



## Flower morpho-anatomy in *Epiphyllum phyllanthus* (Cactaceae)

### Morfo-anatomía de la flor de *Epiphyllum phyllanthus* (Cactaceae)

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**Abstract.** The aim of this contribution was to analyze the morpho-anatomical floral structure of *Epiphyllum phyllanthus* (L.) Haw., a widely distributed species across South America, occurring in humid forests as an epiphyte. Flowers and flower buds were collected in Maringá, Paraná State, Brazil, fixed, processed, and analyzed under light microscope and scanning electron microscope. The flower is sessile and epigynous with a well-developed hypanthium. All flower whorls have uniseriate epidermis. Secretory cavities containing mucilage and calcium oxalate crystals occur throughout the floral parenchymatous tissue. The androecium has many stamens with tetrasporangiate and bithecal anthers. The wall of the young anther is formed by epidermis, endothecium, a middle layer, and binucleate secretory tapetum that eventually becomes uninucleate. The gynoecium is syncarpous with 9-10 carpels, pluriovulate, and with parietal placentation. The ovary has inverted vascular bundles in a similar pattern as in *Pereskia*. The nectariferous region occurs on the inner surface of the hypanthium. The stigma has 9-10 lobes with a secretory epidermis. The ovules are circinotropous, bitegmic, crassinucelate, and have long funiculus as in many other Cactaceae.

Key words: anther, gynoecium, hypanthium, nectary, ovule.

**Resumen.** El objetivo de esta investigación fue analizar la morfo-anatomía de la flor de *Epiphyllum phyllanthus* (L.) Haw, especie con distribución amplia en los bosques húmedos de América del Sur como epífita. Se recolectaron flores y botones en Maringá, PR, Brasil, fijados, procesados y analizados con microscopio de luz y con microscopio electrónico de barrido. La flor es sésil, epígina con hipanto desarrollado. Todos los verticilos florales presentan epidermis simple. Cavidades secretoras con mucilago y cristales de oxalato de calcio se encuentran en todo el tejido parenquimático de la flor. El androceo posee muchos estambres, con anteras bitecas y tetraesporangiadas. La pared de la antera joven está formada por epidermis, endotecio, una capa mediana y tapete secretor binucleado, eventualmente uninucleado. El gineceo es sincárpico con 9-10 carpelos, pluriovulado y de placentación parietal y el ovario tiene haces vasculares invertidos, en un patrón similar a *Pereskia*. La región nectarífera se encuentra en el lado interno del hipanto. El estigma es 9-10 lobado, con epidermis secretora. Los óvulos son circinótrofos, bitegumentados, crassinucelados y con funículo largo como en otras Cactaceae.

Palabras clave: antera, gineceo, hipanto, nectario, óvulo.

### Introduction

Cactaceae are distributed throughout the American continent, from the south and west of Canada to the south of Patagonia in Argentina and Chile (Kiesling, 1988; Rizzini, 1987). This family belongs to Caryophyllales *sensu* APG II (Angiosperm Phylogeny Group, 2003) and includes around 1 500 species and approximately 100 genera (Anderson, 2001; Judd et al., 2002; Wallace and Gibson, 2002; Souza and Lorenzi, 2005). Three subfamilies have been traditionally recognized: Pereskioideae, Opuntioideae and Cactoideae (Barthlott and Hunt, 1993; Wallace and

Gibson, 2002). However, molecular evidence supports the recognition of a fourth subfamily, the Mainhuenioideae (Anderson, 2001; Nyffeler, 2002; Griffith, 2004).

The flowers are generally lateral, solitary, formed in the areoles of stem branches, and frequently large and showy. They are pollinated by bats, hummingbirds, moths, or bees. Tepals are numerous, more or less brightly colored, spirally arranged, petaloid and/or sepaloid; although not clearly differentiated, they are all united at their bases to the hypanthium (Cronquist, 1981).

*Epiphyllum* Haw. (Cactoideae, Hylocereeae) includes about 19 species found mainly in Central America and Mexico, but a few species extend into the Caribbean and South America (Anderson, 2001). *Epiphyllum phyllanthus*

(L.) Haw. which is known as “rainha-da-noite” (Joly, 1998) and “orchid cactus” (Judd et al., 2002) has a wide distribution in South America, extending from southern Mexico to Paraguay, northwestern Argentina and southern Brazil (Kimmach, 1964; Kiesling, 1975; Anderson, 2001; Bauer and Waechter, 2006). This species is an obligate holoepiphyte with branched, flattened and crenated stems, sometimes trigonal at the base (Anderson, 2001; Zappi et al., 2007). Conservation studies on native species, such as *E. phyllanthus*, require basic information on reproductive organs, mainly morpho-anatomical knowledge.

The reproductive biology has been investigated in less than 10% of taxa within the Cactaceae, and the limited amount of data impedes a better understanding of reproductive mechanisms in the family (Cota-Sánchez and Abreu, 2007). In the literature, floral morpho-anatomic studies focusing on cacti are a few (Buxbaum, 1953; Boke, 1963, 1966, 1968; Leins and Schwitalla, 1988; Strittmatter et al., 2002; Terrazas et al., 2008; Fuentes-Pérez et al., 2009). Thus, the present study has the main objective to carry out a morpho-anatomic analysis of the flower of *Epiphyllum phyllanthus*.

## Material and methods

Flowers and flower buds from *E. phyllanthus* (Fig. 1A) were collected in Ingá Park (a fragment of Atlantic Forest) and its surroundings in Maringá, Paraná State, Brazil. Observations of anthesis were carried out at night. Voucher materials were deposited at the Universidade Estadual de Maringá Herbarium (HUEM) and Rio Claro Herbarium (HRCB), collection numbers: 12.673 HUEM, 48.936 HRCB and 48.937 HRCB.

For the floral morphological analysis, fresh and/or fixed material was evaluated under a Leica® stereoscope microscope. The material was fixed in formalin acetic alcohol (FAA) 50 from 2 to 5 days (Johansen, 1940). Illustrations of flowers and flower buds were made through drawing and photomicrographs taken with a digital camera.

For the anatomical study, flowers and flower buds were fixed in FAA 50 and later transferred into alcohol 70%, following the protocol of Johansen (1940). Samples were dehydrated in an ethyl alcohol series, embedded in historesin (Gerrits, 1991) and sectioned (cross and longitudinal sections) at 7 to 9 µm thickness with a rotary microtome. Sections were stained with toluidine blue at 0.05%, pH 4.7 (O'Brien et al., 1965), and mounted in Entellan® synthetic resin. Anatomical illustrations were made with photomicrographs obtained by image capturing under a Leica® photomicroscope using the software Leica

IM50 version 5.

In addition, microchemical tests were done for starch (iodine-potassium iodide test); phenolic substances (ferric chloride added of calcium carbonate test; Johansen, 1940), calcium carbonate crystals (concentrated acetic acid test), calcium oxalate crystals (10% hydrochloric acid test; Souza et al., 2005), mucilage (methylene blue test; Costa, 1972), several polysaccharides and pectins (ruthenium red test; Jensen, 1962), secretory tissue (neutral red test), and reducing sugars (Fehling reagent; Sass, 1951).

The flower and flower bud surfaces were analyzed using a Zeiss® DSM 940A, scanning electron microscope (SEM) equipped with image capturing system at the laboratory of Center in Electron Microscopy Applied to Agricultural Research (NAP/MEPA) of the Escola Superior de Agricultura Luiz de Queiróz (ESALQ) of the Universidade de São Paulo (USP), Brazil.

It should be noted that in the inferior ovary of such species, hypanthium, ovary and perianth tissues are not clearly delimited, since they are adnate. Thus, these regions were named according to their topographic position in the flower of *E. phyllanthus* (Fig. 2E, 8A).

