

Floral anatomy of *Tristerix longibracteatus* (Loranthaceae)

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ABSTRACT. Introduction: Most of the New World members of the Loranthaceae comprise a clade that corresponds to the tribe Psittacanthae. Previous studies on floral anatomy and development in this tribe have concentrated on the highly diversified subtribe Psittacanthinae, while the smaller subtribe Ligarinae has received less attention. A detailed anatomical description of *Tristerix longibracteatus* helps to fill this information gap. **Objective:** The present research analyzes the anatomy of *Tristerix longibracteatus* flowers, detailing the structure of androecium and gynoecium, including megasporogenesis and microsporogenesis. **Methodology:** Anatomical serial sections of flowers at different stages of development were prepared, following processing with fixation techniques, incorporation in paraffin, microtome sectioning and staining with Astra-blue and basic fuchsin. **Results:** The large-sized flowers of *Tristerix longibracteatus* present a complex pattern of vascularization with 18-20 vascular bundles at the base of the inferior ovary. A group of three vascular bundles irrigate the 4-5 petals and associated stamens, and ten bundles continue through the gynoecium. The androecium is composed of four or five anthers with simultaneous microsporogenesis. The gynoecium as a single ovarian cavity with a central mamelon in which the archesporial tissue is oriented towards the style. The base of the style forms a nectary similar to that found in the sister genus *Ligaria*. **Conclusions:** The gynoecium with a single ovarian cavity and central mamelon is a condition shared by *Tristerix* (subtribe Ligarinae) and all the genera of the subtribe *Psittacanthinae*, except *Tripodanthus*. The base of the style forms a nectary similar to that found in the sister genus *Ligaria*. This type of stylar nectary is of taxonomic value for grouping species of the subtribe Ligarinae and differs from the annular nectary of subtribe Psittacanthinae.

Key words: androecium, gynoecium, microsporogenesis, megasporogenesis, stylar nectary.

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The family Loranthaceae comprises 73 genera and around 900 species of hemiparasitic plants distributed mainly in the tropics (Nickrent, Malécot, Vidal-Russell, & Der, 2010; Kuijt, 2015). In the aforementioned most of

the New World genera comprise a clade corresponding to the tribe Psittacanthae, which also includes *Tupeia antarctica* (New Zealand), but in Nickrent, Anderson, and Kuijt (2019) this species is excluded from the tribe.

Gaiadendron, a genus of widespread distribution in the New World, is outside of this clade, being basal in the family.

The Psittacanthae tribe is made up of four subtribes: Psittacanthinae, Ligarinae, Notantherinae (South of Chile), and Tupeinae (New Zealand), that form a polytomy. Almost all Psittacanthinae species are of tropical distribution, spanning a wide altitudinal range. Ligarinae includes two genera, *Ligaria* with species distributed in the temperate and sub-temperate zone of South America and *Tristerix*, which includes a Southern clade of species from the temperate zone of Argentina and Chile and a northern clade with tropical high Andean species (Amico, Vidal-Russell, & Nickrent, 2007).

The Kuijt monograph of *Tristerix* (1988) describes the flowers as bisexual and penta- or tetramerous. The isomorphic stamens are yellow-red, even black or dark violet; their anthers are medifixed, versatile and long (Kuijt, 2015). Kuijt (1988) subdivided the genus *Tristerix* into two subgenera: *Tristerix*, with only two species, each with bracteoles and tetramerous flowers, and *Metastachys*, which includes the remaining species and is characterized by pentamerous flowers that lack bracteoles. In contrast, Amico et al. (2007) generated a molecular phylogeny in which they differentiated two main clades within *Tristerix*, the first consisting of six species, distributed in northern South America (Colombia, Peru and Ecuador), and the other clade composed of species that inhabit the southernmost areas of the continent (Bolivia, Chile and Argentina). Their study indicates that speciation in this genus is related to the emergence of the Andes and the origin of cloud forests.

Tristerix longibracteatus is the highest altitude parasitic plant in the world, reaching 4 618 m above sea level in the cordillera of Vilcabamba in Peru, parasitizing trees of *Polylepis pepeii* and *Gynoxys cuzcoensis* (Sylvester, Sylvester, & Kessler, 2014). According to Graves (1982), this species is pollinated by hummingbirds and presents highly synchronous flowering in Northern Peru. According to Kuijt (1988), *Tristerix longibracteatus* is closely related to

T. peytonii and *T. pubescens*. This is consistent with the results obtained by Amico et al. (2007) in whose phylogeny the three species make up a clade. Studies in the genus *Tristerix* have concentrated mainly on aspects such as morphology, phylogeny, biogeography and pollination, as in the recent work of González and Pabón (2017) where some floral and inflorescence characters were studied in relation to their ornithophilous pollination.

Within the tribe Psittacanthae, the structure of the gynoecium presents variation, including: (1) trilocular ovaries with ategmic ovules in the genus *Tripodanthus* (Cocucci, 1983; Venturelli, 1983), (2) unilocular ovaries with a central conical structure called a mamelon or collective ovules in the genera *Peristethium* (Robles, Raz, & Marquinez, 2015), *Pasovia* (= *Phthirusa*; Kuijt, & Weberling, 1972) and *Strutanthus* (Venturelli, 1984a, 1984b) and (3) gynoecium formed by five congenitally fused carpels with a central solid ovary around a simple mamelon in *Tristerix* (González & Pabón, 2017).

