

Floral Anatomy of *Peristethium leptostachyum* (Loranthaceae)Alejandra Robles¹, Lauren Raz² & Xavier Marquínez¹

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Abstract: **Floral anatomy of *Peristethium leptostachyum* (Loranthaceae).** *Peristethium leptostachyum* is a hemiparasite species of the family Loranthaceae, distributed in Colombia, Costa Rica, Ecuador, Peru, Venezuela and Panama. Previously treated as *Struthanthus leptostachyus*, the species was recently transferred to *Peristethium* together with other species of *Cladocolea* and *Struthanthus*. The present research describes the inflorescence and floral morphoanatomy of *Peristethium leptostachyum*, detailing the structure of the androecium and gynoecium and the processes of microgametogenesis and megagametogenesis, thus allowing comparison with *Struthanthus* and *Cladocolea*. Flowering material was collected in February and August 2012, in Santa María, Boyacá, Colombia. Histological sections were prepared and stained with astrablue-fuchsin and floral dissections were performed under a stereomicroscope. *Peristethium leptostachyum* shares inflorescence characters with *Cladocolea* (determinate inflorescence, ebracteate terminal flower), but also with *Struthanthus* (pairs of triads along the axis, deciduous bracts and actinomorphic flowers). The flowers of *P. leptostachyum* from Santa María are clearly hermaphrodites with androecium and gynoecium fully developed. This observation contradicts the description by Kuijt who reported this species to be dioecious. The androecium was observed to be similar to that of *Struthanthus vulgaris*, with a glandular *tapetum* and simultaneous microsporogenesis; in contrast, *Cladocolea loniceroides* has a periplasmodial *tapetum* and successive microsporogenesis. The gynoecium of *P. leptostachyum*, like that of *Cladocolea*, *Struthanthus* and *Phthirusa*, has a unilocular ovary with a mamelon and arquesporial tissue isoriented towards the style, which in turn is solid and amyliferous. *Peristethium leptostachyum* is similar to *Cladocolea loniceroides* and differs from *Struthanthus vulgaris* in presenting multiple embryo sacs and an unlignified pelvis (hipostase). The presence of a solid stylar canal is proposed as a synapomorphy of the tribe Psittacanthinae. Given that *P. leptostachyum* shares characters with both *Cladocolea* and *Struthanthus* generic placement cannot be clearly determined on the basis of anatomical evidence. Phylogenetic studies that include representative species of all three genera are desirable to test hypotheses of monophyly. The sexual system observed here in *P. leptostachyum* is different from that reported by Kuijt and more studies are needed to identify the factors (geographic, ecological, etc.) that influence this variation. Rev. Biol. Trop. 64 (1): 357-368. Epub 2016 March 01.

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The order Santalales, consisting of 18 families, 160 genera and approximately 2,200 species of hemiparasitic or holoparasitic plants (Mathiasen, Nickrent, Shaw, & Watson, 2008; Nickrent, Malecot, & Vidal, 2010), makes up the main group of parasitic plants of the tropical flora (Dueñas, 2001). The family Loranthaceae is the most representative of the order, with about 73 genera and 960 species (Dueñas, 2001; Nickrent et al., 2010).

Loranthaceae differ from related families being hemiparasitic plants of roots and

branches through primary and secondary haustoria, and the presence or absence of epicortical roots. The curvi-nerved leaves have opposite arrangement (rarely alternate or whorled), the inflorescences are terminal or axillary, arranged in spikes, racemes, umbels or heads, and the basic unit is usually a dichasium. Flowers are bisexual or unisexual, radial or bilaterally symmetric with a reduced calyx (simulating a ring, tube or short tooth) the corolla choripetalous or gamopetalous, and the stamens are free or epipetalous. The anthers are basifixed or dorsifixed with 2 or 4

locules. The ovary is inferior and lacks differentiated ovules and in their place develops a structure called a mamelon consisting of an undifferentiated mass of tissue containing the embryo sac. The fruits are always berries (Nickrent et al., 2010).

Peristethium leptostachyus (Kunth) Tiegh. is part of the tribe Psittacanthae Horan. with *Aetanthus*, *Cladocolea*, *Dendropemon*, *Desmaria*, *Ixocactus*, *Ligaria*, *Notanthera*, *Oryctanthus*, *Oryctina*, *Panamanthus*, *Phthirusa*, *Psittacanthus*, *Struthanthus*, *Tripodanthus*, *Tristerix* and *Tupeia*. The monophyly of the tribe is supported by morphological, karyological and molecular characters (Vidal & Nickrent, 2008; Nickrent et al., 2010).

The genus *Peristethium* Tiegh was originally proposed by Van Tieghem (1895), but was not recognized until later research by Kuijt (2012). The species studied here, *P. leptostachyum*, was originally described as *Loranthus leptostachyus* Kunth (1820), which is also the basionym of the combination *Struthanthus leptostachyus* (Kunth) G. Don (1834).

When Don (1834) carried out the transfer, *Struthanthus* had recently been monographed by Martius (1830) who recognized 25 South American species defined by the following generic characters: inflorescence a spike or raceme formed by pairs of triads, bisexual flowers, and dorsifixed anthers on slender filaments. Eichler (1868) in his treatment of the genus corrected the generic diagnosis of Martius, indicating that the dioecious species whose flowers are unisexual have aborted remaining organs of the opposite sex. Moreover, Eichler added nine Mesoamerican species and 10 South American ones to the genus. Currently there is no monograph of *Struthanthus* but is estimated to have between 50 and 75 species.