## Results

**Floral morphology.** The flower is white (Fig. 1B, 2F), opens at night and emits a sweet odor; anthesis lasts only for a short time during 1 night. It is sessile, cyclic, actinomorphic, hermaphroditic, epigynous and has long tubular hypanthium with bracteoles also present on the pericarpel (the stem-tissue enclosing the inferior ovary in cacti) (Fig. 1C, 2E). The perianth has lanceolate tepals arranged in 2 whorls: 1 is sepaloid and light greenish yellow, and the other is petaloid and light yellow to white. The androecium is multistaminate (Fig. 1B,D,E, 2F) with white filaments of different lengths; the anthers are light brown (Fig. 1D, E) with complete longitudinal dehiscence. The style is light yellow, as long as the hypanthium, and the stigma is white with 9-10 lobes (Fig. 1E, 10A).

**Bracteole structure.** At early developmental stages, the flower bud is almost completely enclosed by lanceolate bracteoles (Fig. 2A) with a uniseriate, glabrous and amphistomatic epidermis. The parenchymatous mesophyll of the bracteoles is homogeneous, has secretory cavities and several small collateral vascular bundles (Fig. 5A, D). During development the bracteoles spread apart from one another due to expansion of the hypanthium and ovary (Fig. 2A-E). The bracteoles differ in the size of parenchymatous cells of the mesophyll (smaller on the abaxial side).

**Pericarpel structure.** The pericarpel has a more or less circular shape with indentations forming small ribs on

the outside (Fig. 1C, 10B, C). The external epidermis of the pericarpel is uniseriate and glabrous (Fig. 3A-C). Seen in surface view the epidermis shows slightly sinuous anticlinal cell-walls and parallelocytic stomata (Fig. 4D, E). The parenchymatous tissue is multiseriate, chlorophyllous, with cells which have a broad lumen, more or less isodiametric shape, and many starch grains (Fig. 4A-C). In the parenchyma there are idioblasts containing calcium oxalate crystals (Fig. 4B), vascular bundles of differing diameters, vascular traces directed toward the bracteoles (Fig. 3A), and abundant secretory cavities (Fig. 7B). The pericarpel is delimited internally by collateral vascular bundles of large circumference arranged in a ring around the ovary (Fig. 3B, 10C). The base of the flower has a vascular cambium and parenchymatous pith (Fig. 3A).

*Tubular hypanthium structure.* The outer hypanthium epidermis is glabrous (Fig. 4F) with parallelocytic stomata. In the lower part of the tube, just above the pericarpel, the separation from the style begins (Fig. 10A, F), where 2 distinct regions can be differentiated: an outer region with large parenchymatous cells with secretory cavities, and an inner region, more compact, and nectariferous (on the inner surface of the hypanthium) (Fig. 4F). Close to the perianth, the hypanthium does not have nectary (Fig. 10G) but has protrusions, both on the inner and outer surfaces; the lobes on the inner surface are smaller but more numerous, being the filament insertion sites (Fig. 4G, 10H). The parenchyma is homogeneous, the inner epidermis is glabrous with some stomata and, just below the inner epidermis there is mucilage accumulation through cellular lysis (Fig. 4G). *Nectary.* The nectariferous region occurs on the inner wall of the hypanthium, where it extends from the base to about midway up the hypanthium. It presents small secretory cells of dense cytoplasm, large nuclei and many adjacent vascular bundles with more phloem than xylem. The inner epidermis is formed of thick-walled secretory trichomes rich in reducing sugars (Fig. 4F, 10F).

*Perianth structure.* The shape and structure of the sepaloid perianth is similar to those of the bracteoles (in cross section); however, the sepaloid tepals are larger and have more stomata on the abaxial side. The mesophyll is homogeneous with secretory cavities and several collateral vascular bundles. The parenchyma is more compact than that of the bracteoles (Fig. 5B, E). The petaloid perianth differs from the sepaloid one in its larger epidermic cells, thinner cell-walls, mesophyll with smaller number of cell layers and fewer stomata on the abaxial side (Fig. 5C, F).

*Androecium structure.* The stamen has a filament formed by uniseriate epidermis with large cubic to slightly cylindrical shaped cells, parenchymatous tissue with secretory cavities and an amphicribal concentric central bundle (Fig. 6C). The connective region is composed of parenchymatous

tissue and a vascular bundle, in which the phloem almost completely envelopes the xylem (Fig. 6D, G).

The anther is bithecal (Fig. 6D, G), tetrasporangiate and longitudinally dehiscent (Fig. 6A). The wall of the young anther is formed of epidermis, endothecium, a middle layer and binucleate secretory tapetum that eventually becomes uninucleate (Fig. 6F-H). In the mature anther, epidermal cells are somewhat papillose with phenolic content and the endothecium has secondary thickening in strips on the outer periclinal and anticlinal walls (Fig. 6D). At dehiscence (Fig. 6B), in addition to the endothecium, large epidermic cells containing phenolic substances persist in the anther (Fig. 6E).

*Gynoecium structure.* The inferior ovary is enclosed by the pericarpel (Fig. 2G, 8A, B). The parenchymatous tissue is composed of non-chlorophyllous cells (Fig. 1C), which become gradually more elongated the closer they are to the inner epidermis in which there are some idioblasts with phenolic contents and many starch grains, as in the pericarpel (Fig. 3B). Vascular bundles of smaller diameter occur in the inner region, including vascular elements of diverse orientation, some inverted, and the xylem is directed toward the outer side (Fig. 3C-E). The inner epidermis is also uniseriate with sparse trichomes (Fig. 3B, 7C). The syncarpous ovary consists of 9-10 carpels, and is multiovulate with parietal placentation.

Ovules are circinotropous (Fig. 7A, C), bitegmic, crassinucellate and have a long funiculus of parenchymatous (Fig. 7C) cells and a single vascular bundle. The outer integument is composed of a variable number of cell layers with more layers in the apical region. The epidermis has unicellular long trichomes with a round tip in the region directed to the micropyle, along the funiculus (Fig. 7C). The inner integument has 3 cell layers that are smaller than those of the outer integument. The micropyle is only delimited by the inner integument, in which cells are larger (Fig. 7C, D). The nucellus is composed of thin-walled, highly vacuolated cells, and there is evidence of division in more superficial cells (Fig. 7D).

The column corresponds to the region of constriction of the pericarpel with the floral tube (Fig. 10A, D, E), where the carpels fuse, forming the roof of the ovarian cavity, and the differentiation of the style (Fig. 8A, B). The column includes parenchyma, secretory cavities and 9-10 dorsal vascular bundles which surround the transmitting tissue. This tissue penetrates the ovarian cavity covering its roof (Fig. 8A, B). In the column region, as well as in the uppermost region of the ovary recurrent bundles can be found (Fig. 8C-D). The cylindrical style (Fig. 9C) is composed of a glabrous uniseriate epidermis with stomata. It has 1-2 layers of collenchymatous tissue and relatively loose parenchyma, including secretory cavities, 9-10

collateral bundles and central transmitting tissue (Fig. 9C, D). The style, in the basal region, is united with the hypanthium and the epidermis differs from the uppermost region in the presence of papillose cells (Fig. 9A, B) rich in reducing sugars, similar to the trichomes on the inner epidermis of the hypanthium in this same region of the flower (Fig. 4F). The transmitting tissue is formed of epidermal and subepidermal tissue with cells containing dense cytoplasm. The stigma has 9-10 lobes and each lobe has a secretory epidermis with uni- and bicellular trichomes. Beneath this is parenchymatous tissue with secretory cavities and a vascular bundle (Fig. 9E, F).

## Discussion

The characteristics of the flower of *E. phyllanthus*, such as an elongate hypanthium, white perianth and nocturnal anthesis with sweet odor suggest that these flowers are sphingophilous, according to Barthlott and Hunt (1993). Silva and Sazima (1995) reported sphingophily for *Cereus peruvianus* Miller, a species that has flowers with attributes for moth visits, similar to those of the flowers of *E. phyllanthus*. Many genera of epiphytic cacti, such as *Hylocereus*, *Echinopsis* and *Selenicereus* (besides *Epiphyllum*) bear large, nocturnal, white and disc-shaped flowers, with a large nectar chamber (Pimenta-Barrios and del Castillo, 2002).