The tribe Psittacanthae also presents variation in the structure of the floral nectaries: (1) annular nectaries between the petals and style in species of the Psittacanthinae subtribe (for example in *Peristethium*, Robles et al., 2015), (2) stylar nectaries in *Ligaria* (Galetto, Bernardello, & Juliani, 1990; Kuijt, 1990) and (3) supraovary nectary disks in *Tristerix* spp. (González & Pabón, 2017).

The objectives of this research are to describe the floral anatomy of *Tristerix longibracteatus*, compare the results with studies of other genera in the Psittacanthae, including vascularization pattern, nectary, androecium and gynoecium structure, megasporogenesis and microsporogenesis.

MATERIALS AND METHODS

Floral buds and mature individual flowers of *Tristerix longibracteatus* were collected near laguna La Cubierta, department of Risaralda, Colombia at 3 400 m in August 2015, voucher specimens were deposited in

HUQ (F. Castaño 560 Fig. 1A). The determination of the material was carried out using taxonomic keys, descriptions and comparison with herbarium specimens.

Flower buds and open flowers from 1.7 to 7.0 cm in length, were fixed in FAA (formaldehyde: acetic acid: 70 % ethanol, 10:5:85), stored in 70 % ethanol, and subsequently treated following the protocol of Robles et al. (2015). This involved standard methods of dehydration using a clearing agent (Histoclear), paraffin infiltration, sectioning with a rotary microtome (820 Spencer, American Optical Company, NY) and attachment to microscope slides. The slides were stained with astra-blue and basic fuchsin and deposited in the Department of Biology collection at the Universidad Nacional de Colombia, Bogotá. They were analyzed and photographed using an Olympus BX-50 microscope with a Moticam Pro 282B camera. Fixed flowers were studied with a Leica M205A stereoscope using multi-focus mode and photographed with a Leica MC 170 HC camera. Digital images were processed and edited with the Adobe programs Photoshop CC and InDesign.

RESULTS

Morphology of the inflorescence and the flower

Tristerix longibracteatus is a profusely branched hemiparasite with terminal simple racemose inflorescences whose flowers develop in an acropetal order (Fig. 1A, Fig. 1B). The perianth is biseriate, actinomorphic, although with the apex slightly curved in the floral bud; the maximum length of the flower is 7 cm, including a 6.1 cm corolla tube and a 0.9 cm inferior ovary (Fig. 1B, Fig. 1G).

Each flower is subtended by a single lanceolate foliaceous bract that is approximately 4 x 0.7 cm at anthesis (Fig. 1C), with reddish-purple coloration at the apex. The bracts are fused at the base with the floral pedicels. The pedicels reach about 6 mm in length, surmounted by the ovoid inferior ovary of ca.

3-5 mm, which is, in turn, surrounded by the hypanthium. The calyculus is reduced to a ring with toothed margins (Fig. 1C), which surrounds the base of the corolla tube and is very prominent in young fruit (Fig. 1F). The corolla is scarlet red at the apex and base, and orange in the central part (Fig. 1C, Fig. 1G). The number of petals varies between 4 and 5, even in the same inflorescence, although the pentamerous condition is the most frequent. Shortly before anthesis, the petals are fused for a third of their length by interdigitation of the epidermal cells, while the distal two thirds are slightly separated forming a fenestrated floral tube. The apical lobes of the corolla are strongly reflexed at anthesis displaying yellow and pink tonalities on the adaxial surface (Fig. 1D).

The flower is bisexual, with an inferior ovary (Fig. 1G). The androecium is composed of 4-5 epipetalous stamens with reddish, cylindrical, papillate filaments and with yellow, versatile, bithecal, and tetrasporangiate anthers. They are medifixed with longitudinal dehiscence (Fig. 1E). The dentate calyculus is very prominent in the fruit (Fig. 1F).

Anatomy of the bract and pedicel: Each pedicel measures approximately 6 mm in length (Fig. 1C, Fig. 1G), and is covered by a uniserial epidermis of quadrangular cells and thin cuticle, the cortex and medulla are parenchymatic with 6-9 vascular bundles arranged concentrically (Fig. 2A). At the base of the bract, a large vascular bundle that is divided in the laminar portion and form a large central vascular bundle and one to two small lateral bundles on each side. The epidermis is unistratified, formed by quadrangular coating cells with a thin cuticle and the parenchymal mesophyll consists of 6-8 homogenous layers of rounded cells (Fig. 2A).

Anatomy of the ovary, floral vascularization: The mature ovary of *T. longibracteatus* is about 4-5 x and 2-3 mm and, being inferior, consists of a projection of the receptacle (hypanthium), which supports the outer whorls of the flower and surrounds the ovary

