REVISTA DE
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Floral ontogeny and anatomy of the hemiparasitic shrub *Gaiadendron punctatum* and a comparison of floral characters in the tribes Gaiadendrinae and Nuytsiae (Loranthaceae)

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ABSTRACT

Introducction: *Gaiadendron punctatum* is a hemiparasitic species of Loranthaceae (Tribe Gaiadendreae) that is widely distributed in mountainous regions of Central and South America. Embryological and phylogenetic studies in the family indicate a trend towards reduction of the gynoecium and ovules, the morphology of which supports the current circumscription of Tribe Gaiadendreae (*Gaiadendron* and *Atkinsonia*). Molecular phylogenetic studies suggest that *Nuytsia, Atkinsonia* and *Gaiadendron* diverged successively, forming a grade at the base of the Loranthaceae, but support values are low.
Objetive: In the present study, the floral anatomy of *Gaiadendron punctatum* was investigated in order to provide additional data to permit comparisons among the three basal-most genera in the Loranthaceae and reevaluate their relationships.
Methods: Flowers of *G. punctatum* were collected at different developmental stages and serial sections were prepared and analyzed by light microscopy.
Results: Inflorescence development is acropetal; the flowers are bisexual with an inferior ovary surmounted by a calyculus, a ring-shaped structure lacking vascular tissue; the ovary is comprised of seven basal locules, each with an ategmic, tenuinucellate ovule. Above the locules is a mamelon that is fused with the adjacent tissues. The androecium is comprised of seven epipetalous stamens, the anthers with fibrous endothecium dehiscence through a single longitudinal slit, releasing tricolpated pollen.
Conclusions: The results of this study show that *Gaiadendron* and *Atkinsonia* share versatile, dorsifixed anthers, while *Gaiadendron* and *Nuytsia* share the same mode of anther dehiscence. On the other hand, *Gaiadendron* shares with members of Tribe Elytrantheae an amyliferous mamelon and an unvascularized calyculus. Combined phylogenetic analyses of morphological and molecular data are desirable to determine whether Tribe Gaiadendreae comprises a clade, a grade or if the two genera are more distantly related.

Key words: *Atkinsonia*; embryo sacs; floral development; gynoecium; hypostase; mamelon; *Nuytsia*.

Loranthaceae Juss. is the most diverse family of parasitic plants in the tropics, comprising approximately 73 genera and 960 species (Dueñas-Gómez & Franco-Roselli, 2001; Nickrent et al., 2010; Dueñas-Gómez, 2015). The monophyly of the family is strongly supported by molecular and morphological evidence, with the molecular data also providing robust support for the genus *Nuytsia* R.Br. as sister to the rest of the family (Vidal-Russell & Nickrent, 2008a; Nickrent et al., 2010; Su et al., 2015). In the aforementioned studies, *Nuytsia* forms a grade with two other early-diverging genera: (*Nuytsia* (*Atkinsonia* F.Muell. (*Gaiadendron* G. Don (rest of the Loranthaceae)))), although Nickrent et al., (2019) recovered an alternate topology: (*Nuytsia* (*Gaiadendron* (*Atkinsonia* (rest of the Loranthaceae)))). Grimsson et al., (2017) recovered a completely different topology in which *Atkinsonia* was resolved as sister to an entirely Australasian clade, but with a low bootstrap support value. More data are desirable to resolve the divergence order in this basal part of the tree.

The current classification of the family includes five tribes and 11 subtribes (Nickrent et al., 2010; Kuijt, 2015). This study focuses in tribe Nuytsieae Tiegh., including only *Nuytsia floribunda* (Labill) R.Br., endemic to Western Australia, and tribe Gaiadendreae Tiegh., comprising *Atkinsonia ligustrina* (Lindl.) F. Muell., also from Australia, plus the Neotropical *Gaiadendron*, which includes *G. punctatum* (Ruiz & Pav.) G. Don, distributed in high montane ecosystems of South and Central America, and *G. coronatum* Kuijt, narrowly endemic to high-Andean dwarf forests in Peru (Kuijt & Graham, 2015). Although Gaiadendreae are not monophyletic, its two component genera share the following characters: versatile, dorsifixed anthers, fruits forming pseudodrupes and seeds with sulcate endosperm. The inflorescences of *Atkinsonia* are composed of monads, while those of *Gaiadendron* form triads, but the floral morphology is similar in both genera (Nickrent et al., 2010).

The first embryological studies in the family, including the first interpretation of the reproductive organs, were published by Treub (1883). A century later, Bhatnagar and Johri (1983) studied the floral structure and embryology of an expanded sample of 13 genera, culminating in the publication of their series titled "Morphological and embryological studies in the family Loranthaceae". Contemporaneously, Cocucci (1982) and Venturelli undertook studies of some Neotropical genera including *Struthanthus* Mart. (Venturelli, 1981) and Tripodanthus (Eichl.) Tiegh. (Venturelli, 1983). Based on their work, these authors had different interpretations of the process of reduction of the gynoecium and ovules observed in the family (Cocucci, 1982; Bhatnagar & Johri, 1983). The "primitive" condition was represented by *Nuytsia* (Bhatnagar & Johri, 1983) or by *Tripodanthus* and *Nuytsia* (Cocucci, 1982), in both cases with ategmic ovules completely separated from the ovary wall and enclosed in differentiated locules with the embryo sacs pointing towards the base of the gynoecium.