The pericarpel is the stem tissue enclosing the inferior ovary in cacti (Buxbaum, 1953; Barthlott and Hunt, 1993). Histologically, between the pericarpel and the ovary wall a ring of collateral vascular bundles can be seen, as in *Opuntia* (Fuentes-Pérez et al., 2009). *Epiphyllum phyllanthus* pericarpel is similar to that of *Opuntia*, but the vascular bundles are noticeably smaller. In *E. phyllanthus* this tissue is conspicuous because of the presence of chlorophyllous parenchyma, the larger number of secretory cavities and larger size of the parenchymatous cells. The base of the flower has a structural arrangement similar to stem (Dettke and Milaneze-Gutierrez, 2008), except that it lacks a collenchymatous hypodermis.

The abundant starch found in the flower tissues, as well as the remaining substances synthesized by the plant are necessary for the floral development and, after fertilization, for fruit and seed development. Erdelská and Ovečka (2004) noted that in cases where the flower is not fertilized, a high proportion of these nutrients are recycled by the plant through reallocation of substances via phloem during flower senescence. In *Epiphyllum* hybrids analyzed by Erdelská and Ovečka (2004), around 42% flower dry matter could be reutilized by the parent plant, which is considered part of the life strategy of Cactaceae to survive

in xeric environments. Calcium oxalate crystals such as those found in idioblasts and secretory cavities of *E. phyllanthus* flower tissues are common in other species of this family, especially in vegetative organs (Metcalf and Chalk, 1979; Harti et al., 2007).

Due to fusion of the pericarpel with the ovary, it was difficult to define the number of carpels in this species. However, 9-10 carpels were established based on the number of bundles that occur in the apical region of the ovary, the number of style bundles, and the number of stigma lobes. Similar methods were adopted by Saunders (1939), Boke (1964) and Roth (1977) for Cactaceae species.

The nectary structure is an important systematic character (Bernardello, 2007). In the Cactaceae the nectar is secreted by a disc (*Pereskia*, *Rhipsalis*) or along the basal portion of the hypanthium. In the latter case, distinct nectar chambers may occur, more or less closed by the formation of a dense ring of filament bases, or filamental or hypanthial appendices (*Schumbergera*), and in some *Opuntia* species by an annular or even cup-like outgrowth at the base of the style (Buxbaum, 1953; Barthlott and Hunt, 1993). In the species of *Opuntia* studied by Fuentes-Pérez et al. (2009), the nectar occurs below the place of insertion of the inner filaments around the style base. In *E. phyllanthus* the nectary is hypanthial type *sensu* Bernardello (2007), as the nectary of *Opuntia* (Fuentes-Pérez et al. 2009). According to Buxbaum (1953) in Cactaceae there are 3 basic types of "nectarial zones": nectar-furrow, disc, and nectar-chamber. The hypanthial type nectary of *E. phyllanthus* corresponds to nectar-chamber type *sensu* Buxbaum. However, a comparative study of nectary development in Cactaceae is necessary in order to determine the possible taxonomic value of this character (Fuentes-Pérez et al., 2009).

In most angiosperms the anther epidermal cells collapse at maturity (Mariath et al. 2006), whereas the anther epidermal cells of *E. phyllanthus* become papillose, occluded with phenolic contents. After dehiscence, the sporangium inner surface is exposed through longitudinal slits, whereas the outer surfaces, with persistent epidermis, become closer together, almost touching each other.

In the literature, the ovule type described for Cactaceae varies. Corner (1976) considered it to be anatropous to more or less campylotropous, Maheshwari (1971) and Fuentes-Pérez et al. (2009) campylotropous, Johri et al. (1992) as anatropous, hemianatropous, campylotropous or circinotropous, while Strittmatter et al. (2002) and Paoli (2006) deemed it circinotropous, and Rosa and Souza (2003) described it as amphitropous. In *E. phyllanthus* the ovule is circinotropous and there is a long funiculus with unicellular and long trichomes of funicular and placental origin, extending to the micropyle. These trichomes



may function as an obturator during fertilization. Rosa and Souza (2003) also observed funicular trichomes in the ovule of *Pereskia aculeata* Mill. However, *Pereskia* funiculus is highly reduced and thus differs from that of *E. phyllanthus*.

The placenta region in angiosperms is normally supplied by ventral or adaxial vascular bundles. In Cactaceae, there is no distinct ventral carpellary vascular bundle, but rather a complex reticulum of bundles that supply the placenta region (Boke, 1964; Roth, 1977). In the studied species, this region has a similar vascularization, with bundles of variable xylem orientation, relative to the phloem, including totally inverted bundles.

The style of *E. phyllanthus* is of a spongy consistency, without an open stylar cavity and with an inner epidermis of papillose cells, such as in *Opuntia* (Fuentes-Pérez et al., 2009) and *Pereskia* (Boke, 1963, 1966, 1968). According to Fuentes-Pérez et al. (2009) a description of the transmitting tissue, as well as the identification of its function, would be important to understand the variation of this tissue on the family.

The axial character of the inferior ovary in cacti can be identified by formation of appendages, such as leaves or scaly bracts, and the vascularization of the flower. The vascular bundles enter the base of the receptacle, and ascend to a level just above the androecium, forming “the ascending receptacular system”, which provides traces to the receptacular bracts and perianth segments. The receptacular system then turns downwards, thus producing the “recurrent receptacular system” from which traces to the stamens and to the gynoecium diverge (Roth, 1977). Rosa and Souza (2003), for instance, considered the ovary of *Pereskia aculeata* to be of axial nature due to the presence of green bracteoles and areoles bearing spines and hairs.

Two theories have been presented to explain the origin of inferior ovary in angiosperms. The appendicular theory suggests that the inferior ovary originates from a gradual fusion of flower pieces (sepals, petals and stamens) with the ovary, whereas the receptacular or axial theory suggests that the ovary becomes immersed into the receptacular tissues (Smith and Smith 1942; Douglas, 1944; Roth, 1977; Dickison, 2000). The receptacular nature of *E. phyllanthus* inferior ovary agrees with the receptacular or axial theory. Although this study did not include a floral ontogenetic study, the 2 vascularization systems (an ascending and a recurrent system: see figures 9 and 10) and bracteoles on its surface indicate the axial origin. The ascending vascularization system, responsible for the vascularization in the whole flower, is formed by collateral bundles. The recurrent vascularization system, observed in the ovary, has inverted vascular bundles of smaller diameter, where

the primary xylem is directed toward the external surface, in a similar pattern to that presented by Boke (1963, 1966, 1968), but without the formation of the columella as in *Pereskia*.