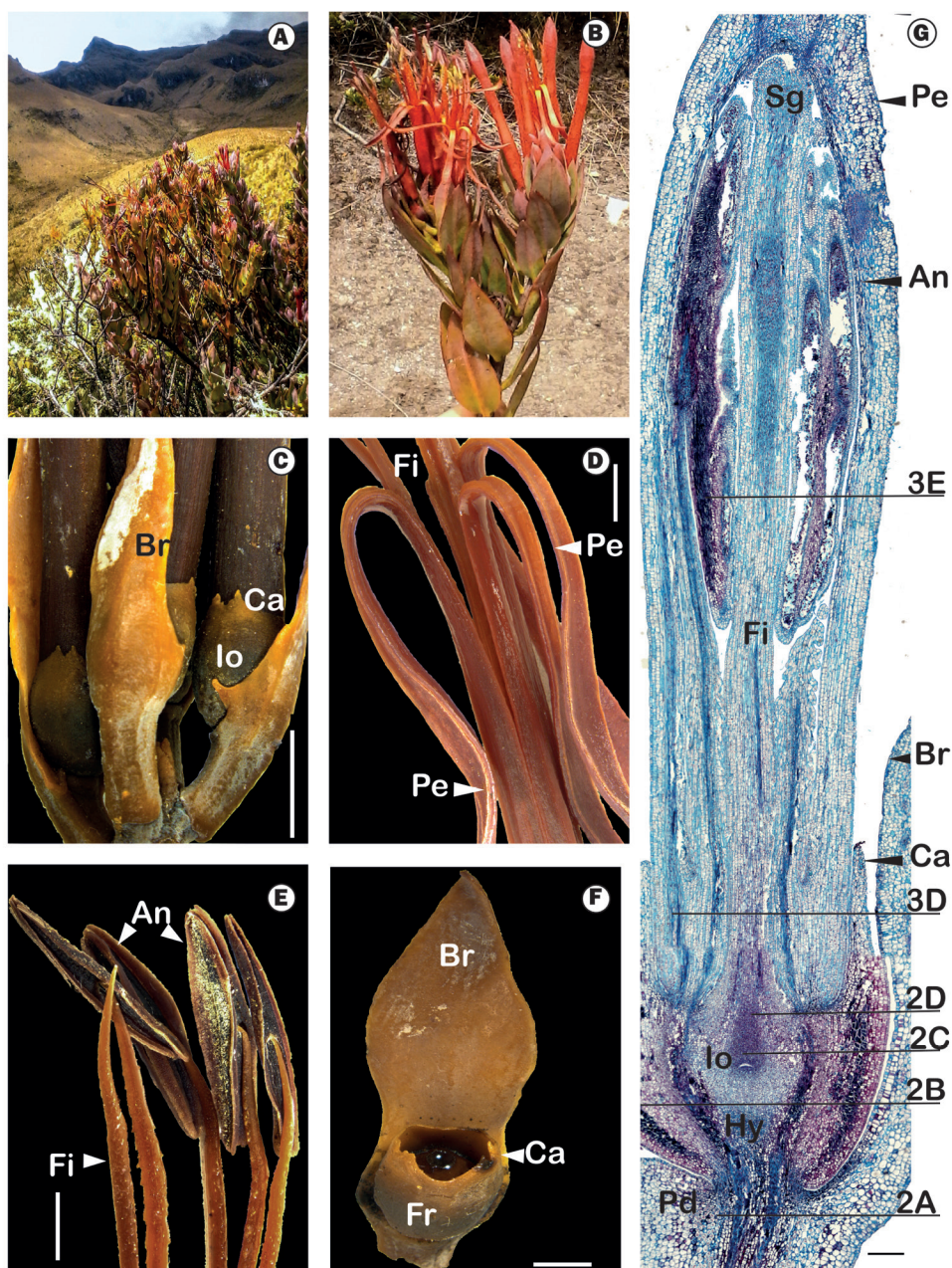


Fig. 1. Adult growth habit, macroscopic view of the inflorescence, morphology and anatomy of the flower in *Tristerix longibracteatus*. **A.** Adult specimen in its natural habitat, the *paramo* ecosystem (Colombia); **B.** Inflorescence at anthesis; showing acropetal development; **C.** Flower at late bud stage showing the inferior ovaries and subtending foliaceous bracts (lateral view); **D.** Flower at anthesis showing the reflected petals and the bases of the filaments (lateral view); **E.** Detail of the stamens revealing anther dehiscence (lateral view); **F.** Fruit at an intermediate developmental stage showing the persistent bract and the toothed calyx (upper view); **G.** Median longitudinal section of the flower shortly before anthesis (the lines correspond to the approximate level of figures 2A-D, 3D-E). Scale bars: 5 cm in **B**, 1 cm in **C**, 5 mm in **D**, **E**, **F**, 2 mm in **G**. Abbreviations: **An**: Anther, **Br**: Bract, **Ca**: Calyx, **Fi**: Filament, **Fr**: Fruit, **Hy**: Hypostase, **Io**: Inferior ovary, **Pd**: Pedicel, **Pe**: Petal, **Sg**: Stigma.