Kuijt (1975), in his monograph of the genus *Cladocolea* Tiegh., said the distinction between this genus and *Struthanthus* is

uncertain given the presence of species with intermediate characteristics. Recent phylogenetic studies confirm this relationship of the sister genera (Vidal & Nickrent, 2008). Currently, more than 30 species of *Cladocolea* are recognized (The Plant List, 2013), characterized by its simple and determinate inflorescences, as well as the absence of bracteoles, and sessile anthers adnate to the petals. The sexuality of flowers varies within this genus.

Kuijt (2012) revived the genus *Peristethium* Tiegh. including four species transferred to *Struthanthus*, among them *S. leptostachyus*, five from *Cladocolea* and five new species discovered in the Andes of Colombia, Ecuador and Peru. With the recent transfer of the species *Struthanthus reticulatus* (Simões, Gomes, & Barnes, 2014), described by Rizzini (1980), the genus *Peristethium* totals 15 species.

Although *Peristethium* species were not sampled in phylogenetic analyses, the genus was preliminarily included in Tribe Psittacanthae, and specifically in the subtribe Psittacanthinae (Vidal & Nickrent 2008 Nickrent et al. 2010). This sub-tribe has also been called the “small flowered New World taxa” (Vidal & Nickrent 2008) and the “*Struthanthus* complex” (Kuijt, 2012).

The restoration of the genus *Peristethium* (sensu Kuijt, 2012) is based on three structural features: (1) development of papery bracts at the base and along the axis of the inflorescence subtending monads and triads; the bracts tend to be persistent at the base of the inflorescence and caducous along the floral axis; (2) inflorescence with a solitary terminal flower followed by one or more pairs of ebracteate monads, and (3) the presence of epipetalous, sessile or almost sessile anthers that are inserted on the upper half of the petal.

Kuijt (2012) describes the following diagnostic reproductive characters for *P. leptostachyum*: one or two axillary inflorescences of about 12 cm in length, with a

peduncle to 2 cm, several sterile internodes and some brown colored lenticels, with ≤ 12 pairs of sessile triads, one or two pairs of sessile and solitary monads, and a terminal flower; monads and triads not sunken in nodal cups, each inflorescence subtended by numerous pairs of deciduous bracts at early stages, floral bracts and bracteoles caducous; flowers hexamerous, unisexual, not sunken in nodal cups, buds 4-5 mm; the female flower is slender, the male flower is wider and shorter; male flower anthers sessile, sometimes slightly twisted, in two different series above the middle of the petals, sometimes with white hairs directly below; anthers in the female flower sterile, strap-shaped; stigma capitate, distinct, oblique; style essentially straight, also present in the male flower; fruit 7 x 5 mm, ellipsoidal, the color initially reddish maturing to a dark blue tone.

In species of some genera of subtribe Psittacanthinae that are related to *Peristethium*, such as *Cladocolea loniceroides* (Cid, 2006), *Phthirusa pyrifolia* (Kuijt & Weberling, 1972), *Struthanthus vulgaris* (Venturelli, 1981, 1984a) and *Struthanthus flexicaulis* (Venturelli, 1984b), floral structure and embryology have been studied. Similar studies have also been performed on more distant genera, but belonging to the tribe Psittacanthae, such as *Psittacanthus* (Gómez, Sánchez & Salazar, 2011), *Tripodanthus* (Cocucci & Venturelli, 1982, Venturelli, 1983; Cocucci, 1983), and *Tupeia* (Smart, 1952). Finally, the series entitled “morphological and embryological studies in the family Loranthaceae” records studies of 13 species of Loranthaceae, these having been compiled and analyzed by Bhatnagar and Johri (1983) and Cocucci (1983).

This study describes and analyzes the morphology and anatomy of flowers and inflorescences of *Peristethium leptostachyum*, detailing the structure of the androecium and gynoecium and the microgametogenesis and megagametogenesis processes. Comparisons

were also made with related species of the tribe Psittacanthinae, looking for informative characters for later taxonomic and phylogenetic studies, and to provide clarification in relation to the previous diagnosis of the species, in particular to determine if it is dioecious as Kuijt had proposed, or if it is hermaphroditic as other researchers have noted.

MATERIALS AND METHODS

Flower buds, young and mature inflorescences, and individual mature flowers of *Peristethium leptostachyum* were collected in February and August 2012 in the municipality of Santa Maria, the town of Cascada la 70 (4°56'24.57" N - 73°20'10.69"; Boyacá - Colombia); along with voucher specimens in the Colombian National Herbarium (A. Robles-Sanchez, 001-002 COL). Micropreparations are in the collection of 101 Laboratory of the Biology Department, National University of Colombia, Bogotá.