These interpretations were based on detailed studies of embryogenesis in *Nuytsia floribunda* (Narayana, 1958a). Detailed embryological studies have also been conducted on *Atkinsonia ligustrina* by Prakash (1961), who suggested that it would be useful to study the embryology of *Gaiadendron* in order to test the relationships of the Gaiadendreae and Elytrantheae Engl. (considered subtribes at that time). More recently, Suaza-Gaviria et al., (2016) studied the floral structure of *Gaiadendron* along with that of other Neotropical Loranthaceae species, but this work was mostly focused on inflorescence morphology.

Here we present a detailed study of floral development in *Gaiadendron punctatum* in order to fill in existing gaps in the data and thereby allow direct comparison of floral morphology and especially embryological characters among the three basal genera (*Nuytsia*, *Atkinsonia* and *Gaiadendron*) in Loranthaceae, data that may help shed light on the as-yet unresolved relationships among these taxa.

MATERIALS AND METHODS

Plant material: Flowers at different phenological stages (buds, anthetic and post-anthetic

flowers) were collected in the field and immediately fixed in FAA (formaldehyde: acetic acid: 70 % ethanol, 10:5:85) from a plant of a small population of *Gaiadendron punctatum* from the following locality: Colombia. Dept. Cundinamarca, Municipality la Calera, locality "Los Patios", North of the tollboth, paramo ca. 3 000 m. (voucher specimen Raz 1742, COL). The determination was confirmed using keys and descriptions by Kuijt (1981) and, Dueñas-Gómez and Franco-Roselli (2001).

In order to facilitate the description of the floral developmental processes, we established six stages of flower development taking into account the phenological phase (bud: 1 to 4; anthesis: 5; post-anthesis: 6), the total length of the flower measured in longitudinal section and the observed phases of microgametogenesis and megagametogenesis in the androecium and gynoecium, respectively (Table 1).

The flowers, fixed in FAA for 48 hours, were subsequently stored in 70 % ethanol, and treated following the Robles et al., (2016) protocol. This involved standard methods of dehydratation, 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 fuchsine and deposited in the Department of Biology collection at the Universidad Nacional de Colombia, Bogotá.

The slides 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 multifocus mode and photographed with a Leica MC 170 HC camera. Digital images were processed and edited with the software Adobe Photoshop CC and InDesign.

RESULTS

Flower and inflorescence morphology: *Gaiadendron punctatum* is a profusely branched shrub with axillary and/or terminal compound racemose inflorescences, developing acropetally (Fig. 1A). The partial inflorescences are dichasia composed of a central flower and two sessile lateral flowers (Fig. 1B). The dichasia are opposite and decussate along the inflorescence axis, each subtended by bracts. All the flowers are bisexual and subtended by foliaceous bracts.

The flower of *Gaiadendron punctatum* is epigynous, so the ovary is inferior (Fig. 1B, Fig. 1C) and is surrounded by the hypanthium. The inferior ovary subtends the other floral whorls: the calyx (or calyculus), the corolla and the androecium of epipetalous stamens (Fig. 1D).

The anatomical description presented here follows a topological order, from the base to

TABLE 1

Floral developmental stages studied in *Gaiadendron punctatum*, lengths in mm and characteristics associated with the androecium and gynoecium

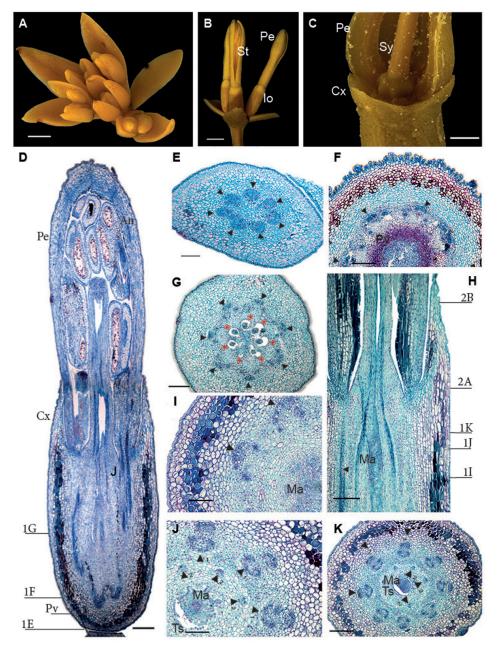| Stage | Length (mm) | | | | Characteristics | |
	Inferior ovary	Calyculus to apex	Total	Style and stigma	Androecium	Gynoecium
1	1.7	2.5	4.2	1.5	Archesporium, dyads and tetrads	Ovules
2	2.1	3.4	5.5	2.0	Tetrads	Meiosis in the ovule
3	3.1	5.4	8.5	4.2	Tetrads, tetrads with triradiate pollen	Meiosis in the ovule
4	3.5	6.5	10	4.8	Mature pollen free uninuclear and binuclear	Lengthening of the embryo sacs
5	3.8	8.1	11.9	6.4	Anthesis flower, anther dehiscence, mature pollen shed	Lengthening of the embryo sacs
6	4.0	-	-	9.5	Post-anthesis flower without petals and stamens	Mature embryo sacs, Complete ovular apparatus