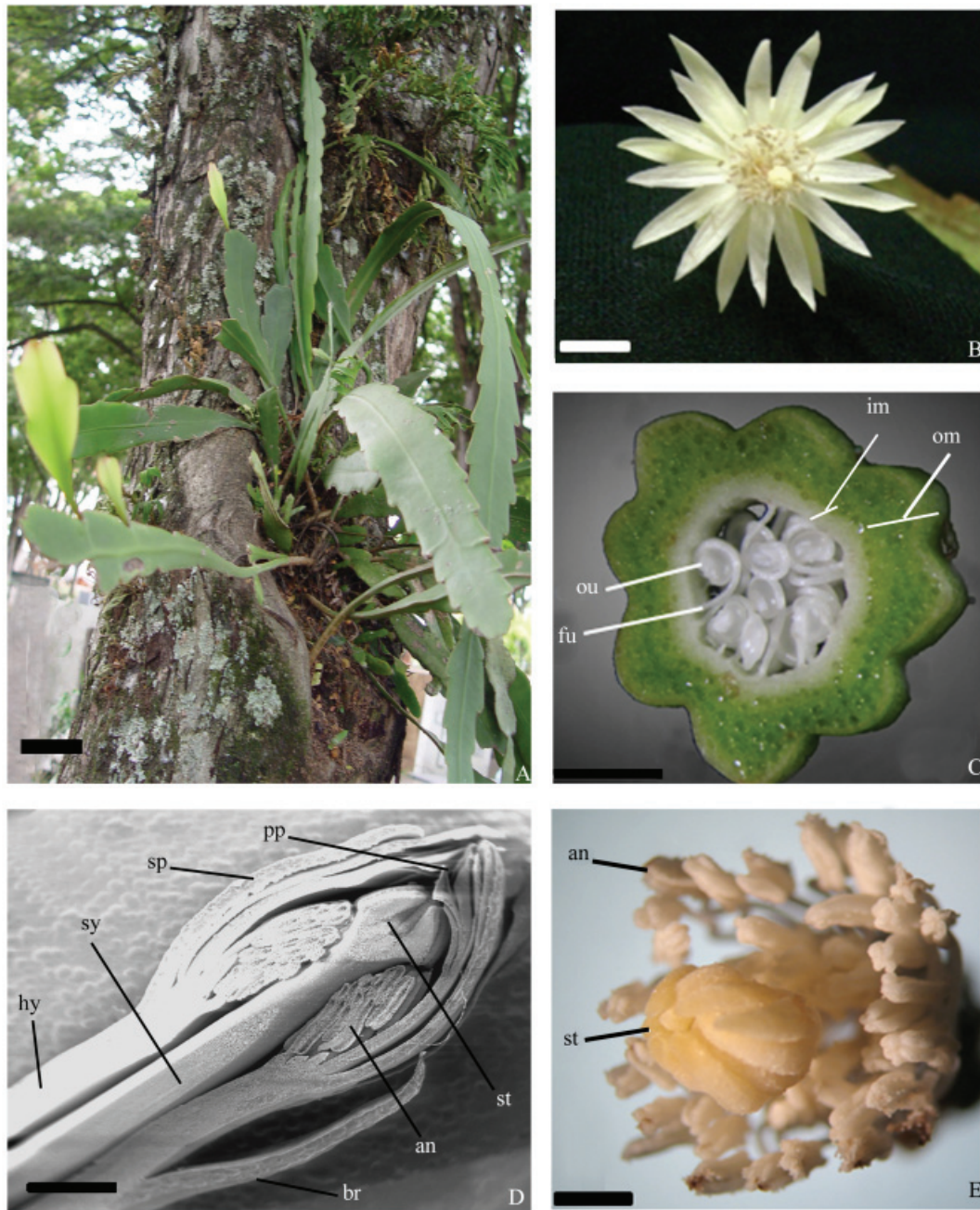
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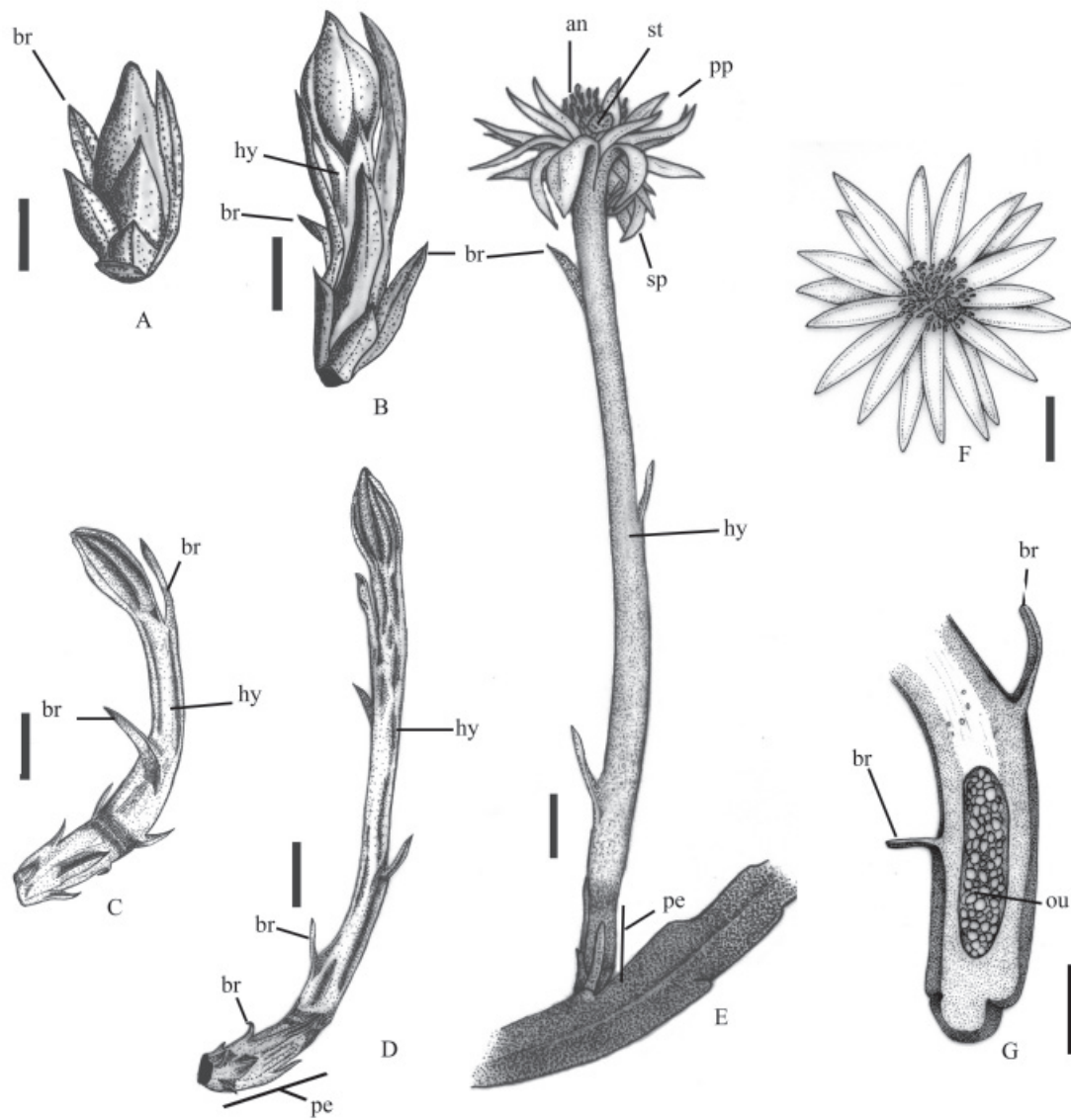
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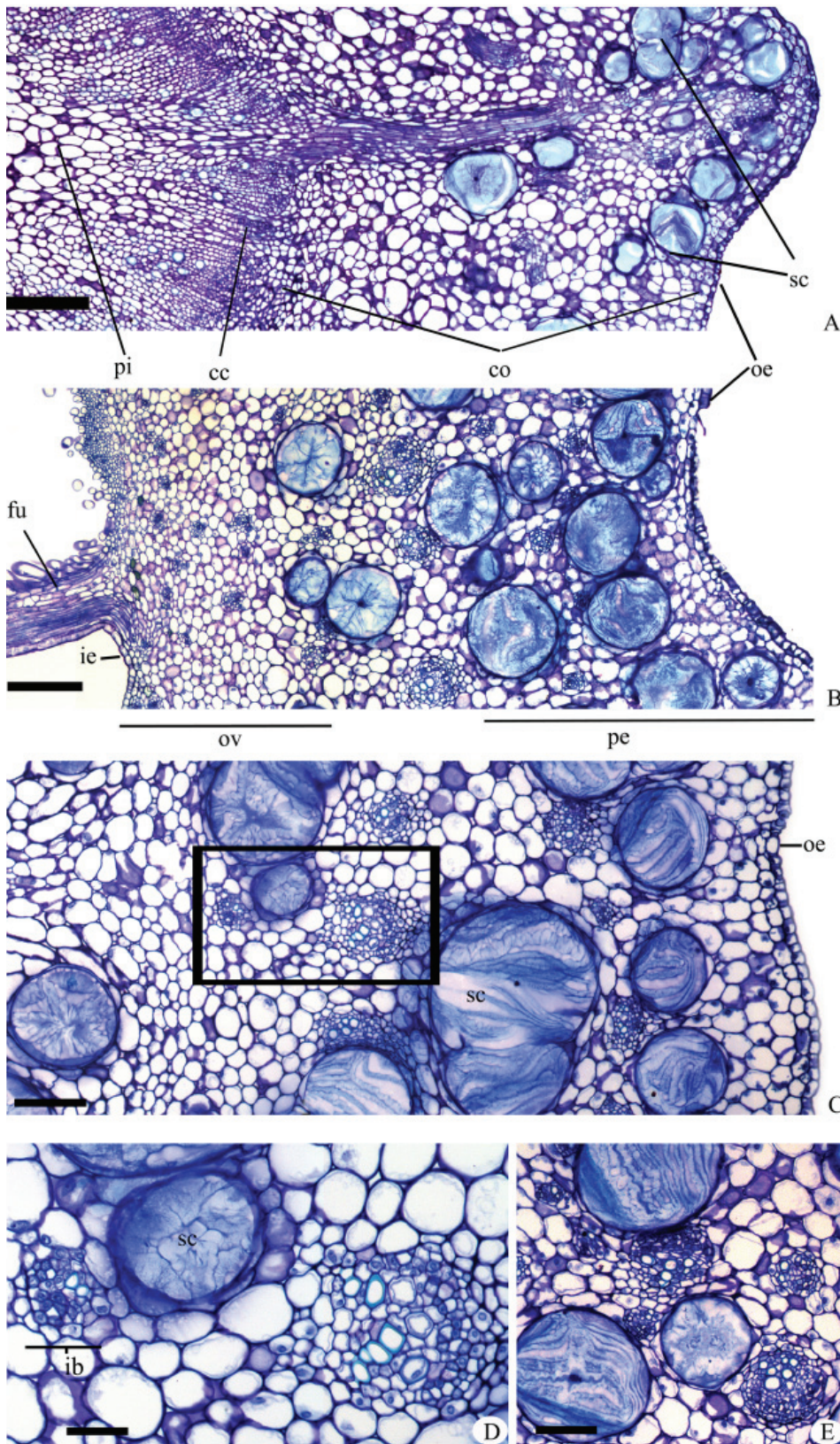
**Figure 1.** General aspect of *Epiphyllum phyllanthus* plant and flower. A. Epiphytic habit on a *Caesalpinia peltophoroides* trunk. B. Frontal view of the flower at anthesis. C. Ovary in a transversal section of a flower in anthesis. D. SEM of a pre-anthetic flower in longitudinal section. E. Anthers and stigma. (an = anthers; br = bracteole; fu = funiculus; hy = hypanthium; im = inner region (ovary); om = outer region (pericarpel); ou = ovule; pp = petaloid perianth; sp = sepaloid perianth; st = stigma; sy = style). Bars = 2 cm (A-B); 1cm (C); 500  $\mu$ m (D); 2mm (E).