Determining the plant to the species level was performed using the keys of Kuijt (2012) and confirmed by the experts Job Kuijt and Daniel L. Nickrent from photographs of plant material in vivo and from collections, respectively. The collected samples were fixed in FAA solution (10:5:85, formalin: acetic acid: 70% EtOH) for 48 hours, and then treated following the conventional protocols of Johansen (1940) modified as follows: EtOH dehydration series, 70% for 24 hours, 90%, 96%, 100% and 100% for 4 hours each; 100% EtOH series and the clearing agent Histoclear (90:10, 70:30, 50:50, 30:70, 100, 100); embedding in paraffin (Paraplast Plus 60° C) three times for 24 hours each; serial or individual sectioning with a rotary microtome (Spencer 820, American Optical Company, NY) and fastening to slide by application of Haupt reagent (Ruzin, 1999). The slides were placed in an oven at 60 ° C for 24 hours, then deparaffinized in two steps with xylene:

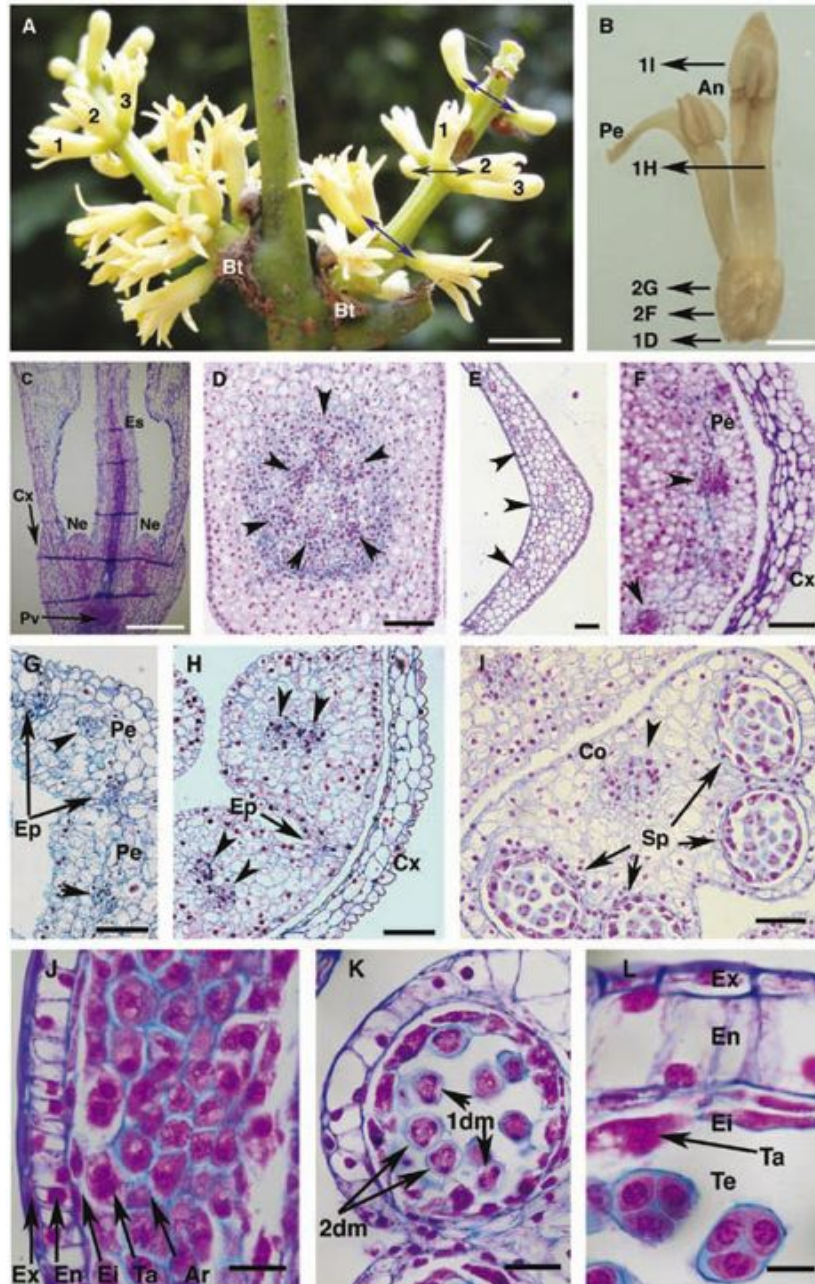


Fig. 1. *A.* *Peristethium leptostachyum* inflorescence with flowers developing acropetally; *B.* Single flower in longitudinal view (arrows indicate the approximate position of the corresponding cross sections to 1D, 1H, 1l, 2F and 2G); *C.* Longitudinal section of the flower; *D.* Flower peduncle; *E.* floral bract; *F.* calyx and petals; *G.* petals; *H.* Calyx and sepals at the base of the filaments; *I.* Young anther with archaesprium; *J.* Detail of young anther pollen sac; *K.* anthers with microspores in the first and second meiotic divisions; *L.* Anther with tetrads. **1,2,3** = triad of flowers, **1dm** = first meiotic division, **2dm** = second meiotic division, **An** = anther, **Ar** = archaesprium, **Br** = bract, **Cx** = calyx, **Co** = connective, **Ei** = intermediate strata, **En** = endothecium, **Ep** = epidermal cells connected by interdigitation; **Es** = style, **Ex** = exothecium, **Ne** = nectary, **Pe** = petal, **Pv** = pelvis, **Sp** = pollen sacs, **Ta** = tapetum, **Te** = tetrads. **Arrow heads** = vascular bundles. *C-L:* Optical microscopy, fast green-safranin. *C,J:* Longitudinal sections; *D,I,K,L.* Cross sections. Scales: 5 mm in *A*; 1 mm in *B*; 500 mm in *C*; 200 mm in *D-I*; 100 mm in *J,K*; 50 mm in *L*.

