpfer	<sup>1</sup> <i>Epiphyllum phyllanthus</i> , <sup>5</sup> <i>E. hookeri</i> (= <i>Phyllocactus hookeri</i> )  <sup>3</sup> <i>Cereus hildmannianus</i> K. Schum., <sup>3</sup> <i>Cereus jamacaru</i> DC., <sup>3</sup> <i>C. peruvianus</i> (L.) Mill., <sup>5</sup> <i>C. spachianus</i> Lem., <sup>5</sup> <i>C. tortuosus</i> J. Forbes ex Otto & A. Dietr., <sup>5</sup> <i>C. triangularis</i> (L.) Haw., <sup>3</sup> <i>Pilocereus exerens</i> K. Schum. [= <i>Cephalocereus exerens</i> (K. Schum.) Rose], <sup>3</sup> <i>P. albispinus</i> [= <i>Cephalocereus albispinus</i> (Salm-Dyck) Borg <sup>5</sup> <i>Echinopsis eyriesii</i> Pfeiff. & Otto, <sup>5</sup> <i>E. multiplex</i> Pfeiff. & Otto, <sup>5</sup> <i>E. tubiflora</i> (Pfeiff.) Zucc. ex A. Dietr.	<sup>5</sup> <i>Echinocactus denudatus</i> Link. & Otto, <sup>5</sup> <i>E. hexaedrophorus</i> Lem.	
Cactoideae - BCT Clade - Trichocereae Cactoideae - RNBCT Clade - Rhipsalideae	<sup>5</sup> <i>Echinopsis oxygona</i> (Link) Zucc. Pfeiff. & Otto  <sup>5</sup> <i>Lepismium warmingianum</i> (K. Schum.) Barthlott (= <i>Rhipsalis warmingiana</i> K. Schum.), <sup>2</sup> <i>Lepismium cruciforme</i> (Vell.) Miq., <sup>2</sup> <i>Rhipsalis cereuscula</i> Haw., <sup>2</sup> <i>R. floccosa</i> Salm-Dyck ex Pfeiff. subsp. <i>hohenauensis</i> (F. Ritter) Barthlott & N.P. Taylor, <sup>5</sup> <i>R. dissimilis</i> (G. Lindb.) K. Schum.			

Table 3. Continued.

Arranged according to Barcenas et al. (2011) phylogeny	Diarch root (typical)	Tetrarch root - <i>Cereus</i> type (4 phloem and 2 xylem poles)	Tetrarch root (typical)	Septarch-octarch
Opuntioideae			<sup>5</sup> <i>Nopalea</i> (= <i>Opuntia</i> ), <sup>5</sup> <i>Opuntia</i> <i>albicans</i> Salm-Dyck, <sup>5</sup> <i>O. bergeriana</i> F.A.C. Weber, <sup>5</sup> <i>O. ficus-indica</i> (L.) Mill., <sup>5</sup> <i>O. imbricata</i> (Haw.) DC., <sup>5</sup> <i>O. maculacantha</i> C.F. Först., <sup>5</sup> <i>O.</i> <i>polyacantha</i> Haw., <sup>5</sup> <i>O. stricta</i> (Haw.) Haw., <sup>5</sup> <i>O. tuna</i> (L.) Mill.	<sup>4</sup> <i>Opuntia</i> <i>basilaris</i> Engelm. & J.M. Bigelow
Pereskioideae		<sup>5</sup> <i>Pereskia pittitache</i> Karw. ex Pfeiff.,		

facilitation of seed hydration, mediation of germination under waterlogged conditions, prevention of seed dispersal or predation by adherence to soil, and promotion of seed dispersal by attachment to animals (Western 2012). Altogether, it is possible that mucilage secretion has similar functions in *E. phyllanthus* and may be advantageous in environments with limited water supply, such as the tree canopies, where a wet exterior layer is needed for successful germination while preventing exposure to drought and desiccation of the embryo. This sticky substance may also assist in establishment and dispersal of this, and possibly other tropical species, by adhering to the host plant surface and the animals' fur feeding on this fruit.