Fig. 1. A. Inflorescence and flower anatomy of *Gaiadendron puntantum*. **A.** Inflorescence with flowers developing acropetally; **B.** Lateral flowers in side view; **C.** Detail of flower with inferior ovary; **D.** Longitudinal section of the flower (the lines indicate the approximate position of the transverse sections corresponding to Fig. 1E, Fig. 1F and Fig. 1G); **E.** Transverse section (TS) of the floral receptacle; **F.** TS at the pelvis level; **G.** TS at the ovary level; **H.** Detail of the mamelon in longitudinal section (the lines indicate the approximate position of the transverse sections corresponding to Fig. 1I, Fig. 1J, Fig. 1K, Fig. 2A and Fig. 2B; **I. J.** and **K.** TS at different levels of the mamelon immersed in amyliferous tissue. An = Anther; Cx = Calyculus, Io = Inferior ovary; Ma = Mamelon; Pe = Petal; Pv = Pelvis; Sy = Style; St = Stamen; Ts = Surrounding tissues; Triangle = Vascular bundle; Triangle with number 1 = External vascular bundle; Triangle with number 2 = Internal vascular bundle; Red asterisks = Locules; **D. E. F. G. H. I. J.** and **K.** Astra blue/fuchsine. Scale bars: 2 mm in **A.** and **B.**; 1 mm in **C.**; 500 **μm in D.**; 300 μm in **E.**; 200 μm in **F.**; 150 μm in **G.**; 100 μm in **H. I.** and **J.**

the distal parts of the flower. We begin with the receptacle, then the inferior ovary and the hypanthium, following the vascularization as it continues into the style and stigma and the perianth: calyculus and corolla, ending with the epipetalous androecium. The processes of microsporogenesis and megasporogenesis are described in association with the description of the gynoecium and androecium, respectively.

Gynoecium and floral vasculature: Seven traces enter the sessile flowers via the receptacle from the inflorescence axis (Fig. 1E). The mature hypanthium has a single epidermal layer composed of elongate, papillose cells covered by a thick cuticle; after the epidermis, 6-9 inner layers of tanniniferous parenchymatic tissue are observed, followed by 10-11 layers of parenchyma towards the central region; the vascular bundles that irrigate the petals and stamens are embedded in parenchymatic cells (Fig. 1F).

The ovary is situated above the base of the receptacle, which is in turn composed of sclerenchymatic tissue (when mature) and is referred to as the pelvis (Fig. 1F). It is composed of seven basal locules each with a single ategmic ovule (Fig. 1G). There is a non-vascularized conical structure above the locules called the mamelon (Fig. 1H) which is composed of amyliferous parenchyma that is fused with the surrounding tissues; embryo sacs will eventually grow through this tissue (Fig. 1I). The mamelon is separated from the surrounding tissues just beneath the style (Fig. 1J, Fig. 1K).

At the base of the flower, seven vascular bundles can be seen forming a ring at the periphery of the pelvis (Fig. 1E); above this point, the bundles divide to form two concentric rings (Fig. 1H, Fig. 1I). The interior ring is made up of seven bundles that correspond to the seven locules of the gynoecium (Fig. 1H, Fig. 1J); all or part of these five to seven bundles continue into the style (Fig. 2A, Fig. 2B). There are seven vascular bundles in the exterior ring, each one composed of three to five strands including a large central strand that is external

to the others, plus two to four lateral strands (Fig. 1I, Fig. 1J, Fig. 1K). The lateral strands fuse to form a new bundle that enervates the stamens, while the external strand enervates the petals. No vascular traces enter the calyculus (Fig. 1D, Fig. 1E, Fig. 1F, Fig. 1G, Fig. 1H), in which there is neither evidence of tracheids, nor any other vascular tissue.

Megasporogenesis: The ovary is composed of seven locules (Fig. 1G), each of which contains a single ategmic, tenuinucellate ovule oriented towards the base of the flower (Fig. 2D). The placentation is axillary. In floral developmental stages 2 and 3 (Table 1), meiosis can be observed, resulting in four cells that are similar in size and contour (Fig. 2E, Fig. 2F). In each locule, the developing embryo sacs elongate through the amyliferous tissue of the mamelon until they reach the base of the style (Fig. 2C, Fig. 2J; stage 6 in Table 1) without invading the latter structure.

At the upper end of the mature embryo sac, a small protuberance is observed. It is called the caecum, which functions as a haustorium (Fig. 2G). It is followed by the synergids, the filiform apparatus, the egg cell and the central cell (Fig. 2H). The antipodal cells are found at the base of the embryo sac, near the hypostase and these tend to degenerate (Fig. 2I).