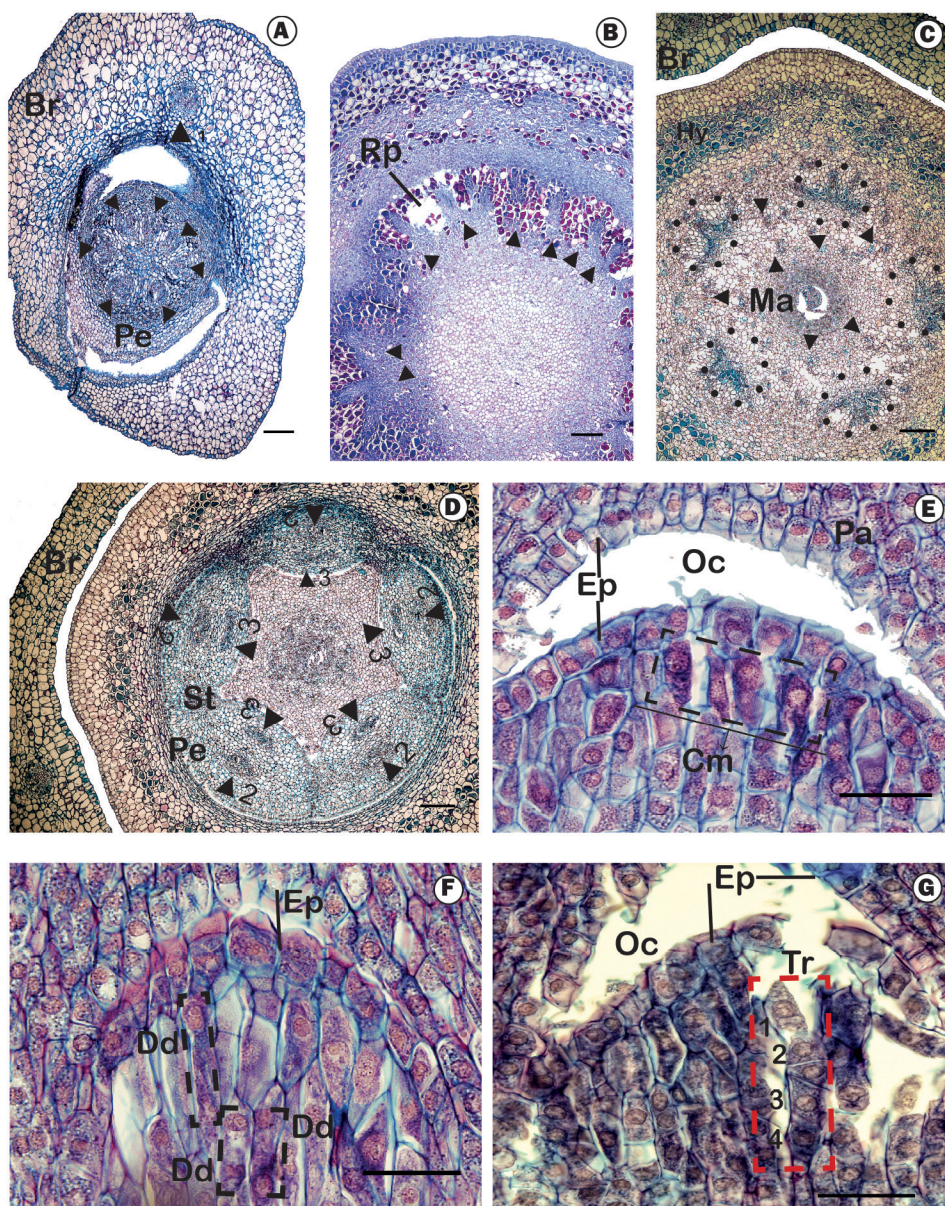


Fig. 2. Serial transverse sections of the flower and longitudinal sections of the mamelon in *Tristerix longebracteatus*. **A.** Transverse section of the basal region of the flower around mid-height of the pedicel (arrowheads = vascular bundles); **B.** Transverse section of the flower corresponding to the basal region of the ovary (arrowheads = vascular bundles of the gynoecium); **C.** Transverse section of the flower at the level of the mamelon (dotted circle = vascular bundles of the hypanthium, arrowheads = vascular bundles of the gynoecium); **D.** Transverse section of the flower towards the basal region of the petals and the style (arrowheads 2 = external vascular bundles of the petals, arrowheads 3 = internal vascular bundles of the petals); **E.** Longitudinal section of the flower showing the mamelon with arquesporial cells (dotted circle); **F.** Longitudinal section of the mamelon showing the dyads (dotted circles); **G.** Longitudinal section of the flower showing the mamelon with tetrads (numbers corresponding to cells 1, 2, 3, 4. **Scale bars:** 1 mm in A, B, C, D. 50 μ m in E, F, G, H. **Abbreviations:** Br: Bract, Cm: Megagametophyte cells, Dd: Dyad, Ep: Epidermis, Es: Embryo sac, Hy: Hypanthium, Ma: Mamelon, Nu: Embryo sac nucleus, Oc: Ovarian cavity, Pa: Amyloiferous parenchyma, Pe: Petal, Rp: Parenchymal rays, St: Stamen, Tr: Tetrad.

itself (Fig. 2A). The base of the ovary, below the pelvis or hypostase, is a uniserial epidermis of quadrangular cells with a thin cuticle, followed by one to two layers of collenchyma and then 13 to 16 layers of tanniniferous parenchyma consisting of large, ovoid cells. Interior to this are 4-6 layers of parenchyma formed by smaller cells that surround about 18-20 concentric vascular bundles separated from each other by tanniniferous parenchymal rays. The medullary parenchyma is composed of about 26 layers of small cells (Fig. 2B).

At the level of the pelvis, towards the sclerenchyma structure on which the ovary develops, the vascular bundles divide again and reorganize. Five groups of three vascular bundles surrounded by tanniniferous parenchyma externally traverse the hypanthium and innervate the five petals, along with their adnate stamens. Another ten gynoeceium vascular bundles are located around the ovary, five of which continue into the style (Fig. 2C, Fig. 2D). In the area above the pelvis is the ovary itself, corresponding to a reduced structure called the mamelon or collective ovary, which means that megagametophytes (embryo sacs) develop directly from an oval, amyliiferous central structure without the formation of ovules (Fig. 1G). The mamelon is located below the canal of the style (Fig. 1G).

The mamelon is initially composed of an epidermis and a layer of 4-6 archesporial subepidermal cells (Fig. 2E), which lengthen and divide meiotically giving rise to dyads (Fig. 2F) and then linear tetrads (Fig. 2G). Before anthesis, the embryo sacs invade the amyliiferous tissue that surrounds the mamelon and the transmitting tissue of the style (Fig. 3A). Unfortunately, no flowers were collected with mature embryo sacs.