Fast deposition of mucilage during germination has also been reported on the seed surface of several terrestrial cacti, namely *Opuntia echios* Howell (Wiggins and Focht 1967), *O. basilaris* (Freman 1969) and *O. bradtiana* (J.M. Coult.) Brandege (Hamilton 1970). In turn, small seeds of other epiphytic cacti, such as *Lepismium cruciforme*, *Rhipsalis cereuscula*, and *R. floccosa* subsp. *hoenauensis*, do not produce it during germination (Secorun and Souza 2011). However, germination tests in *Schlumbergera truncata* seeds, which are among the largest in the Rhipsalideae, secrete mucilage only in the HMR after the third day in contact with water (O.J.G. Almeida and J.H. Cota-Sánchez pers. obs.); that is, the mucilage does not surround the entire seed as in *E. phyllanthus*. A mucilage sheath has been reported in *Schlumbergera* Lem. (Barthlott and Hunt 1993), its potential role in germination and/or dispersal is not addressed. It is possible that buildup of this sticky substance in cactus seeds is correlated with seed size, i.e., the larger seed and embryo, the greater the metabolic activity at the onset of germination and the more seed coat surface area for mucilage to accumulate. Less mucilage is present in seeds smaller than 2 mm, e.g., *Arabidopsis* L. and *Chenopodium* L., whereas larger seeds (4–6 mm) of *Citrus* L. and *Cydonia* Mill. secrete more mucilage (Western 2012). Mucilage, therefore, may be a substance favoring successful seedling establishment and protecting emerging roots from desiccation.

The operculum, a lid- or plug-like structure located in the micropylar region of the seed, detaches during germination. It is typical in seeds of different plant families, and its

ontogeny, structure, shape, opening mechanism, and function vary across angiosperms (Werker 1997). In the Cactaceae the operculum forms at the HMR and facilitates germination because with the release of the seed's caudal part the embryo emerges more rapidly as opposed to the slower dorsal ruptures or deterioration of seed coat (Bregman and Bouman 1983). Even though several epiphytic genera of the Rhipsalideae have operculated seeds (Table 2), the operculum is wanting in *E. phyllanthus*; hence, germination occurs via rupturing/cracking on the dorsal region of the seed coat. Whether seeds of other Hylocereae species lack this structure is unknown, but its absence in *E. phyllanthus* seeds is a shared feature with seeds of some basal cacti, e.g., *Pereskia* and *Opuntia*, two genera in which this structure was not identified by Bregman and Bouman (1983).

**SEEDLING MORPHOLOGY AND ANATOMY.** Variation in body size and seedling anatomy is frequent in all organisms. The Cactaceae is no exception as evidenced by significant variations in the vascular system of vegetative organs, which can be viewed as transitional phenomena with phylogenetic implications. For example, the number of protoxylem and protophloem poles, from two in *Lepismium* and *Rhipsalis* to seven and eight in *Opuntia basilaris* (Table 3). In addition, three kinds of arrangements in the stele of roots have been described in cacti, namely typical tetrarch, typical diarch, and tetrarch-*Cereus* type, the latter characterized by four protophloem poles and only two protoxylem poles (Fraine 1910). Our study reveals that the primary root of the epiphytic *E. phyllanthus* matches the tetrarch-*Cereus*-type (Fig. 20); however, seedlings of epiphytic cacti of the Rhipsalideae, including *Rhipsalis dissimilis*, *R. warmingiana* (Fraine 1910), *Lepismium cruciforme*, *R. cerascula* and *R. floccosa* subsp. *hoenauensis* (Secorun and Souza 2011), have diarch primary root. This suggests that the primary root anatomy in cacti may not be directly related to the epiphytic habit in Hylocereae and Rhipsalideae.

The vascularization in *E. phyllanthus* seedlings, i.e., primary root tetrarch with ramifications of the phloem along the hypocotyl-root axis, is comparable to the configuration observed by Fraine (1910) in several terrestrial basal cacti, such as *Pereskia pittache*, *Opuntia*, *Nopalea*, and *Cereus tortuosos* [= *Harrisia*

*tortuosa* (J. Forbes ex Otto and A. Dietr.) Britton and Rose]. The vascularization in *O. basilaris* seedlings exhibits connection of tracheary elements in the primary xylem, from the lateral cotyledonary traces to the tracheary elements in the primary xylem of the taproot; nonetheless, the other elements of the primary xylem are gradually lost along the hypocotyl (Freeman 1969). Even though Esau (1977) pointed out that in the seedling, the vascular system of the epicotyl is a separate structure from the cotyledon-hypocotyl-root unit, our study indicates that this is not the case in *E. phyllanthus* seedlings, in which the epicotyledonary traces are located quite low in relation to the cotyledonary node (Fig. 23–26) and an evident link between the central cylinder of the hypocotyl and the epicotyledonary traces exists. Thus, the epicotyl and the cotyledon-hypocotyl-root are not disconnected in seedlings; instead, there is a gradual transition in the vascularization of these structures.