Style and stigma: The base of the style is expanded (Fig. 1C, Fig. 1D, Fig. 1H) and seven projections are observed at the periphery (Fig. 1C); these correspond to the outermost layers of the tissues that nourish the embryo sacs during their development (Fig. 2A). The style is hollow (Fig. 1D, Fig. 1H) and it is composed of a single outer epidermal layer interspersed with stomata and covered by a thick cuticle. Beneath the epidermis, there are 10-12 layers of tanniniferous parenchyma and seven collateral vascular bundles are observed towards the inner part of the tissue, surrounded by smaller parenchyma cells, which are, in turn, adjacent to the inner epidermis that surrounds the stylar canal (Fig. 2B).
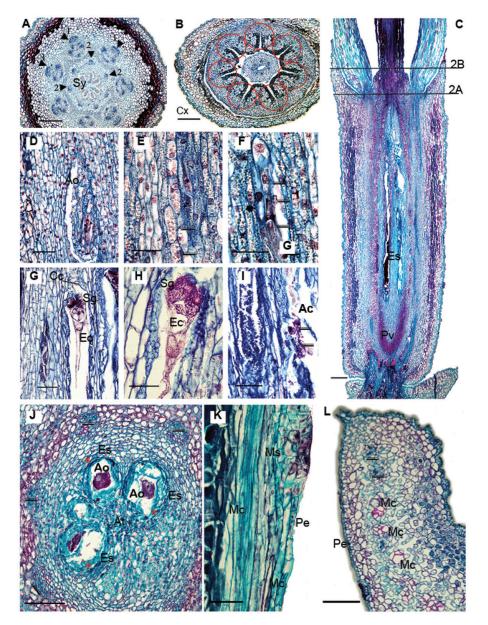
Fig. 2. Floral morphoanatomy of *Gaiadendron punctantum*, megasporogenesis and perianth. **A.** Transverse section of the flower (TSF) at the base of the style; **B.** TSF at the level of the calyculus; **C.** Longitudinal section (LS) of the gynoecium, (the lines indicate the approximate positions of the corresponding transverse sections in **A.** and **B.**); **D.** LS, detail of the ovary and ategmic ovule; **E.** LS after first meiotic division, arrows indicate nuclei; **F.** LS, after second meiotic division, arrows indicate nuclei; **G.** and **H.** LS at the upper level of embryo sac showing the caecum, synergids and egg cell; **I.** LS at the lower level of embryo sac showing degenerated antipodal cells; **J.** Transverse section at the level of the embryo sacs; **K.** and **L.** Detail of the mucilage secreting structures. Ac = Antipodal cells; Ao = Ategmic ovule; At = Amyliferous tissue; Cc = Caecum; Cx = Calyculus; Ec = Egg cell; Es = Embryo sac; Mc = Mucilage canal; Ms = Mucilage secreting structure; Pe = Petal; Pv = Pelvis; Sg = Synergids; Sy = Style. Triangle = Vascular bundle; Triangle with number 1 = External vascular bundle; Triangle with number 2 = Internal vascular bundle; Asterisks = Locules; Red circle = Interdigitated epidermal cells; 2e-3e = Second and third embryo sac divisions; **A. B. C. D. E. F. G. H. I. J. K.** and **L.** = Astra blue/fuchsine. Scale bars: 200 μm in **A. B.** and **L.**; 500 μm in **C**; 50 μm in **D. G. I.** and **K.**; 30 μm in **E. F.** and **H.**; 100 μm in **J.**

There is a trilobate stigma at the end of the style, with interdigitated epidermal cells at the edges of the lobes; stigmatic cells are slightly rounded, whereas the epidermal style cells are flattened (Fig. 1D).

Perianth - calyculus and corolla: The calyculus is short and light green in color (Fig. 1C); it forms a ring above the ovary, being more or less persistent in fruit, with a variable number of irregular teeth at the margin (Fig. 1C, Fig. 2B, Fig. 2C). The epidermis is uniseriate abaxially and is made up of radially elongated cells and structures that secrete mucilage (Fig. 2B), the latter persisting even in fixed material (Fig. 1C). The adaxial epidermis (oriented towards the petals) is composed of quadrangular cells. The mesophyll is composed of undifferentiated parenchyma with small cells and thin walls as well as parenchyma with larger cells and dark, tanniniferous cytoplasm. As mentioned above, no vascular traces were observed (Fig. 2B).

The corolla is composed of seven long, white petals that measure approximately 3.5 x 1.3 mm, with valvate aestivation (Fig. 1B). The petals are postgenitally fused in the flower bud by interdigitation of the external epidermal cells (Fig. 1D, Fig. 2B, Fig. 3K). The bases of the petals are narrow and articulated (Fig. 1H). The corolla lobes are completely free at anthesis, but the basal third of the petals form a tube that surrounds the style; the petals are reflexed in the upper two thirds of their length, where the filaments of the epipetalous stamens are free from the petals (Fig. 3B). The adaxial epidermis of the petals is papillose, followed by 4-7 layers of tanniniferous parenchyma made up of very tightly packed cells; then there is a less compact parenchymatous region toward the center of the petals in which the vasculature is embedded, the latter consisting of a central bundle and one or two lateral bundles. Between the central and lateral bundles, some packets of sclerenchymatic cells with not very thick walls are observed; these are accompanied by cells that stain densely and form longitudinal mucilaginous ducts. Exterior to these cells, another layer of tightly packed parenchymatic cells is seen, some of which are tanniferous; the abaxial epidermis at outer edge of the petals is composed of quadrangular cells and mucilage-secreting structures (Fig. 2K, Fig. 2L).