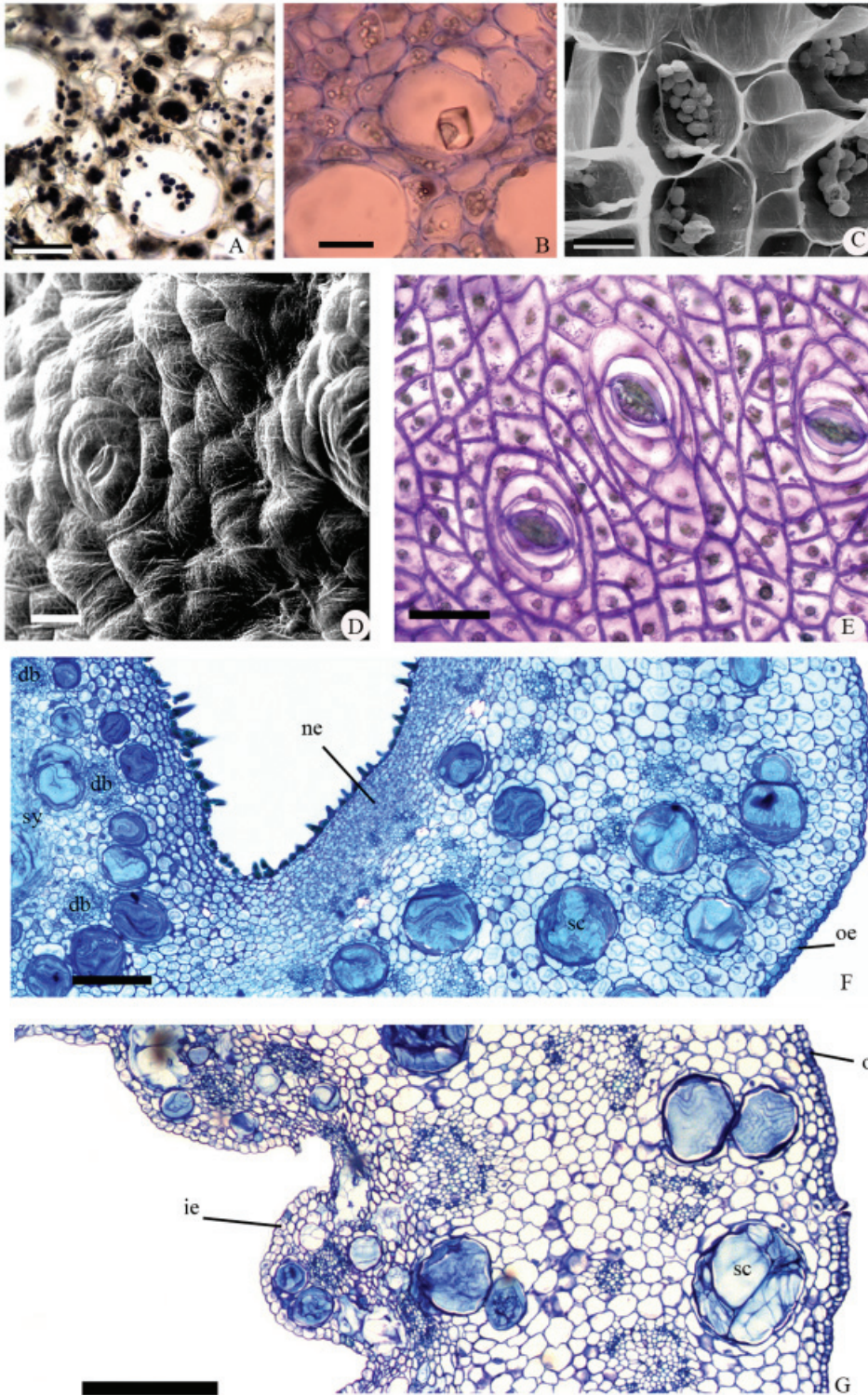
**Figure 2.** *Epiphyllum phyllanthus* flower. A-D. Flower buds. E. Flower in lateral view. F. Flower in frontal view. G. Basal region of the flower in longitudinal section. (an = anther; br = bracteole; hy = hypanthium; ou = ovules; pe = pericarpel; pp = petaloid perianth; sp = sepaloid perianth; st = stigma;). Bars = 2mm (A); 3mm (B); 10 mm (C-F); 5mm (G).