The relevance of using traditional plant anatomy as a powerful tool to refine taxonomic and evolutionary relationships in the Cactaceae has been highlighted in various sources, e.g., Mauseth (1988), Wallace and Gibson (2002), and Abreu et al. (2012). Studies of stem anatomy, e.g., Terrazas and Arias (2003), Calvente et al. (2008), Detke and Milaneze-Gutierrez (2008), Torres-Boeger et al. (2010), and Lemos and Melo-de-Pinna (2011), discuss traits with potential taxonomic and adaptive significance in the Cactoideae, e.g., cuticle, epidermis, stomata, hypodermis, cortex, vascular cylinder, and pith. Nevertheless, Stuessy (2009) and Lemos and Melo-de-Pinna (2011) advocate that some anatomical characters used in systematic analysis, such as vascular and dermal systems, should be revised in view of their plasticity because morpho-anatomical variability can have serious repercussions in phylogenetic inferences as the issue of homology versus analogy come into play. Convergence is a common phenomenon in vegetative and reproductive structures in the cactus family, and factual data indicate that morphological features are environmentally influenced, as in *Cephalocereus columna-trajani* (Karw. ex Pfeiff.) K. Schum., whose stem and cephalium tilt towards the light source (Zavala-Hurtado et al. 1998), and *Lepismium*, in which hypodermis thickness and stomata density are correlated with the epiphytic habitat (Torres-Boeger et al. 2010).



A single character also exhibits structural modification in different conspecific individuals in separate populations, like one to two layers of hypodermis in individuals of *Rhipsalis elliptica* G. Lindb. ex K. Schum. from the Atlantic Forest (Rio de Janeiro state, Brazil) (Calvente et al. 2008), whereas conspecific individuals collected at the Parque Nacional da Serra do Cipó (Minas Gerais state, Brazil) lack this tissue (Lemos and Melo-de-Pinna 2011). Similarly, the stem of *E. phyllanthus* from Parque do Ingá (Paraná state, Brazil) has well-developed hypodermis (Dettke and Milaneze-Gutierrez 2008) as opposed to the lack of hypodermis in conspecific plants from the Serra do Cipó Lemos and Melo-de-Pinna (2011). Remarkably, the seven-month-old seedlings from seeds collected at the Parque do Ingá analyzed in this study do not develop hypodermis in the phylloclade, supporting the plasticity of the hypodermis in *E. phyllanthus*.

The occurrence of sclerenchyma around the stele in the hypocotyl and phylloclade (epicotyl) of the *E. phyllanthus* seedlings was not unexpected. This tissue is present in several terrestrial members of tribe Cereeae (Soffiatti and Angyalossy 2007), in species of epiphytic *Rhipsalis* (Calvente et al. 2008), and in *Epiphyllum*, *Hatiora*, and *Lepismium* (Dettke and Milaneze-Gutierrez 2008, Torres-Boeger et al. 2010, Lemos and Melo-de-Pinna 2011). The sclerenchyma cells originate from the pericycle and exhibit the same pattern in seedlings (as per our results) and in the stem of mature individuals (Dettke and Milaneze-Gutierrez 2008, Lemos and Melo-de-Pinna 2011). These data confirm the existence of pericycle fibers in epiphytic cacti. These fibers provide mechanical support and reinforcement (Metcalf and Chalk 1979) and maintain the integrity of the plant tissue during severe drought (Soffiatti and Angyalossy 2007). In addition, the protection of vulnerable plant parts and organs from water loss and desiccation depends on the cork cells with thick walls. The development of layered sclerenchyma in the cork of the root and hypocotyl of *E. phyllanthus* seedlings is likely correlated with protection in times of limited water. An increase in the cellular layers of the periderm with simultaneous levels of suberization and lignification in adult plants of *E. phyllanthus* and *Rhipsalis baccifera* (J.S. Muell.) Stern. exposed to a 30 day drought period (North and Nobel 1994) supports this idea.

**SEEDLING ESTABLISHMENT.** The tufted hairs (trichoblast cluster) growing shortly after germination in the distal region of the *E. phyllanthus* root originates from elongated cells in the embryo's protoderm. Given the limited growth of the taproot, these hairs represent the initial structures facilitating the attachment and subsequent successful seedling establishment. Another major finding in *E. phyllanthus* seedlings is the determinate growth of the primary root. Determinate root growth is uncommon in flowering plants because the elongation of the primary root is advantageous during the establishment phase (Dubrovsky and North 2002). However, other cacti from tribes Pachycereeae and Cactaceae exhibit determinate primary root growth (Dubrovsky and North 2002). Since the lack of primary root development induces the formation of secondary roots, which are essential structures for fixation and more efficient water uptake (Dubrovsky and North 2002), it is feasible that this growth pattern is beneficial during seedling development. On the other hand, the primary root in epiphytic cacti (*Lepismium cruciforme*, *Rhipsalis cereuscula* and *R. floccosa* subsp. *hoenauensis*) of the Rhipsalideae has indeterminate growth. The roots expand slowly and the collet develops root hairs and adventitious roots facilitating seedling anchorage to the phorophyte (Secorun and Souza 2011).