Androecium and microsporogenesis: The androecium is composed of seven stamens of three different lengths; this variation can be seen in the mature flower as well as in earlier developmental stages (Fig. 3A). The filaments are adnate to the bases of the petals (Fig. 3B) and the anthers are dorsifixed (Fig. 3L, Fig. 3M), each with two thecae measuring 2-3 mm long (Fig. 3K). Two pollen sacs of similar size are observed within each theca, each with a uniseriate exothecium of very thin-walled, unlignified cells (Fig. 3C). The fibrous endothecium is made up of a single layer of large, quadrangular cells plus two thinner layers that do not persist, and a secretory tapetum that persists until maturity. The tapetum is non-invasive (Fig. 3I, Fig. 3K) and is initially composed of uninucleate cells, becoming binucleate at the end of their development. No amyloplast or other type of reserves were observed in the endothecium, or in any of the other tissues of the anther during its development.

Microsporogenesis begins with a solid, diploid archaesporium (Fig. 3C). The uninucleate microspores separate (Fig. 3D) and undergo two meiotic divisions (Fig. 3E), first forming dyads (Fig. 3F) and then tetrads (Fig. 3G). The four nuclei separate simultaneously, creating primarily tetrahedral tetrads (Fig. 3G), near which tapetum and orbicules (Ubisch bodies) can be observed, the latter appearing red in the figure. In the final stage, four trilobate pollen grains can be distinguished within the callose wall of the tetrad (Fig. 3H, Fig. 3I). The free pollen becomes binucleate just before anthesis (Fig. 3J).

Anther dehiscence is longitudinal and occurs via dehydration of the tissues (Fig. 3L, Fig. 3M). The cells of the exothecium near the stomium become elongated in the later developmental stages of the anther to facilitate pollen dispersal. Dehiscence occurs without prior
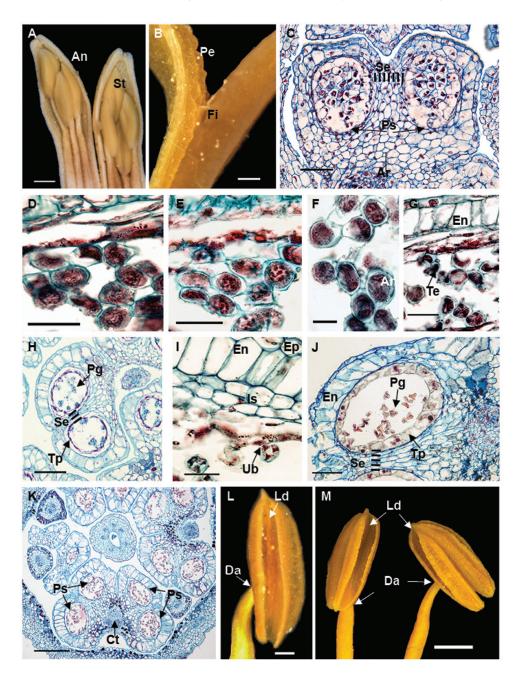
Fig. 3. Floral morphoanatomy of *Gaiadendron puntantum*: Androecium and microsporogenesis; **A.** Anther in longitudinal section in mature stage; **B.** Detail of the anther attachment; **C.** Immature pollen sacs with archaesporium (2n); **D.** Uninucleate microspores; **E.** First meiotic division; **F.** Dyads; **G.** Tetrad formation in the pollen sacs; **H.** Pollen sac with tetrads; **I.** Detail of fibrous endothecium, intermediate strata and tapetum**; J.** Pollen sac with mature pollen and persistent tapetum; **K.** Mature anther; **L.** and **M.** Dorsifixed anthers showing longitudinal dehiscence. An = Anther; Ar =Archesporium; Ct = Connective tissue; Da = Dorsifixed anther; En = Endothecium; Ep = Epidermis; Fi = filament of stamen; Is = Intermediate strata; Ld = Longitudinal dehiscence of the anther; Pe = Petal; g = Pollen grain; Ps = Pollen sac; Se = Septum; t = Stamens; Tp = Tapetum; Ub = Ubisch bodies (orbicules). **C. D. E. F. G. H. I. J.** and **K. =** Astra blue/fuchsine. Scale bars: 2 mm in **A.**; 500 µm in **B., L.**; 50 µm in **C, J**; 20 µm in **D. E. G.** and **I.**; 10 µm in **F.**; 100 µm in **H.**; 200 µm in **K.**; 1 mm in **M.**

TABLE 2
Comparison of characters among the three basal genera of Loranthaceae

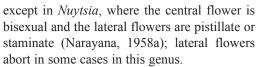| Character	*Nuytsia*	*Atkinsonia*	*Gaiadendron*
Inflorescence	Racemose	Racemose	Racemose
Parcial inflorescence	Dichasium with central flower bisexual, laterals pistillate	Monad subtended by three bracts, bisexual flowers	Dichasium of bisexual flowers
Central mamelon	Present	Present (¿?)	Present
Number of locules	3	4	7
Fusion of the mamelon and adjacent tissues	No	¿?	Yes, in the middle
Tracheids in the mamelon	Yes	¿?	No
Number of vascular bundles at the base	7	7	7
Number of bundles in the internal ring of the ovary and (style)	6 (6)	4 (4)	7 (5-7)
Number of bundles in the internal ring of the petals and (stamens)	7 bundles each with: 1 external (2 laterals that fuse)	7 bundles each with: 1 external (2 laterals that fuse)	7 bundles each with: 1 external (2 to 4 laterals that fuse)
Vascularization of the calyculus	Yes, 5 initial traces that anastomose	Yes; no traces enter from the main stele, but tracheids differentiate in each lobe	No
Ovules	Ategmic, oriented towards the base, tenuinucellar	¿?	Ategmic, oriented towards the base, tenuinucellar
Style	With amyliferous tissue	¿?	Without amyliferous tissue
Intrusion of the embryo sacs	Ovary and style	Ovary	Ovary
Hipostase composition	Collenchyma	Collenchyma	Sclerenchyma
Base of the style	With angular thickenings	With slightly rounded thickenings	With angular thickenings
Anthers	Epipetalous, Basifixed	Epipetalous, dorsifixed, Versatile	Epipetalous, dorsifixed, Versatile
Sources	Narayana (1958a) Narayana (1958b)	Prakash (1961) Garg (1958) Cocucci (1982)	This study