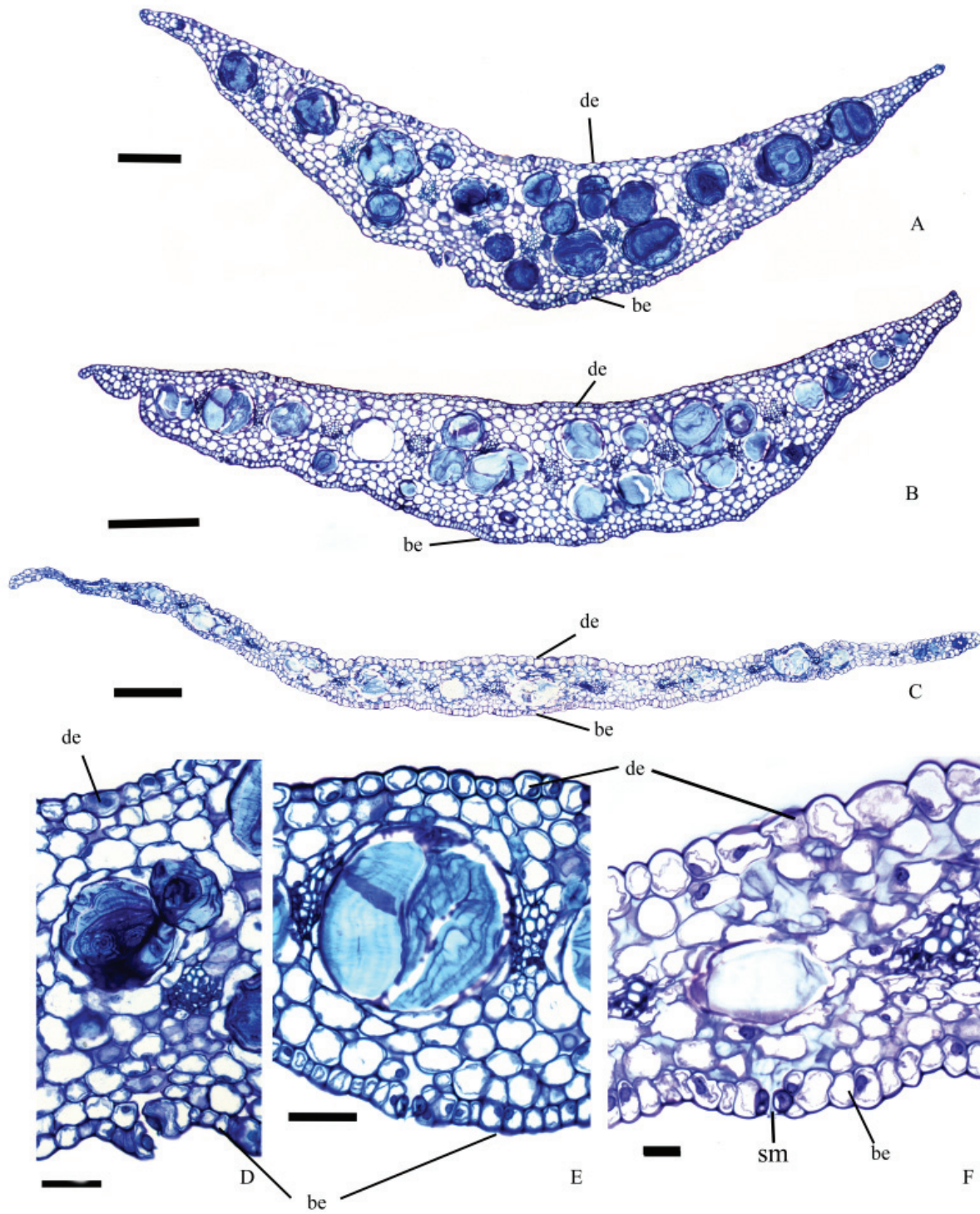
**Figure 3.** *Epiphyllum phyllanthus* flower in transverse sections. A. Basal region of the flower. B. Ovary and pericarpel. C. Ovary and pericarpel, apex. D and E. Detail of the inverted vascular bundle and vascular bundles with diverse orientation of the xylem relative to the phloem. (cc = central cylinder; co = cortex; fu = funiculus; ib = inverted bundle; ie = inner epidermis; oe = outer epidermis; ov = ovary; pe = pericarpel; pi = pith; sc = secretory cavity). Bars = 300µm (A), 150µm (B), 100 µm (C), 30 µm (D-E).



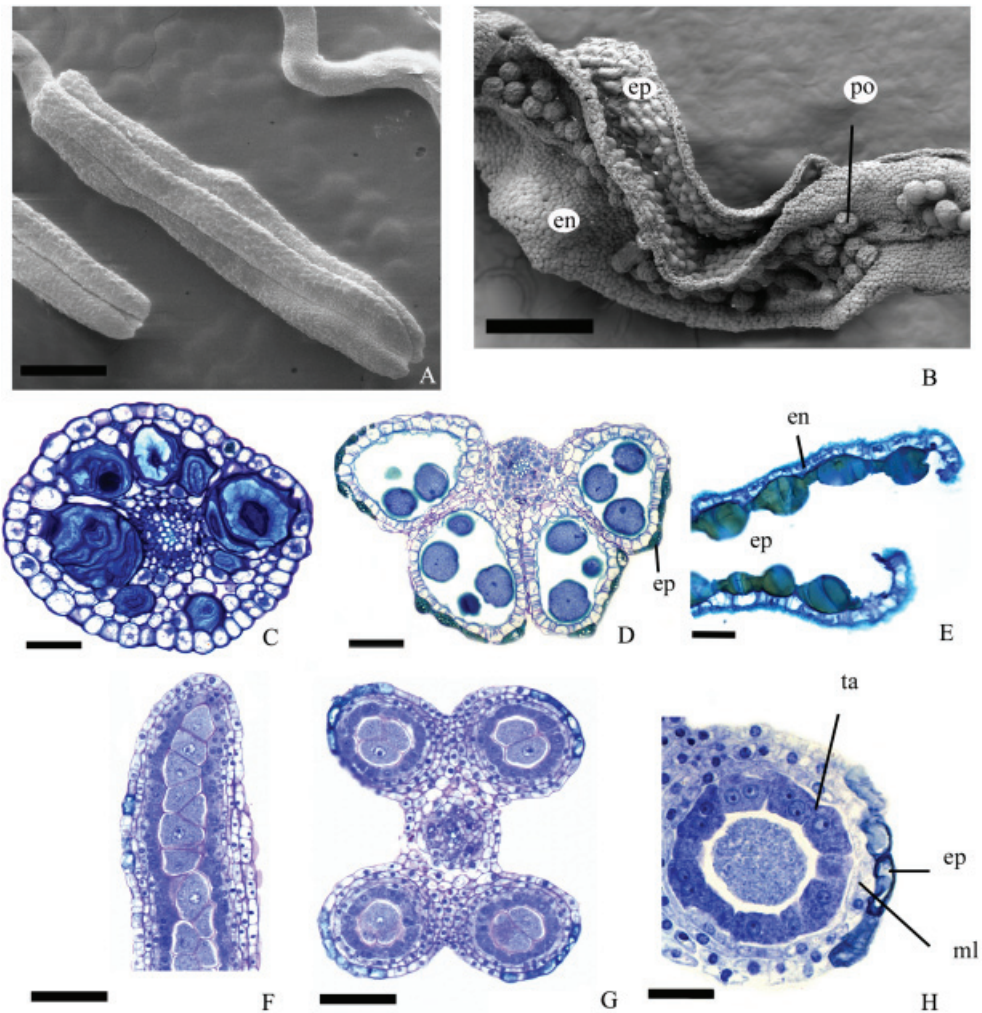


**Figure 4.** *Epiphyllum phyllanthus* ovary, epidermis of the pericarpel and hypanthium. A and B. Ovary mesophyll, with starch and a prismatic calcium oxalate crystal. C. Ovary mesophyll with some cells containing starch (SEM). D. Pericarpel outer surface (SEM). E. Pericarpel exoderm paradermal section. F-G. Transverse sections. F. Hypanthium, lower region, site of style separation; note the nectariferous region. G. Hypanthium, upper region. (db = dorsal vascular bundles; ie = inner epidermis; ne = nectariferous region; oe = outer epidermis; sc = secretory cavity; sy = style). Bars = 60  $\mu$ m (A-B), 5  $\mu$ m (C), 20  $\mu$ m (D), 60  $\mu$ m (E), 150  $\mu$ m (F-G).