The style is long and straight, being almost the same length as the petals (ca. 6 cm, Fig. 1G). At its widened base is a nectary formed by an epidermis with abundant sunken stomata, below which there is parenchymal tissue with dense cytoplasm and abundant intercellular spaces surrounding the vascular bundles (Fig. 3A, Fig. 3B). The distal portion of the style is

formed by a unistratified epidermis composed of rounded cells, 6-8 layers of parenchyma, among which there are five collateral vascular bundles and a central, solid, thick-walled collenchymal tissue with abundant amyloplasts (Fig. 3D). The stigma is capitate, reddish, approximately 1 mm thick; the epidermis consists of elongated cells with a thick reddish cuticle (Fig. 1G, Fig. 3C).

Perianth anatomy: The calyculus or calyx has a single layer of epidermal cells, which are small and elongated on the adaxial side and large and rounded on the abaxial side. The mesophyll consists of 11-12 undifferentiated layers of parenchyma, without any vascularization. On the abaxial side the cells of the parenchyma are rounded and on the adaxial face they are cubic and smaller.

The corolla is formed by four or five petals joined by interdigitation of the epidermal cells in the floral bud. Towards the basal half the petals are thicker, since they include the adnate filaments of the corresponding stamens (Fig. 3D). The distal half of the petal is laminar. Anatomically, the petals are formed by an adaxial epidermis formed by quadrangular cells and few poorly differentiated stomata and the adaxial epidermis is formed by round cells (Fig. 3E).

Toward the basal half, the mesophyll includes about 22 layers of tanniniferous parenchyma, two vascular bundles of the stamen and the vascular bundle of the petal (Fig. 3D, Fig. 3E). The distal portion of the petals has 9-11 layers of parenchyma cells of similar size and shape. The vascular bundle of the epipetalous portion divides into three toward the distal laminar portion. Some sclereids are also isolated or in small groups in the second or third layer below the adaxial epidermis (Fig. 3D).

Androeceum: This whorl consists of 4-5 epipetalous, isomorphic stamens, with irregularly shaped filaments vascularized by two bundles. The filaments are formed by a uniseriate epidermis of more or less quadrangular, thick-walled cells, followed by four to seven