coalescence of the pollen sacs in each theca, via rupturing of the central septum and the walls of the two pollen sacs along their entire length (Fig. 3J).

DISCUSSION

Given the lack of robust support for a specific branching order among the three basal genera in the Loranthaceae (*Gaiadendron*, *Nuytsia* and *Atkinsonia*) according to the phylogenetic proposals available for the family, we consider the utility of comparative floral morphoanatomy to help resolve these relationships. With the data reported here for *Gaiadendron*, such a comparison is now possible and is presented below. Table 2 summarizes some of the characters and their states in the basal genera of Loranthaceae: *Nuytsia*, *Atkinsoni*a and *Gaiadendro*n. Comparisons with some members of the nearby tribe Elytrantheae are also considered here.

The inflorescences of the three basal genera in the Loranthaceae are indeterminate racemes; the parcial inflorescences are dichasia in *Gaiadendron*, but these are reduced to monads in *Atkinsonia*. The flowers are generally bisexual,

except in *Nuytsia*, where the central flower is bisexual and the lateral flowers are pistillate or staminate (Narayana, 1958a); lateral flowers abort in some cases in this genus.

Gaiadendron punctatum has a septilocular ovary, which is the greatest number of locules in the Loranthaceae. *Nuytsia* (Narayana, 1958a) and *Lepeostegeres* (Dixit, 1958) each have three locules, while *Atkinsonia, Amylotheca* (Raj, 1970), *Lysiana* (Narayana, 1958b) and *Peraxilla* (Prakash, 1960) each have four. The ovary is unilocular in the remaining genera of the family (Kuijt, 2015).*Nuytsia, Gaiadendron* and the genera of the tribe *Elytrantheae* all have a mamelon, being amyliferous in *G. punctatum* and in the tribe *Elytrantheae* (Johri et al., 1992) and not being amyliferous in *Nuytsia* (Narayana, 1958a). The mamelon in *G. puntatum* is retained within the ovary without entering the style, a condition also reported in *Peraxilla* Tiegh. and *Amylotheca* Tiegh. (Prakash, 1960; Raj, 1970), while in *Nuytsia, Lysiana* Tiegh. and *Lepeostegeres* Blume (Dixit, 1958; Narayana, 1958a; Narayana, 1958b), the mamelon invades the hollow style. In *Nuytsia* the mamelon remains separate from the adjacent tissue, but in *Lysiana* and *Lepeostegeres* this structure is fused with the ovary wall and the tissues of the stigma.

The mamelon of *Nuytsia* does not have amyliferous tissue, but the style does have amyliferous parenchyma. Thus, the embryo sacs can invade the style for quite a good part of its length. The embryo sacs of *Lysiana* and *Lepeostegeres* also enter the style due to the invasion of the amyliferous tissue of the mamelon into the lumen of the stylar canal (Bhatnagar & Johri, 1983). The embryo sacs of *Gaiadendron*, just like those of *Peraxilla* y *Macrosolen* (Blume) Rchb.*,* develop only up to the base of the style because the mamelon is short and there is no amyliferous tissue in the style. The mamelon is fused with the ovary wall in *Gaiadendron*, a condition also seen in *Lysiana* and *Lepeostegeres* (Dixit, 1958; Narayana, 1958b) but not in *Nuytsia*. The aforementioned characters suggest that *Gaiadendron* has

a greater affinity with the tribe Elytrantheae than with *Nuytsia*.

Although Cocucci (1982) indicated that *Atkinsonia ligustrina* has an amyliferous ovary like that found in genera of the tribe Elytrantheae, there is no direct evidence of this condition in *Atkinsonia* according to the studies cited by Cocucci (Garg, 1958; Prakash, 1961).

Calyculus, base of style and nectary: The vascularization of the calyculus is different in each of the three basal genera of Loranthaceae (Table 2). The absence of bundles or tracheids in the calyculus of *Gaiadendron punctatum* is the state found in the rest of the Loranthaceae, while only *Nuytsia* and *Atkinsonia* present alternative states. This result supports the Su et al., (2015) topology of (*Nuytsia* (*Atkinsonia* (*Gaiadendron* (rest of the Loranthaceae)))). It should be noted that the inconspicuous calyculus of *G. punctatum* contrasts with that of *G. coronatum*, in which the lobes are dentate, well differentiated, and persistent in fruit (Kuijt, 2015).