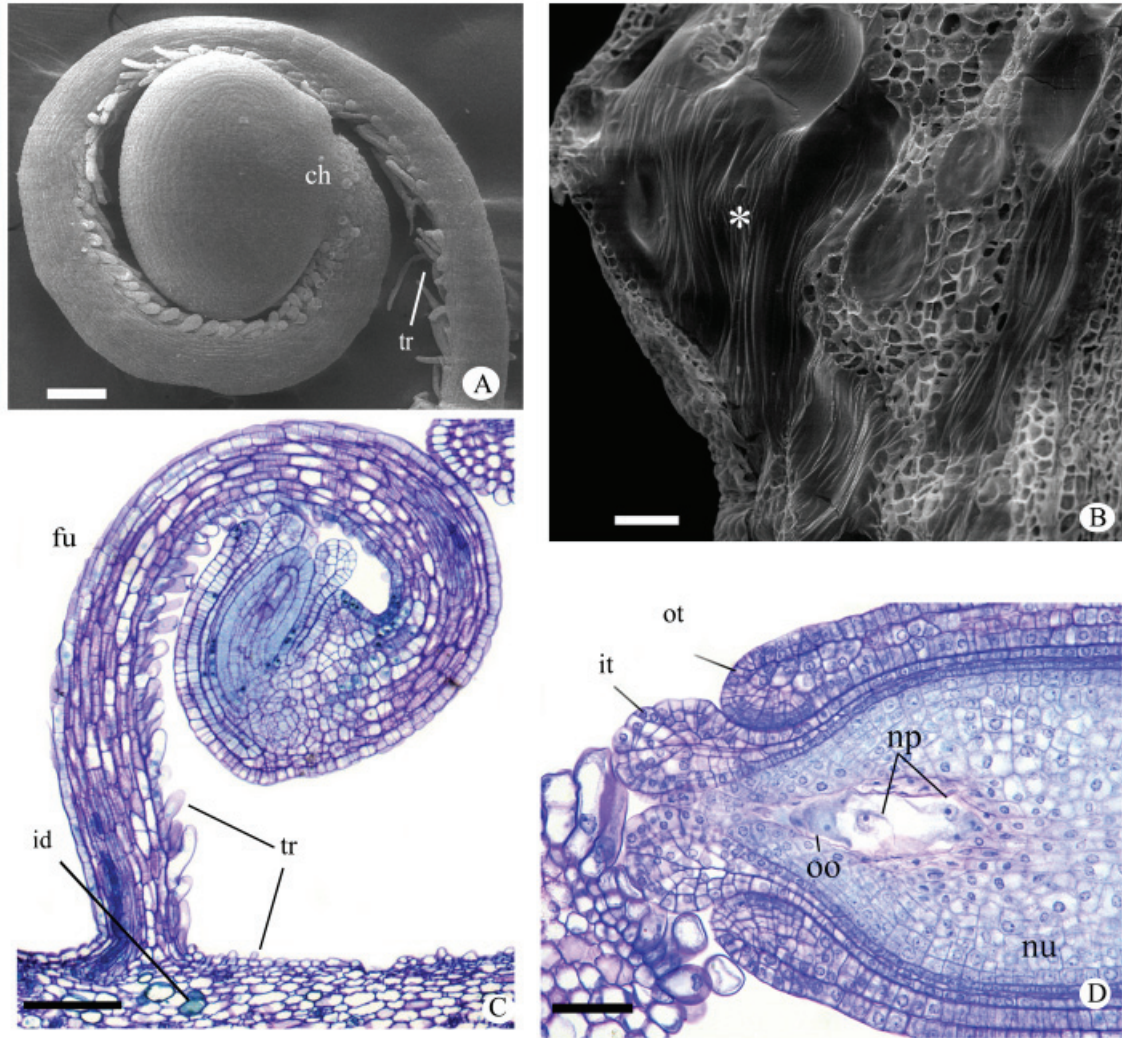


**Figure 5.** *Epiphyllum phyllanthus* bracteole and perianth in transverse sections. A. Bracteole. B. Sepaloid perianth. C. Petaloid perianth. D-F. Details of bracteole, sepaloid perianth, petaloid perianth, respectively. (be = abaxial epidermis; de = adaxial epidermis; sm = stomata). Bars = 300 $\mu$ m (A-B), 500  $\mu$ m (C), 60  $\mu$ m (D-F).