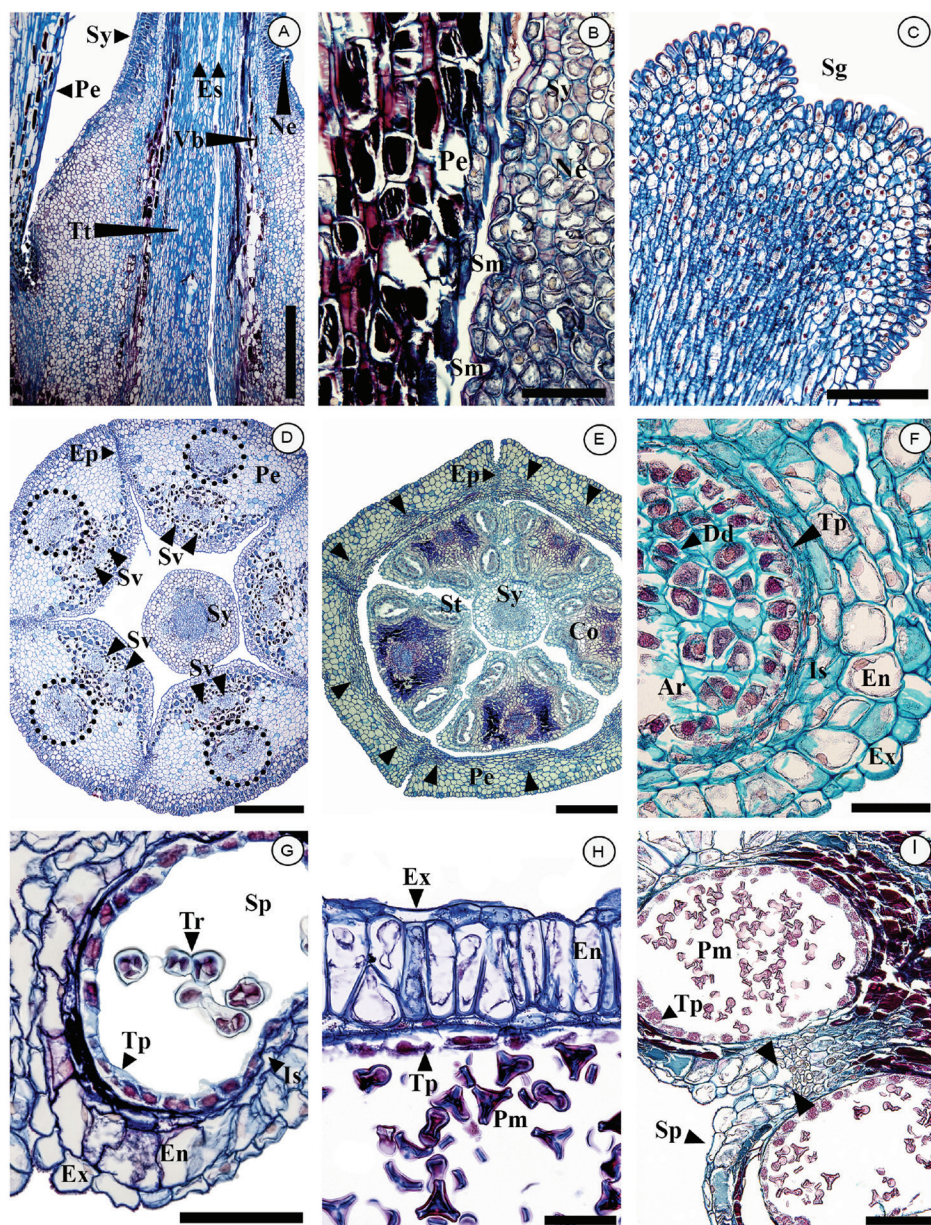


Fig. 3. Anatomy of the flower in *Tristerix longebracteatus*. **A.** Longitudinal section of the flower towards the center of the gynoecium revealing the stylar nectary; **B.** Detail of the nectary stomata (longitudinal section); **C.** Detail of the capitate stigma (transverse section); **D.** Transverse section of the flower around corolla mid-height (dotted circles = central vascular bundles of the petal); **E.** Transverse section of the flower at the level of the anthers (arrows indicate the vascular bundles of the petals); **F.** Detail of a pollen sac showing archesporial tissue (transverse section); **G.** Detail of a pollen sac showing tetrads (transverse section); **H.** Detail of a pollen sac with mature pollen (transverse section); **I.** Transverse section of the anther revealing longitudinal dehiscence of the anther (arrows indicate rupture point of the septum). Scale bars: 1 mm in **A**, **D**, **E**, 200 µm in **B**, **C**, 50 µm in **F**, **H**, 100 µm in **G**, **I**. Abbreviations: **Ar**: Archesporium, **Co**: Connective, **Dd**: Dyad, **En**: Endotecium, **Ep**: epidermal cells joined by interdigitation, **Es**: Embryo sac, **Ex**: exothecium, **Is**: Intermediate strata, **Ne**: Nectary, **Pe**: Petal, **Pm**: Mature pollen, **Sg**: Stigma, **Sm**: Stoma, **Sp**: Pollen Sac, **St**: Stamen, **Sv**: Stamen vascular bundles, **Sy**: Style, **Tp**: Tapetum, **Tr**: Tetrad, **Tt**: Transmitting tissue, **Vb**: Vascular bundle.

layers of parenchymal tissue with tannins in the innermost layers that surround the two collateral vascular bundles. The filaments are not completely cylindrical, presenting lobes orientated toward the base (Fig. 1E, Fig. 1G).

The anthers are about 5-8 x 1.2 mm, being medifixed, bithecal and tetrasporangiate (Fig. 1E, Fig. 3E, Fig. 3I). The exothecium consists of a single layer of quadrangular cells, elongated and flat, followed by a fibrous endothecium with O-shaped thickenings and 2-3 intermediate layers of elongated cells. No amyloplasts were observed in any of the layers of the wall at any stage of anther development; however, the connective presents tanniferous parenchyma (Fig. 3E).

The secretory-type tapetum is unistratified, consisting of uni or binuclear rectangular cells with dense cytoplasm, that remain in the original position and maintain their identity during microsporogenesis until their eventual degeneration *in situ* at the end of pollen development (Fig. 3F, Fig. 3I). The cells of the archesporium are polygonal and thin-walled, with prominent nuclei and dense cytoplasm (Fig. 3F). Simultaneous microsporogenesis occurs, generating dyads (Fig. 3F) and then tetrahedral tetrads (Fig. 3G). Subsequently, the four trilobate pollen grains are formed inside the callose wall of the tetrad. (Fig. 3H). The dehiscence of the anthers is longitudinal and occurs by rupture between the central septum and the walls of the pollen sacs of each theca.

DISCUSSION

Taxonomic identification and morphological variability: The material studied corresponds to the species *Tristerix longebracteatus*, according to the criteria established by Kuijt (1988), Dueñas-Gómez and Franco-Roselli (2001), Amico et al. (2007). The axes of the inflorescences and pedicels are terete with foliaceous bracts, mainly pentamerous flowers, petals up to 6 cm long and fruits ripening black with a prominent calyculus and conical apex. The maximum size of the petals of our material (6.1 cm) is close to the

maximum length indicated by Kuijt (1988) in his identification key.

Nevertheless, the presence in the same inflorescence of pentamerous and tetramerous flowers makes this character less useful for distinguishing among *Tristerix* species, and even less so for separating the subgenera *Tristerix* (tetramerous) and *Metastachys* (pentamerous) proposed by Kuijt (1988) and Dueñas-Gómez and Franco-Roselli (2001).

On the other hand, it should be said that the characteristic floral coloration in alternating bands of red-yellow-red, reported by Amico et al. (2007) for *T. longebracteatus* and closely related species, did not appear clearly in our material, in which the general reddish coloration acquires orange, but not yellow tones in the floral tube. The proposed coloration of the stamens, yellow in the filament and reddish in the anther (Kuijt, 1988), which are clearly observed in Contero and Dehesa (2007) for *T. longebracteatus* specimens from Cayambe (Ecuador), contrasts with the reddish coloration of the filaments and yellow of the anthers in our material. Also, the pendant position of the inflorescences in González and Pabón (2017) contrasts with the erect position observed in this study. Kuijt (1988) described variation in leaf size and floral length across this species' broad geographic distribution, but it should be noted that the characters reported above are also variable.