Diverse types of nectaries have been reported in Loranthaceae. In the Neotropical clade Psittacanthinae, (*sensu* Nickrent et al., 2010), the nectaries often form a disk or ring between the base of the style and the base of the petals. This ring usually has stomata and subepidermal parenchyma with dark-staining cellular contents, as can be seen in *Peristethium leptostachyum* Kunth (Tiegh.) (Robles et al., 2016), *Psitacanthus* Mart. (Robayo et al., 2020), *Passovia* Karst., *Aetanthus* (Eichl.) Engl. and *Oryctanthus* Eichl. (Suaza-Gaviria et al., 2016). A different type of nectary found at the base of the petals has been described in *Cladocolea* Tiegh. (Cid-Villamil, 2006).

Nevertheless, it has been reported that the basal genera *Nuytsia* (Narayana, 1958a), *Atkinsonia* (Prakash, 1961) and few species of the Tribe Elytrantheae have thickenings at the base of the style (*Amylotheca, Lepeostegeres*: respectively, Dixit, 1958; Raj, 1970). In some of these cases, they have been interpreted as possible nectaries (*Lepeostegeres, Nuytsia*: respectively Narayana, 1958a; Raj, 1970)

TABLE 3
Anther dehiscence in *Nuytsia*, *Atkinsonia*, *Gaiadendron* and some genera of the tribe Elytrantheae

Genus	Endothecium	Dehiscence type	Scheme of dehiscence	Source
Atkinsonia	Fibrous	A		Prakash, 1961
Nuytsia *Gaiadendron*	Fibrous	B		Narayana, 1958a This study
Lysiana *Peraxilla*	Fibrous	C		Narayana, 1958b Prakash, 1960
Lepeostegeres *Amylotheca*	Non-Fibrous	D		Dixit, 1958 Raj, 1970

and only in *Lepeostegeres*, the presence of a dark-staining subepidermal tissue has been confirmed (Schaeppi & Steindl, 1942). Also, Lamilla et al., (2020) recently found that the base of the style conforms a nectary in *Tristerix longebracteatus* (Desr.) Barlow & Wiens, a basal member of the tribe Psittacantheae Horan

Suaza-Gaviria et al., (2016) suggested that *G. punctatum* presents a nectary disk. However, no disk was observed in the present study (Fig. 1D, Fig. 1E, Fig. 1F, Fig. 1G, Fig. 1H; Fig. 2B, Fig. 2C). Our longitudinal sections of *G. punctatum* found a widened base of the style with some stomata in the epidermis and typical parenchyma beneath the epidermis (Fig. 1D, Fig. 2B); nor did we find sunken stomata at the base of style, and therefore the evidence is insufficient to confirm or reject that this structure is a nectary. In contrast, our research team found a widened style in *Tristerix* formed by an epidermis with abundant sunken stomata, underlain by parenchymatic tissue with dense cytoplasm and abundant intercellular spaces surrounding the vascular bundles (Lamilla et al., 2020). Nevertheless, the dark-staining subepidermal parenchyma at the base of the petals

and stamens suggests the possibility of a different type of nectary (Fig. 2B).

Anther dehiscence: The endothecium in Loranthaceae can be fibrous or non-fibrous. When is it fibrous, it is composed of thickened cells that surround the pollen sacs, interrupted by cells that lack thickenings. The position of these weaker cells determines the type of dehiscence (Table 3).

In type A dehiscence, present in *Atkinsonia ligustrina* (Prakash, 1961), the two pollen sacs of each theca undergo coalescence due to the rupturing of the septum between them, followed by a single longitudinal rupture of the stomium.

In type B dehiscence, present in *Nuytsia* (Narayana, 1958a) and *Gaiadendron* (this study), the septum that divides the pollen sacs within each theca remains intact initially, and dehiscence occurs via simultaneous rupture of the septum and the outer wall of the theca (stomium), at the precise point where they connect, thereby creating a single longitudinal opening (Table 3).

In the genera *Lysiana* and *Peraxilla* of the tribe Elytrantheae, the interruption of the fibrous endothecium occurs in the middle of each pollen sac (Table 3) such that each theca opens via two longitudinal slits (type C dehiscence). On the other hand, in *Amylotheca* and *Lepeostegeres,* also of the tribe Elytrantheae, the endothecium has no thickened zones and therefore dehiscence may occur at any point (type D dehiscence).

According to Fig. 7F of Suaza-Gaviria et al., (2016), anther dehiscence in *Gaiadendron punctatum* occurs via two longitudinal slits in each theca, one for each pollen sac, corresponding to our type C. The results of the present study do not corroborate type C dehiscence in this taxon. The septum that separates the two pollen sacs within each theca is thickened and the weakest zone (Fig. 3K) is localized to the point where it fuses with the outer wall of the theca (the stomium, Table 3). Dehiscence occurs via a single longitudinal slit between the two pollen sacs (Fig. 3M). Therefore, *Gaiadendron punctatum* has type B anther dehiscence, just like *Nuytsia floribunda*.

***Atkinsonia* and *Gaiadendron*: clade or grade:** In traditional classifications of Loranthaceae as well as in recent molecular systematic studies, *Nuytsia*, *Atkinsonia* and *Gaiadendron* have been considered basal genera. The character traditionally used to support this position is root parasitism through secondary haustoria, whereas in the rest of the family, it is the primary haustorium that parasitizes the stems of the host plants (Vidal-Russell & Nickrent, 2008b). It should be noted, however, that *Gaiadendron punctatum* can occasionally parasitize epiphytes (Kuijt, 2015).