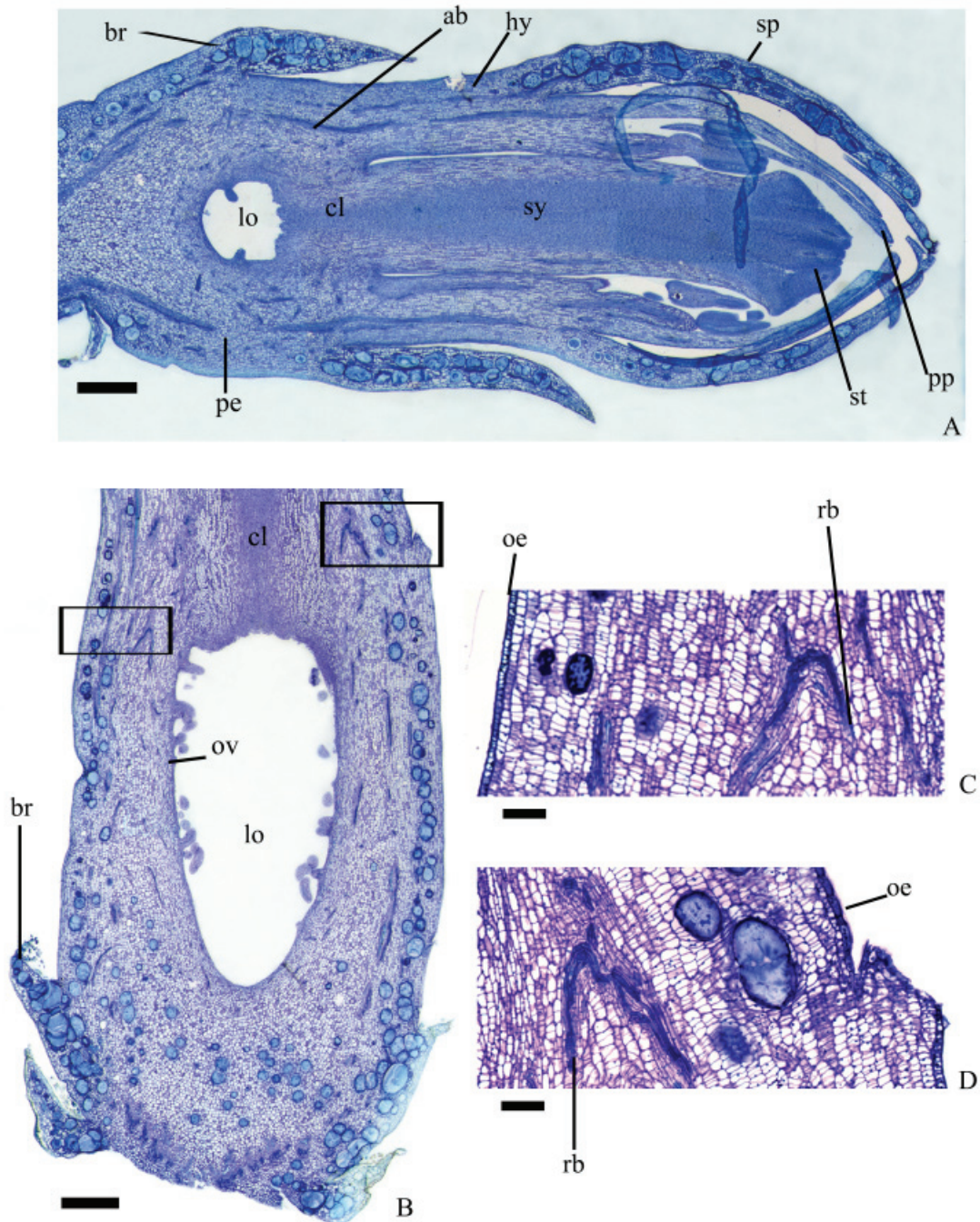


**Figure 6.** *Epiphyllum phyllanthus* pre-anthetic and anthetic stamen. A. General view of stamens (SEM). B. Stamen with pollen grains after opening of the longitudinal slit (SEM). C to E. Transverse sections. C. Filament. D. Mature anther. E. Part of the dehiscent anther. F. Detail of part of the theca of a young anther in longitudinal section. G and H. Young anther and detail of part of the theca in transversal sections. (en = endothecium; ep = epidermis; ml = middle layer; po = pollen; ta = tapetum). Bars = 100  $\mu\text{m}$  (A-B, E), 60  $\mu\text{m}$  (C-D), 75  $\mu\text{m}$  (F-G), 30  $\mu\text{m}$  (H).



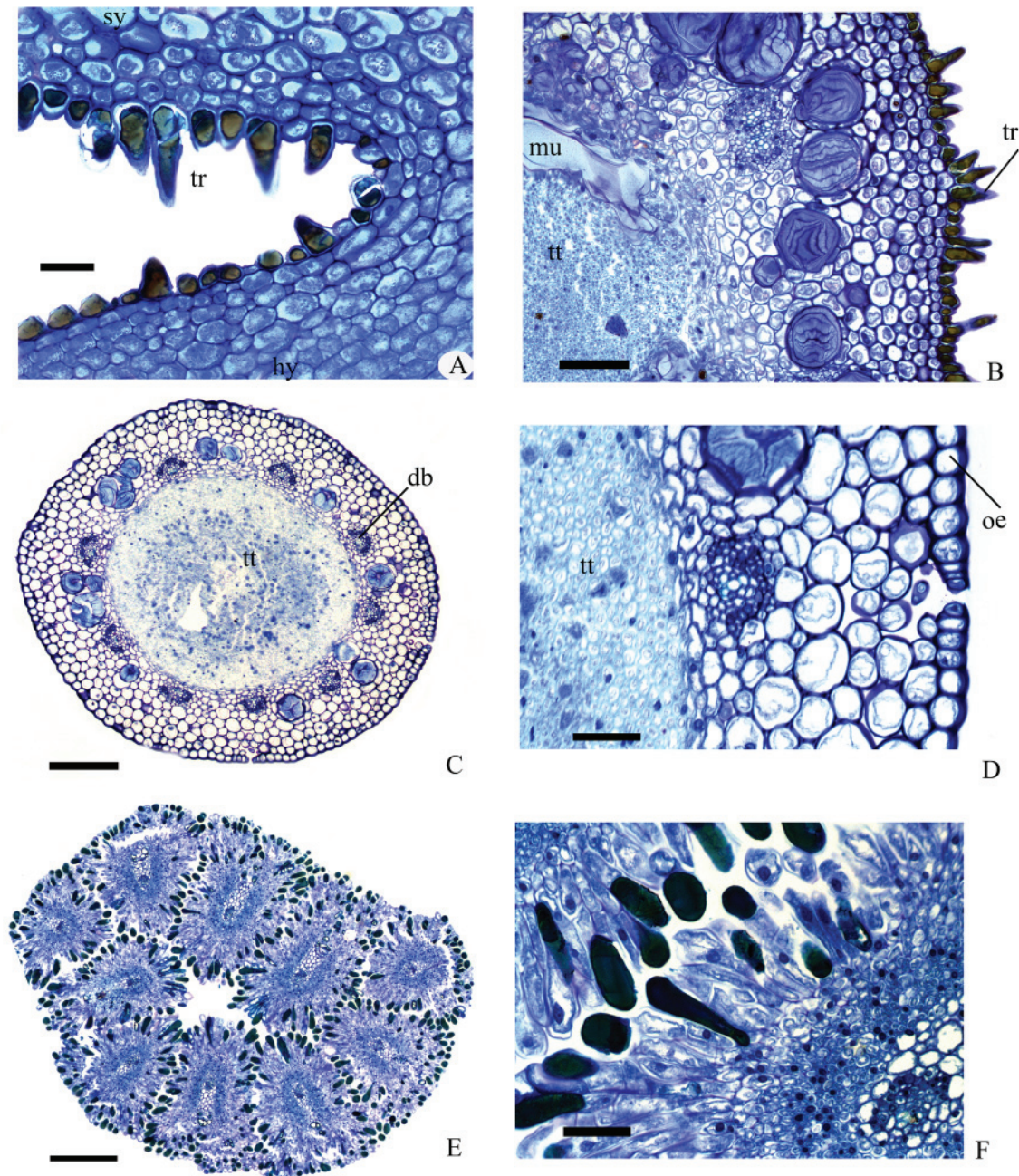


**Figure 7.** *Epiphyllum phyllanthus* ovule and pericarpel. A. SEM of the ovule in lateral view. B. SEM of the ovary pericarpel; note the secretory cavities (\*). C. Circinotropous ovule in longitudinal section. D. Ovule detail (ch = chalaza; fu = funiculus; id = phenolic idioblast; it = inner integument; np = polar nuclei; nu = nucellus; oo = oosphere; ot = outer integument; tr = trichome; \* = secretory cavities). Bars = 80  $\mu$ m (A), 100 $\mu$ m (B-C), 50  $\mu$ m (D).



**Figure 8.** *Epiphyllum phyllanthus* flower buds in longitudinal sections. A. Lateral view of a very young flower bud. B. Flower bud inferior region. C-D. Details of the region just above the ovary cavity, left and right sides, respectively, showing recurrent vascular bundles. (ab = ascending vascular bundles; br = bracteole; cl = column; hy = hypanthium; lo = locule; oe = outer epidermis; ov = ovary; pe = pericarpel; pp = petaloid perianth; rb = recurrent vascular bundle; sp = sepaloid perianth; st = stigma; sy = style). Bars = 150  $\mu\text{m}$  (A-B), 60  $\mu\text{m}$  (C-D).





**Figure 9.** *Epiphyllum phyllanthus* style and stigma in transverse sections. A. Style, basal region, site of hypanthium separation, with secretory trichomes. B. Style in the basal region. C-D. General view of the style, apical region and detail. E-F. Stigma and detail of the lobes (db = dorsal vascular bundles; hy = hypanthium; mu = mucilage; oe = outer epidermis; sy = style; tr = trichomes; tt = transmitting tissue). Bars = 60µm (A-B, D), 100µm (C), 300 µm (E), 50 µm (F).



**Figure 10.** Section diagrams of *Epiphyllum phyllanthus* flower in anthesis. A. Flower longitudinal section. B-H. Transversal sections. B. Flower base. C. Ovary and pericarpel. D-E. Column region. F. Region where the style starts detaching. G. Hypanthium middle region. H. Hypanthium apex. (ab = vascular bundles of the ascending system; bt = bracteole trace; bv = vascular bundles ring; cc = central cylinder; db = dorsal vascular bundles; et = sepaloid trace; mt = stamen trace; mu = mucilage; ne = nectary; ou = ovule; ov = ovary; pe = pericarpel; pt = petaloid trace; rb = vascular bundles of the recurrent system; sy = style; tt = transmitting tissue). Bars = 10 mm (A), 1 mm (B-H).