Vascularization of the flower: González and Pabón (2017) indicate that in the *Tristerix* species that they studied, "five vascular bundles enter the base of the pedicel [...] above which they radially split into an outer ring of five traces that irrigate the common petal-stamen bases and an inner ring that serves the gynoecium." In our specimens we observed a more complex pattern of vascularization, with 6-7 pedicel bundles that subdivide into 18-20 vascular bundles separated from each other by tanniferous parenchyma (Fig. 2B) that González and Pabón (2017) identify as a collenchyma cap. The traces that irrigate the petal-stamens bases in our case are not solitary,

but are formed by three minor vascular bundles (Fig. 2C); and there are ten gynoecial bundles of which five continue in style (Fig. 3D). This pattern of floral vascularization is much more complex than that observed in other species of the subtribe Psittacanthae, such as *Passovia* (= *Phthirusa* in Kuijt and Weberling, 1972), *Struthanthus* (Venturelli 1981, 1984a, 1984b), *Peristethium* (Robles et al, 2015), *Passovia*, *Aetanthus* and *Oryctanthus* (Suaza, Pabón, & González, 2016), and can be explained by the large size of the flowers and the adaptation to pollination by hummingbirds that requires increased production of nectar.

Nectary: The most diverse subtribe is the neotropical Psittacanthinae, characterized by flowers with annular nectaries between the petals and the base of the floral style in the genera *Peristethium* (Robles et al., 2015), *Passovia*, *Aetanthus*, *Oryctanthus* (Suaza et al., 2016), *Struthanthus* (Venturelli, 1981, 1984a), and *Cladocolea* (Kuijt, 1975); although Cid-Villamil (2006) reports nectaries at the base of the petals in *Cladocolea loniceroides*.

On the other hand, nectaries are also reported as being due to modification and thickening of the base of the style proper in *Nuytsia*, the basal genus of the family (Narayana, 1958) and genera of the tribe Elytrantheae such as *Lepeostegeres* (Schaeppi & Steindl, 1942; Raj, 1970). In the present study we found a nectary formed by thickening of the base of the style in *T. longebracteatus* (named supraovary nectary disk by González and Pabón (2017) in *Tristerix* spp.). In the genus *Ligaria*, the other genus that together with *Tristerix* makes up the subtribe Ligarinae, this kind of nectary is also present (Galletto et al., 1990; Kuijt, 1990).

Although the type of nectaries in the subtribes Psittacanthinae (annular nectary) and Ligarinae (stylar nectary) appear to be of taxonomic importance, unfortunately, the absence of information in the subtribe Nothantharinae does not allow a clear interpretation of the evolution of this character in the tribe.

Gynoecium: González and Pabón (2017) affirm “The ovary [of *Tristerix* spp.] is solid and served by a poorly differentiated vascular ring around a simple mamelon” and that “the gynoecium is formed by five congenitally fused carpels, which are evident by the five vascular bundles and the edges alternating the petals and stamens”. According to our observations, the ovary of *T. longebracteatus* has a more complex vascularization compared to the rest of the tribe Psittacanthae or even the rest of the family. We also observed a mamelon clearly separated from the surrounding parenchymatic tissues in the ovary chamber, both in cross section (Fig. 2C), and in longitudinal section (Fig. 2E, Fig. 2G), across all developmental stages, which implies a single ovarian cavity, not a solid ovary.

The number of vascular bundles or their position alone is insufficient to determine the number of carpels that make up the gynoecium of this or other Lorantheae. The evolution of the gynoecium in Lorantheae and the identity of the ovary-ovule complex and mamelon (*sensu* Johri, Ambegaokar, & Srivastava, 1992), or the “collective ovary” *sensu* Cocucci (1982) is currently unclear and new approaches in developmental biology, such as those used in the pioneering work of Brown, Nickrent, and Gasser (2010), will be required to shed light on the matter.

Ethical statement: authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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RESUMEN

Anatomía floral de *Tristerix longibracteatus* (Loranthaceae). **Introducción:** La mayoría de los miembros de la familia de Loranthaceae del nuevo mundo comprenden un clado que corresponde a la tribu Psittacanthae. Estudios previos de la anatomía floral y desarrollo en esta tribu se han concentrado en la alta diversidad de la subtribu Psittacanthinae, en tanto que la subtribu Ligarinae ha presentado menor atención. Una descripción detallada de la anatomía de *Tristerix longibracteatus* contribuye a llenar vacíos de información. **Objetivo:** la presente investigación analiza la anatomía floral de *Tristerix longibracteatus* detallando la estructura del androceo, gineceo, incluyendo los procesos de megasporogenesis y microsporogenesis. **Metodología:** Se prepararon secciones anatómicas seriadas de flores en diferentes etapas de desarrollo, con técnicas de fijación, incorporación en parafina, corte en micrótopo y doble tinción con azul de astra y fucsina. **Resultados:** Las flores de gran tamaño de *Tristerix longibracteatus* presentan un complejo patrón de vascularización con 18-20 haces vasculares en la base del ovario inferior. Un grupo de tres haces vasculares irrigan los 4-5 pétalos y estambres asociados, y 10 haces vasculares continúan a través del gineceo. El androceo está compuesto por cuatro o cinco anteras con microsporogénesis simultánea. El gineceo presenta una sola cavidad ovárica con un mamelón central en el que el tejido arqueosporial está orientado hacia el estilo. La base del estilo forma un nectario similar al que se encuentra en el género hermano *Ligaria*. **Conclusiones:** El gineceo con una sola cavidad ovárica y un mamelón central es una condición compartida por *Tristerix* (subtribu Ligarinae) y todos los géneros de la subtribu Psittacanthinae, excepto *Tripodanthus*. La base del estilo forma un nectario similar al que se encuentra en el género hermano *Ligaria*. Este tipo de nectario estilar tiene valor taxonómico agrupando las especies de la subtribu Ligarinae en contraste con el anillo nectarífero presente en la subtribu Psittacanthinae.

Palabras claves: androceo, gineceo, nectario estilar, microsporogenesis, megagametogenesis.