100% EtOH 50:50 for 3 minutes (x2), followed by hydration in EtOH series 100%, 95%, 70% , 50% distilled H₂O for 2 minutes, and Astra-blue staining acidified (1g / 100 mL of 2% tartaric acid) for 10 minutes, followed by washing in distilled H₂O for 2 minutes, and counterstained with ethanolic basic fuchsin (0.1 g / 100 mL of 50% ethanol) for 20 minutes, washed in distilled water for 15 seconds. After dehydration staining was performed in series of EtOH (50%, 70%, 95%, 100%, 100%, 1 minute c / u), and ethanol: xylene (70:30, 50:50, 2 min c / u), 100% xylene 2 times for 3 minutes. Finally cytoresin was placed on the slides, the coverslips were mounted and allowed to dry at room temperature.

Micropreparations are analyzed and photographed using a Nikon Eclipse E100 microscope and an Olympus BX50 with AmScope MU300 and Motic cameras Moticam Pro 282B respectively. The digital images were processed and edited in the PhotoScape program. The organs collected and fixed were kept in 70% ethanol. Observations and measurements of inflorescences and flowers were performed with a Nikon SMZ745T stereoscope, taking photographs with a Opticam PRO5 camera. The digital images were edited with the PhotoScape program.

RESULTS

Inflorescence: *P. leptostachyus* shows a racemose inflorescence with a solitary terminal ebracteate flower (determinate inflorescence), surrounded by pairs of bracteate triads that are distributed in a decussate manner along the axis (Fig. 1A); the bracts that cover the triads in the floral bud state fall during anthesis. Both short axes that support the triads, as well as the pedicels of the flowers are articulated. The

development of flowers in the raceme is acropetal, that is, first the triads at the base of the inflorescence open, continuing in order to the inflorescence apex; all the flowers in anthesis are hermaphrodites, hexamerous, with slender body and a size of ca. 6.7 x 1.5 mm (length x width; Fig 1B and 1C). The fruits are berries ca. 7 x 5 mm, ellipsoidal in shape and a dark reddish color blue.

The pedicels are short and are covered by a uniseriate epidermis consisting of quadrangular cells with thin cuticles; cortical and medullary parenchyma is without idioblasts and make six (terminal flower) or seven (lateral flower) vascular bundles arranged concentrically (Fig. 1D). In the lateral flowers, one of them is innervated by the floral bracts. Anatomically, the abaxial and adaxial uniseriate epidermis of the floral bracts, is formed by elongated cells without stomata; an undifferentiated mesophyll composed of three rounded parenchyma cell layers and a branching type of collateral vascular bundle (Fig. 1E).

Perianth - calyx and corolla: Since the ovary is inferior, the pale green calyx is located on the ovary in the shape of a ring (calyculus; Fig 1F.). In longitudinal section, the calyx has a maximum width of 0.1 mm and 0.27 mm in length (Fig. 1C). The epidermis is uniseriate, the adaxial lined with small and elongated cells, while the abaxial with larger round cells (Figure 1C, Figure 1F); the mesophyll parenchyma is undifferentiated with a maximum 7-8 cells at the base without vascularization.

The corolla has six petals (occasionally seven) adnate to 1/3 of its length (4.2 x 0.8 mm), yellowish white in color (Fig 1A, 1B, 1C); the base connecting the gamopetalous ring with the floral receptacle is thin (articulated; Fig. 1C), followed by a widened zone that includes