**PHYLOGENETIC IMPLICATIONS.** A comprehensive understanding of morpho-anatomy in *Epiphyllum phyllanthus* is significant because it illustrates chronological developmental phases and external and internal attributes with potential phylogenetic implications. When our own data are combined with literature information, some anatomical features of this species are relevant in assessing degree of putative phylogenetic relationship. There are, in fact, certain characters of *E. phyllanthus* seedlings shared with those of basal cacti from the Pereskioideae and Opuntioideae as well as other species from the Cactoideae.

Several traits suggest a strong correlation of *E. phyllanthus* with terrestrial basal and columnar lineages of the family. Characters such as the presence of mucilage on the seed coat, the lack of operculum, and the large cotyledons in *E. phyllanthus* are comparable to basal cacti, specifically *Pereskia* and *Opuntia* (Table 2), but the root anatomy is more

similar to columnar relatives, e.g., *Cereus* (Table 3). Putative plesiomorphic seedling features are seemingly retained (but reversals should not be ruled out) in *Epiphyllum phyllanthus*, *Hylocereus*, and *Peniocereus*, as evidenced by their relatively medium to large embryo and expanded cotyledons, which are also present in basal lineages of Opuntioideae and Pereskioideae (Table 2). The presence of large leafy cotyledons is consistent in other members of the Hylocereeae as described by Ganong (1898) for several species of the former genus *Phyllocactus*, an assemblage now encompassing the species of *Disocactus*, *Epiphyllum* and *Weberocereus* (sensu Anderson 2001) in modern cactus taxonomy. These three genera and *Hylocereus* are distantly related to *Pereskia* and *Opuntia* but possess seeds with medium to relatively large-sized embryos and seedlings with bigger cotyledons (Table 1). *Disocactus*, *Epiphyllum*, *Hylocereus*, and *Weberocereus* represent assemblages that have abandoned the open, dry desert for a lifestyle in more mesic conditions, which favors the spread of larger leaf surface area and the evolution of larger seeds with larger embryos with corresponding larger cotyledons. From the phylogenetic viewpoint, these genera are part of the subfamily Cactoideae and members of the Hylocereeae, which along with the Leptocereeae and Pachycereeae form Nyffeler's (2002) HLP Clade, an assemblage comprising mainly terrestrial species.

We found another atypical anatomical feature in relation to the vascular system in seedlings of the Cactaceae. There is an apparent trend in decreasing number of phloem and xylem poles in the stele of the primary root. From a phylogenetic perspective and considering recent phylogenies proposed for the family, e.g., Bárcenas et al. (2011) and Hernández-Hernández et al. (2011), the decreasing number in vascular bundles is correlated with degree of specialization and advanced phylogenetic position or terminal branches in the cladograms. That is, tetrarch to septarch-octarch in basal lineages, e.g., *Opuntia* and *Pereskia*, tetrarch *Cereus*-type in columnar species of *Cereus*, *Pilocereus*, *Echinopsis*, to the diarch vascular system in Rhipsalideae and some species with cylindrical growth habit, such as *Mammillaria*, *Echinocereus*, *Echinocactus*. It is also noteworthy that the primary root in *Epiphyllum phyllanthus* and *E. hookeri* is tetrarch *Cereus*-type, a trait shared

with other columnar cacti from the *Brownningia*, *Cereaceae*, and *Trichocereaceae* (BCT) Clade *sensu* Bárcenas et al. (2011) (see Table 3). While we can infer that the BCT Clade is probably distinguished by tetrarch *Cereus*-type vascular system and the monophyletic core, Rhipsalideae has diarch primary root system; no inferences can be made for the core Notocactaceae, also part of the RNBCT Clade of Nyffeler (2002) and Bárcenas et al. (2011), due to the lack of information. Finally, the fact that *E. phyllanthus* shares the epiphytic lifestyle and tree canopies with members of the Rhipsalideae does not imply that these two lineages necessarily share structural traits. While several phenotypic features, presumably the result of convergent evolution, are shared by members of the Hylocereeae and Rhipsalideae, the morphological differences of seedlings between members of these two tribes are extreme (Tables 2, 3). The Rhipsalideae is distinguished by relatively small operculated seeds, seedlings with tiny cotyledons, reinforcing the idea that these two subfamilies have evolved independently probably from different stocks, as proposed in several studies of the Cactaceae, e.g., Nyffeler (2002), Bárcenas et al. (2011) and Hernández-Hernández et al. (2011).

In summary, this study provides an extensive description of seedling development in *E. phyllanthus*. Limited data on seedling morphology in the Hylocereeae and the Cactaceae challenges the precise interpretation of morphological and anatomical patterns and/or evolutionary directionality of character state change. Several features, such as seed germination (mucilage build-up and inoperculate seed), anatomy of the transition zone in hypocotyl, primary root, and morphology of cotyledons in seedlings of *E. phyllanthus*, can be used as a model in future comparative investigations to determine the putative plesiomorphic character of large cotyledons in the Hylocereeae and decipher the putative evolutionary paths of other morphological characters in the cactus family.

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