REFERENCES

Amico, G. C., Vidal-Russell, R. & Nickrent, D. L. (2007). Relationships and ecological speciation influence of *Tristerix* (Loranthaceae): the influence of pollinators, dispersers, and host. *American Journal of Botany*, 94(4), 558-567.

Brown, R. H., Nickrent, D. L., & Gasser, C. S. (2010). Expression of ovule and integument-associated genes in reduced ovules of Santalales. *Evolution and Development*, 12(2), 231-240.

Cid Villamil, R. M. (2006). *Biología del desarrollo de Cladocolea loniceroides* (Van Tieghem) Kuijt (Loranthaceae) (Doctoral dissertation). Universidad Nacional Autónoma de México, México, DF, México.

Cocucci, A. E. (1982). El óvulo y el gineceo en Loranthaceae. *Boletín de la Sociedad Argentina de Botánica*, 218(1-4), 131-141.

Cocucci, A. E. (1983). New evidence from embryology in angiosperm classification. *Nordic Journal of Botany*, 3(1), 67-73.

Contero, R., & Dehesa, M. A. (2007). Estudio etnobotánico del “mingari”: *Tristerix longibracteatus* (Desr.) Barlow & Wiens. *La Granja: Revista de Ciencias de la Vida*, 6(2), 25-29.

Dueñas-Gómez, H. C., & Franco-Roselli, P. (2001). Sinopsis de las Loranthaceae de Colombia. *Caldasia*, 23, 81-99.

Galetto, L., Bernardello, L. M., & Juliani, H. R. (1990). Acerca del nectario, nectar y visitantes florales en *Ligaria cuneifolia* (Loranthaceae). *Darwiniana*, 30, 155-161.

González, F., & Pabón, N. (2017). Inflorescence and floral traits of the colombian species of *Tristerix* (Loranthaceae) related to hummingbird pollination. *Anales del Jardín Botánico de Madrid*, 74(2), e061.

Graves, G. R. (1982). Pollination of a *Tristerix* mistletoe (Loranthaceae) by *Diglossa* (Aves, Thraupidae). *Biotropica*, 14(4), 316-317.

Johri, M., Ambegaokar, B., & Srivastava, S. (1992). *Comparative Embryology of Angiosperms*. Berlin: Springer-Verlag.

Kuijt, J. (1975). The genus *Cladocolea* (Loranthaceae). *Journal of the Arnold Arboretum*, 56(3), 265-335.

Kuijt, J. (1988). Revision of *Tristerix* (Loranthaceae). *Systematic Botany Monographs*, 19, 1-61.

Kuijt, J. (1990). A second species of *Ligaria* (Loranthaceae). *Brittonia*, 42(1), 66-69.

Kuijt, J. (2015). Santalales. In J. Kuijt & B. Hansen (Eds.), *The families and genera of vascular plants*, (Vol. 12) *Flowering plants: Eudicots; Santalales, Balanophorales* (pp. 1-189). Springer: Switzerland.

Kuijt, J., & Weberling, F. (1972). The flower of *Phthirusa pyrifolia* (Loranthaceae). *Berichte der Deutschen Botanischen Gesellschaft*, 85(7-9), 467-480.

- Narayana, R. (1958). Morphological and embryological studies in the family Loranthaceae - *Lysiana exocarpi* (Behr) Van Tieghem. *Phytomorphology*, 8, 146-168.
- Nickrent, D. L., Anderson, F., & Kuijt, J. (2019). Inflorescence evolution in Santalales: integrating morphological characters and molecular phylogenetics. *American Journal of Botany*, 106(3), 1-13.
- Nickrent, D. L., Malécot, V., Vidal-Russell, R., & Der, J. P. (2010). A revised classification of Santalales. *Taxon*, 59(2), 538-558.
- Raj, B. (1970). Morphological and embryological studies in the family Loranthaceae-XIII. *Amylothea dictyophleba* Van Tiegh. *Plant Systematics and Evolution*, 118(5), 417-430.
- Robles, A., Raz, L., & Marquinez, X. (2015). Floral anatomy of *Peristethium leptostachyum* (Loranthaceae). *Revista de Biología Tropical*, 64(1), 341-352.
- Schaeppi, H., & Steindl, F. (1942). Blütenmorphologische und embryologische Untersuchungen an Lorantheiden. *Vierteljahrsschr Naturf Ges Zürich*, 87, 301-337.
- Suaza, V., Pabón, N., & González, F. (2016). Development and morphology of flowers in Loranthaceae. *International Journal of Plant Sciences*, 177, 559-578.
- Sylvester, S. P., Sylvester, M. D., & Kessler, M. (2014). The world's highest vascular epiphytes found in the Peruvian Andes. *Alpine Botany*, 124(2), 179-185.
- Venturelli, M. (1981). Estudos sobre *Struthanthus vulgaris* Mart.: anatomia do fruto e semente e aspectos de germinação, crescimento e desenvolvimento. *Revista Brasileira de Botânica*, 4, 131-147.
- Venturelli, M. (1983). Estudos embriológicos em Loranthaceae: Gênero *Tripodanthus*. *Kurtziana*, 16, 71-90.
- Venturelli, M. (1984a). Estudos embriológicos em Loranthaceae: *Struthanthus flexicaulis* Mart. *Revista Brasileira de Botânica*, 7, 107-119.
- Venturelli, M. (1984b). Morfologia e anatomia floral de *Struthanthus vulgaris* Mart. (Loranthaceae) *Revista Brasileira de Botânica*, 7, 121-128.