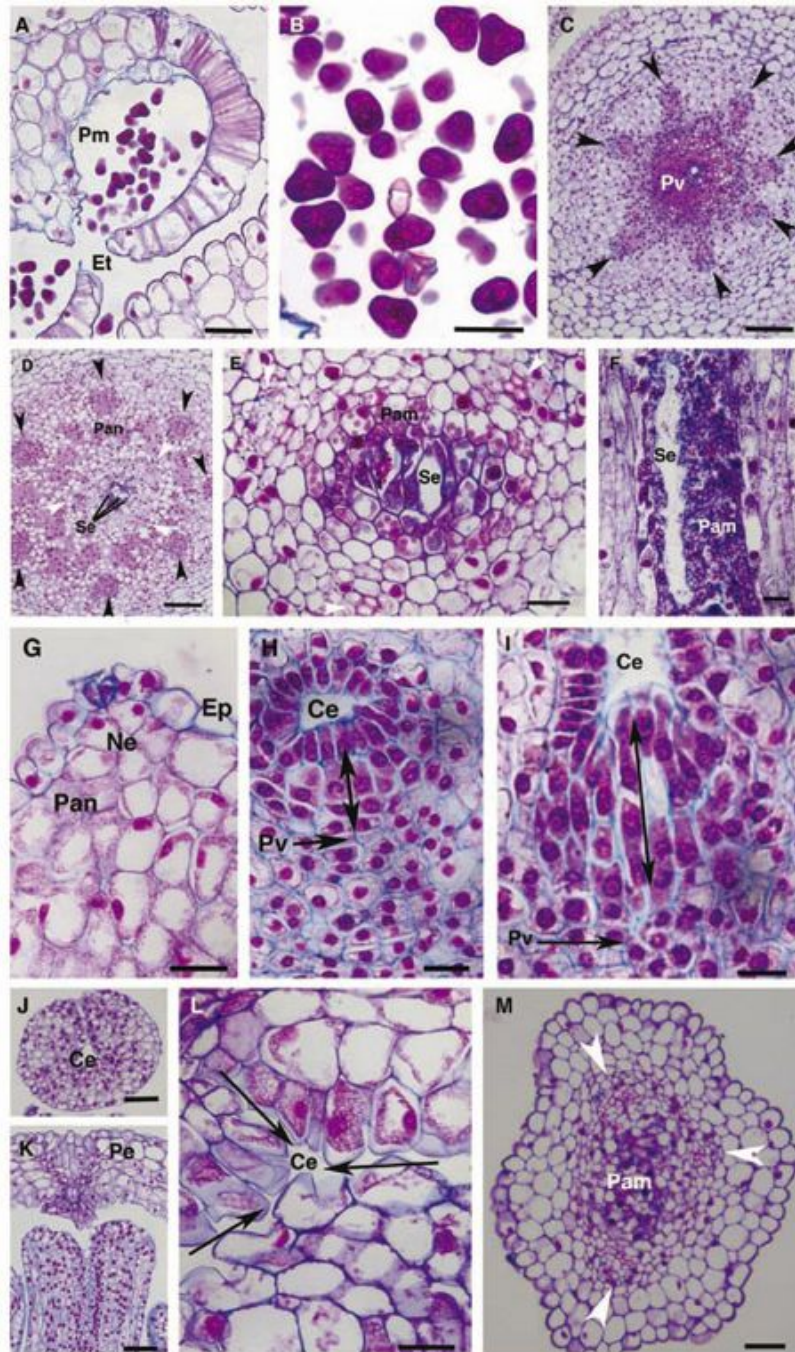


Fig. 2. **A.** Mature anther in dehiscence; **B.** mature pollen; **C.** ovary cross section at the level of the hypostase; **D.** ovary cross section at the level of embryo sacs; **E-F.** detail of embryo sac immersed in starchy parenchyma; **G.** nectary; **H.** mamelon with dyads (double arrow); **I.** mamelon with tetrads (double arrow); **J-K.** stigma of flower in bud; **L.** Detail of suture in the stigma of flower in bud; **M.** stigma of flower at anthesis. **Ce** = style canal, **Ep** = epidermis, **Et** = stomium, **Pm** = mature pollen, **Pv** = pelvis, **Pam** = starchy parenchyma of the mamelon, **Pan** = starchy parenchyma of the nectary, **Pe** = petals, **Se** = embryo sac, **white arrow heads** = vascular bundles of the mamelon, **black arrow heads**: vascular strands of the hypanthium. **Colouring:** safranin fast green. **F-I, K:** Longitudinal sections; **A-E, J, L, M:** Cross sections. Scales: 200 mm in **C,D,J-L**; 100 mm in **A,E-H,M**; 50 mm en **B**.

the petals and stamens adnate to the basal third (Fig. 1C); the terminal two thirds corresponds to the free surface of the petals.

The abaxial uniseriate epidermis formed by a covering of rounded cells with thin cuticle and adaxial epidermis of more square cells, but of a similar size; 7 to 8 mesophyll cells thick, homogeneous and composed of parenchymal cells, almost quadrangular and robust consistency and one central immersed vascular bundle, with central xylem and peripheral phloem. In the flower buds, the petals are joined by interlocking of the outer epidermal cells except at the distal end where their separation permits identification of the start of the floral opening (Fig. 1G, Fig. 1H and Fig. 2K).

Androecium and microsporogenesis: The androecium consists of six epipetalous stamens, three long filaments (1.3 mm) and with three short filaments (1 mm), intercalated with each other; in both cases, the base of the filament is wide, about 0.6 mm at the insertion site and 0.2 mm in the center. The sole vascular bundle of each petal branches and it is introduced into the filament of the anther providing the corresponding stamen vascularization (Fig. 1H). The filament of the anther presents a uniseriate epidermis, quadrangular cells, cortical parenchymal, composed of rounded cells and a central vascular bundle. The connective is anatomically similar to the filament (Fig. 1H, Fig. 1I).

The anthers are dorsifixed, bithecal, 1 mm long and 0.6 mm wide. In each theca two pollen sacs of similar size are located (Fig. 1I). The exotheca is uniseriate, with cells with very thin walls, without lignified thickenings, with a thickness of 10 μm and a length of no more than 50 μm . The endothecial consists of large cells, of quadrangular shape, and uniseriate appearance with lignified mesh shaped thickenings, that is, with the external, internal and lateral walls thickened, which is especially

visible in mature and dehiscent anthers (Fig. 2A). The intermediate strata are reduced to a single, long, thin cell layer. During development no starch reserves accumulate in any of three layers of the anther (Fig. 1I, Fig. 1J, Fig. 1K and Fig. 1L, Fig. 2A).

The tapetum is secretory and comprises quadrangular cells, robust and binucleate surrounding the sporogenous tissue since it is archesporial until the end of meiosis (Fig. 1I, Fig. 1J, Fig. 1K); once the tetrads are formed, the tapetum and intermediate layers are degraded (Fig. 1L and Fig. 2A). During the developmental process small but visible orbiculas (corpuscles of Ubisch) are formed. The anther dehiscence is the longitudinal type and occurs by dehydration of tissues which generate stress rupture the septum between the pollen sacs of each theca and along the stomium cells (Fig. 2A). The cells of the exothecium near the stomium elongate at the end of anther development to facilitate dispersal of pollen.