The grouping of the genera *Atkinsonia* and *Gaiadendron* in the tribe Gaiadendreae was based on the following characters: vascular bundles in the calyculus, drupaceus fruit, furrowed endosperm and dorsifixed and versatile anthers (Nickrent et al., 2010). Regarding the first character, the vascularization of the calyculus occurs in three different states in each of these three basal genera (Table 2)

with *Gaiadendron punctatum* lacking vascular traces or tracheids, a state shared with the rest of the Loranthaceae. It would be interesting to study this character in *Gaiadendrum coronatum*, the calyculus of which is much larger and persists in the fruit (Kuijt, 2015). Regarding the drupaceous fruit, *Atkinsonia* and *Gaiadendron* have pseudodrupes in which the embryo is surrounded by a hard endocarp. This condition is also found in fruits of the tribe Elytrantheae, including the genera *Amylotheca* (Raj, 1970), *Lepeostegeres* (Dixit, 1958), *Lysiana* (Narayana, 1958b) and *Peraxilla* (Prakash, 1960). The fruit of *Nuytsia* is completely distinct, being a winged nut (Kuijt, 2015). Discarding the first two characters, only furrowed endosperm and dorsifixed versatile anthers remain as possible synapomorphies of tribe Gaiadendreae as it is currently circumscribed.

In most molecular phylogenetic studies published to date (Nickrent et al., 2010; Su et al., 2015; Nickrent et al., 2019), *Gaiadendron* and *Atkinsonia* form a grade rather than a clade, although Grimsson et al., (2017) recovered *Atkinsonia* as sister to a clade comprising mostly genera of the tribe Elytrantheae. As can be seen, the morphological evidence is not unequivocal either, with some characters supporting *Gaiadendron* as sister to *Atkinsonia* (dorsifixed versatile anthers) and/or to *Nuytsia* (type of anther dehiscence), while the combination of characters 1. non-vascularized calyculus and 2. amyliferous mamelon would appear to support a sister relationship of *Gaiadendron* to the tribe Elytrantheae or to rest of the Loranthaceae. In order to definitively resolve the matter, it will be necessary to collect the last bits of missing data for *Atkinsonia ligustrina* (Table 2) and ideally, perform a combined analysis of molecular and morphological data to determine if the two genera of Gaiadendreae really do form a clade, a grade or are more distantly related.

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

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RESUMEN

Ontogenia floral y anatomía del arbusto hemiparasítico *Gaiadendron punctatum* y comparación de caracteres florales en las tribus Gaiadendrinae y Nuytsiae (Loranthaceae)

Introducción: *Gaiadendron punctatum* es una especie hemiparásita perteneciente a uno de los tres géneros basales de la familia Loranthaceae, siendo los otros dos *Nuytsia* y *Atkinsonia*. El género está conformado por dos especies distribuidas en regiones montañosas de Sudamérica y Centroamérica. Tanto los estudios embriológicos, como los filogenéticos, indican una tendencia hacia la reducción del gineceo y de los óvulos en la familia, cuya morfología respalda la circunscripción de la tribu Gaiadendreae (*Gaiadendron* y *Atkinsonia*). Estudios filogenéticos moleculares sugieren que *Nuytsia*, *Atkinsonia* y *Gaiadendron* divergieron sucesivamente, formando un grado en la base de la familia Loranthaceae, pero los valores en los que se sustenta son bajos.

Objetivo: En el presente trabajo se describe la anatomía floral de la especie *Gaiadendron punctatum* con el objetivo de complementar la información embriológica, de manera que se pueda comparar directamente la morfología floral y los caracteres embriológicos entre los tres géneros basales de la familia Loranthaceae y reevaluar sus relaciones.

Métodos: Las flores de *G. punctatum* fueron recolectadas en diferentes estados de desarrollo, se realizaron cortes histológicos seriados, se tiñeron con azul de astra y fucsina, y se analizaron mediante microscopía óptica.

Resultados: Las inflorescencias mostraron un desarrollo acrópeto, las flores bisexuales presentaron ovario ínfero con presencia de una estructura en forma de anillo, carente de tejidos vasculares llamada calículo; el ovario se compone por siete lóculos basales, cada uno con un óvulo atégmico tenuinucelar. Por encima de los óvulos, el mamelón se fusiona con los tejidos adyacentes. El androceo está conformado por siete estambres epipétalos, las anteras presentan un endotecio fibroso y granos de polen tricolpados. La dehiscencia de las anteras es mediante una sola apertura longitudinal.

Conclusiones: Los resultados del presente trabajo demuestran que *Gaiadendron* y *Atkinsonia* comparten anteras dorsifijas y versátiles, mientras *Gaiadendron* y *Nuytsia* comparten el tipo de dehiscencia anteral y por otro lado *Gaiadendron* comparte los caracteres de mamelón amilífero y calículo no vascularizado con la tribu Elytrantheae. La clasificación del género *Gaiadendron* con respecto a los dos géneros basales de la familia debe ser objeto de investigación (análisis filogenético combinado) que permita dirimir si la tribu Gaiadendreae es un clado, un grado o dos géneros más lejanamente emparentados.

Palabras clave: *Atkinsonia*; sacos embrionarios; desarrollo floral; gineceo; hipostasa; mamelón; *Nuytsia*.

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