The mother cells of pollen grains that make up the archesporial cells are rounded, with prominent nuclei and dense cytoplasm, with smooth walls composed primarily of callose (Fig. 1I and Fig. 1J), the mother cells remain loosely linked during the process of microsporogenesis (first and second meiotic division; Fig. 1K); generating at the end of the process tetrahedral tetrads (rarely decussate) by simultaneous division forming smooth walls centripetally (Fig. 1L). The resulting microspores are ovoid. Only when the wall of the mother cell are released and starts forming exine and intine; acquiring the final morphology of the pollen grain, a size of ca. 20 microns, tricolpate, triangular in shape with three small arms recurs a median groove of isopolar symmetry, psilate surface, some slightly concave; binucleate (vegetative and generative nuclei) at the end of their development within the anther (Fig. 2A and Fig. 2B).

Floral vasculature, gynoecium and megasporogenesis: Before describing the structure of the gynoecium (inferior ovary), one should describe the floral vasculature. The pedicel has six vascular bundles, exceptionally seven (Fig. 1D). At the height of the hypostase (Fig. 2C), the structure on which the ovary develops, are six vascular bundles in an external position (Fig. 2D, Fig. 2E), going over the hypanthium and innervating the petals, bifurcating again to feed the anthers (Fig. 1H). While three vascular bundles disconnected from the receptacle (Fig. 2D, Fig. 2E) are located around the mamelon and continue along the pistil, surrounding the central transmission tissue, to the base of stigma.

The floral receptacle is covered by a uniseriate epidermis and parenchymatous tissue comprised of large, rounded parenchymal cells (8 to 12 layers, by cells with periclinal division), followed by smaller parenchyma cells and cells elongated periclinally (6-7 layers) that surround the six open colateral conductors, separated radially by parenchymatous cells from one to five cells thick and around a medular parenchyma 4-6 cells in diameter (Fig. 1D).

The receptacle forms a cup-shaped structure (hypanthium) surrounding the ovary, which is located on the hypostase (Fig. 1C and Fig. 2C); the hypanthium has an outer area with very vacuolated parenchyma cells including six vascular bundles, culminating in the petals, and an inner zone formed by parenchyma with denser cytoplasm and abundant amyloplasts culminating in the nectar ring around the style (Fig. 2D); in longitudinal section is seen apical stomata topping the nectar ring (Fig. 2G).

In the center of the flower and above the hypostase, maintaining histological continuity with the hypanthium, one sees the ovary, fed by three bundles that run through externally (Fig. 2D and Fig. 2E) and continue into the style. The ovary is a mamelon type of structure sensu Brown, Nickrent and Gasser (2010) or

“collective ovary” (sensu Venturelli, 1984a), this means that megagametophytes (embryo sacs) develop directly from a central gynoecial structure without forming ovules (Fig. 2D, Fig. 2E).

At the beginning of development, the mamelon is seen as an ovoid mass of meristematic cells, higher than the surrounding parenchyma and actively dividing (see pairs of nuclei, Fig. 2H), located below the styler canal. The mamelon initially presents an epidermis and a line of 4-6 subepidermal archesporial cells, which enlarge and divide meiotically resulting in dyads and then linear tetrads (Fig. 2I). The hypostase (sensu Johri & Raj, 1969) or pelvis (sensu Venturelli, 1984a) is formed by cells with thickened walls (Fig. 1C and Fig. 2C, Fig. 2H, Fig. 2I) and is located towards the base of archesporium, separated from it by two or three layers of cells.

In the flower at anthesis, the mamelon has been transformed into an elongated structure, bordered by three vascular bundles and formed by a starchy (amyliferous) parenchyma with strong blue staining surrounding the embryo sac (2-4) located from the hypostase to the style base (Fig. 1C, Fig. 2I). The starchy parenchyma continues in the style forming the transmission tissue (Fig. 1C, Fig. 2M).

Style and stigma: In the early stages of ontogeny of the style-stigma one observes a trimeric structure that is by fused epidermal interdigitation, forming initially a hollow style that has continuity with the mamelon (Fig. 2J, Fig. 2K, Fig. 2L). In the flower at anthesis, the style is thick (0.3 mm at the base and 0.4 mm at the center) and is formed by a uniseriate epidermis with quadrangular cells, an external parenchymal tissue composed of irregular cells, three vasculares bundles and one solid parenchymal central transmission tissue with abundant amyloplasts (Fig. 2F). The stigma is capitate, with a thickness of 0.45 mm, with a uniseriate epidermis of rounded but not glandular cells and without an apparent cuticle (Fig. 2K).

DISCUSSION

Among the morphological characteristics proposed by Kuijt (2012) for the circumscription of the species within the *Struthanthus* complex, one sees characters such as: determinate or indeterminate inflorescences, units of the inflorescences as monad or triad types, presence or absence of bracteoles in monads, and presence (in *Peristethium*) or absence (in other genera) of basal papery bracts on the inflorescence.

The proposed character states for *Peristethium* (Kuijt, 2012) are present in *P. leptostachyum* where the inflorescence is determinate, comprising an ebracteolate terminal flower (monad) and followed by bracteolate triads; however, it is clear that the floral development is acropetal unlike the basipetal condition indicated by Kuijt (2012) for the species. The inflorescence structure is more akin to that of *Cladocolea*, being determinate and composed of ebracteolate monads (Cid, 2006) and differs from *Struthanthus* spp. that are indeterminate and made of triads with all bracteolate flowers (Venturelli, 1981, 1984a, 1984b); however, shares with *Struthanthus vulgaris* (Venturelli, 1984a) the presence of pairs of triads along the floral axis with caducous bracts and actinomorphic flowers.

Among the interpretations of floral structure of *P. leptostachyum* by Kuijt (2012) and the present study there are important differences. Kuijt (2012) described the species as dioecious; the male flowers as short and stout, with short trichomes at the base of the filaments that have six sessile anthers, three of them large, sterile and three short, viable; and in female flowers long and slender with sterile anthers. In this work all the observed flowers are hermaphrodite, the same size and appearance, and with functional androecium and gynoecium. The anthers are not sessile but adnate to the petals.

In this work all the flowers observed were the same size and appearance, hermaphroditic, with development of viable pollen in all (short and long) anthers according to the shape and characteristics described by Feuer & Kuijt (1985) and Kuijt (2012); as well as development of a typical mamelon and later, viable embryo sacs, and functional style, highly developed and with three vascular bundles. The anthers were not observed to be sessile but adnate to the petals and with trichomes on the inferior part.

Although Kuijt (2012) notes that genus *Peristethium* has dioecious and monoecious species with hermaphroditic flowers, in some cases he was uncertain owing to insufficient material in the collections; in describing *Peristethium leptostachyum*, based on more than 30 specimens, he pointed to the species as dioecious with unisexual flowers. Van Tieghem (1895) and Engler (1897) reported this species as bisexual. Since bisexual and dioecious conditions could exist in different populations of *P. leptostachyum*, one cannot rule out the possibility that there is interspecific variation in the reproductive system, and if this is the case, the phenomenon requires further study. This highlights the importance of using multiple lines of morphological and anatomical evidence to confirm sexuality in this and other species.

The calyculus (reduced calyx) of *Peristethium leptostachyum* is annular and has no vascularization, a condition typical of the family Loranthaceae except basal genera where vascular tissues are presented (Kuijt, 2013); anatomically it resembles *Psittacanthus* in the absence of trichomes, druses and sclereids in the calyx (Gomez et al., 2011).

Kuijt (2013; see Table 1) when comparing the genera of *Struthanthus* complex, consider the character number of petals, which in the *Peristethium* species usually varies between four and six. The presence of hexa- or heptamerous corolla found in this work in flowers of the same individual of *Peristethium*

leptostachyum raises doubts about the taxonomic utility of this character; variable and odd number of monomorphic petals runs counter to the idea of a biseriata corolla proposed by Wanntorp and De Craene (2009) and supports the classical concept of monoserial corolla defended by Kuijt (2013). The interdigitization of the epidermis of the petals in the flower bud is shared with *Struthanthus vulgaris* (Venturelli, 1984a).

The vascularization of the flower of *Cladocolea loniceroides*, *P. leptostachyum* and *Struthanthus vulgaris* (Venturelli, 1984a) is similar, differing only in the number of bundles that run through the style; presenting three vascular bundles in the first two species, instead of six in *S. vulgaris*, covering the ovary and the style of the flower. In all these species, the vascular bundles surrounding the mamelon itself and traveling through the style (Hm) are not attached to the main vascularization of the flower, at least until anthesis. Since the gynoecium contains three vascular bundles and in the early stages a stigma with three lobes that are fused, it is proposed as a hypothesis that the ovary of *P. leptostachyum* is derived by reduction of a tricarpeolate ovary.

Peristethium leptostachyum is more akin to *Struthanthus vulgaris* due to glandular tapetum and simultaneous microsporogenesis (Venturelli, 1984a), differing from *Cladocolea loniceroides* which has a periplasmial tapetum (amoeboidal) and successive microsporogenesis (Cid, 2006).

By the circumscription of species proposed by Nickrent et al. (2010), the gynoecium in the tribe Psittacanthae would have three different conditions:

- *Tupeia antarctica* (subtribe Tupeinae; Smart, 1952) has a very simple structure, without egg or mamelon and with the archesporial tissue beneath a solid starchy tissue that goes through the style and stigma, which resembles the extreme reduction types found in *Helixanthera* and *Moquiniella* (Bhatnagar & Johri, 1983),

with the difference that they have open stylar channels.

- *Tripodanthus acutifolius*, the earliest diverging genus (Vidal & Nickrent, 2008) of the subtribe Psittacanthinae, presents a trilobular ovary with an open compitum connecting the three cavities, with discrete ovules, free mamelon without integuments, but with a nucellus or megasporangium well differentiated and with an upper placental region, in such a manner as the archesporial tissue is on the pole opposite the style; the stylar channel is closed and with starchy tissue (Venturelli, 1983; cf. Fig 2 Cocucci, 1983.); all these characteristics caused Cocucci (1982) to mistakenly propose an affinity with *Nuytsia* and basal in the family.

- *Cladocolea* (Cid, 2006), *Phthirusa* (Kuijt & Weberling, 1972), *Struthanthus* (Venturelli, 1981, 1984a, 1984b) and *Peristethium* (this investigation) have a unilocular gynoecium, with mamelon whose archesporial tissue is oriented towards the style, that it is solid and with starchy tissue, resembling that seen in *Helicanthes*, *Amyema* and other Lorantheae tribe genera (Bhatnagar & Johri, 1983).

Classical proposals on the evolution of the gynoecium in Lorantheae (Cocucci 1982; Bhatnagar & Johri, 1983), and particularly the tribe Psittacanthae should be reviewed in the light of the phylogeny of Nickrent et al. (2010); however, it is proposed as a synapomorphy for the tribe the presence of a solid stylar channel when the flower is in anthesis; additionally considering the alternative condition, an open stylar channel, as a plesiomorphy in the family, as in *Peristethium* seen in early ontogeny of the flower (cf. Fig. 2J, Fig. 2K, Fig. 2L, Fig. 2M). In Table 1 some other characteristics of the stamens, ovary and embryogenesis in species of tribe Psittacanthinae are compared.

In conclusion, since *P. leptostachyum* shares reproductive traits of both *Cladocolea* as well as with *Struthanthus*, the relationship among these genera is not resolved on the basis of anatomical evidence. Phylogenetic studies

including representative species of the three genera to assess the assumptions of monophyly are required. The sexual system observed in this study differs from that reported by Kuijt, so studies are required to identify the geographical and ecological factors that influence this variation.

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SUMMARY

Peristethium leptostachyum es una especie hemiparásita de la familia Loranthaceae, distribuida en Colombia, Costa Rica, Ecuador, Perú, Venezuela y Panamá. Previamente tratada como *Struthanthus leptostachyus*, la especie fue recientemente reubicada en *Peristethium* junto con otras que previamente estaban en los géneros *Cladocolea* y *Struthanthus*. La decisión de reconocer a *Peristethium* como género es controversial y fue tomada con base en caracteres de la inflorescencia y de la flor; en tanto que la monofilia de los tres géneros nombrados es incierta. En esta investigación se estudió la morfoanatomía de flores e inflorescencias de *Peristethium leptostachyum*, detallando la estructura del androceo y gineceo, así como los procesos de microgametogénesis y megagametogénesis; adicionalmente se realizaron comparaciones con especies afines y precisiones en relación con las diagnósticas previas. Se recolectaron flores en diversas fases de desarrollo en Santa María (Boyacá-Colombia), se prepararon y analizaron bajo microscopio secciones histológicas teñidas con astrablue-fucsina, además de disecciones bajo estereomicroscopio. Los resultados mostraron

que *P. leptostachyum* comparte caracteres inflorescenciales con *Cladocolea* (inflorescencia determinada, flor terminal ebracteada), pero también con *Struthanthus* (pares de tríadas a lo largo del eje, brácteas caducas y flores actinomorfas). Las flores de *P. Leptostachyum* de Santa María son claramente hermafroditas, con androceos y gineceos totalmente desarrollados; lo cual contradice la descripción hecha por Kuijt que reporta una condición dioica para esta especie. El androceo resultó afín al de *Struthanthus vulgaris*, con *tapetum* glandular y microsporogénesis simultánea; en contraste, *Cladocolea loniceroides* presenta *tapetum* periplasmoidal y microesporogénesis sucesiva. El gineceo de *P. leptostachyum*, al igual que en *Cladocolea*, *Struthanthus* y *Phthirusa*, es unilocular con mamelón y tejido arquesporial orientado hacia el estilo, el cual es sólido y con tejido amilífero. *P. leptostachyum* es afín a *Cladocolea loniceroides* y difiere de *Struthanthus vulgaris* por presentar varios sacos embrionarios y pelvis (hipostasa) no lignificada. La presencia de un canal estilar sólido se propone como sinapomorfia de la tribu Psittacanthinae. Dado que *P. Leptostachyum* comparte caracteres anatómicos florales tanto con *Cladocolea* como con *Struthanthus*, la relación entre estos tres géneros no queda resuelta, se requieren estudios filogenéticos para establecer esta relación y poner a prueba las hipótesis de monofilia de cada uno de ellos.

Keywords: Santalales, dioecious, hermaphroditic, hypostase, mamelon, microesporogenesis, Psittacanthinae, glandular tapetum, periplasmoidal tapetum.

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Table 1
Features of the androecium, gynoecium and embryogenesis in species of the tribe Psittacanthinae (Loranthaceae)

	Cells with Swellings	Microsporogenesis	Tapetum	# Styler Vascular Traces	Hypostase	# Embryo sacs	References
<i>Cladocolea loniceroides</i>	Endothecium Endothecium + epidermis	Successive	Periplasmodial	3	Colenchymatous	Various	Cid (2006) Kuijt & Weberling (1972)
<i>Phthirusa pyrifolia</i>	Endothecium + epidermis	Simultaneous	Glandular	4	Colenchymatous	Single (?)	
<i>Struthanthus vulgaris</i>	Endothecium	Simultaneous	Glandular	6	Lignified	Single Various, one single mature	Venturelli (1981, 1984a)
<i>Struthanthus flexicaulis</i>	Endothecium	?	Glandular	?	Lignified		Venturelli (1984b)
<i>Tripodanthus acutifolius</i>	Endothecium	Simultaneous	Glandular	?	Lignified	Various	Venturelli (1983)
<i>Tupeia antarctica</i>	Endothecium	Simultaneous	Glandular	?	Colenchymatous	Various	Smart (1952)
<i>Peristhetium leptostachyum</i>	Endothecium	Simultaneous	Glandular	3	Colenchymatous	Various	This investigation