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TOWARDS AN UNDERSTANDING OF THE BASAL EVOLUTION OF VIOLACEAE FROM AN ANATOMICAL AND MORPHOLOGICAL PERSPECTIVE

Saul Hoyos

Introduction

The violet family, Violaceae, are predominantly tropical and contains 23 genera and upwards of 900 species (Feng 2005, Tukuoka 2008, Wahlert and Ballard 2010 in press). The family is monophyletic (Feng 2005, Tukuoka 2008, Wahlert & Ballard 2010 in press), even though phylogenetic relationships within Violaceae are still unclear (Feng 2005, Tukuoka 2008). The family embrace a great diversity of vegetative and floral morphologies. Members are herbs, lianas or trees, with flowers ranging from strongly spurred to unspurred. The fruits range from indehiscent and fleshy to capsular with small to noticeably large seeds that may be winged or carunculate (Hekking 1988).

Recent phylogenetic studies (Ballard and Walhert 2010 in press) have brought changes in our understanding of evolutionary history of Violaceae by clarifying “basal” groups that are prime candidates for study of morphology and anatomy. Within Violaceae, the *Fusispermum* spp. and *Rinorea apiculata* clades are strongly supported as being successive sisters to the rest of the family (Wahlert and Ballard 2010 in press. Fig 1. 1.00 BP).

Another development that affects our understanding of the evolution of Violaceae is their association with Goupiaceae, which contains *Goupia*, with

two species, one from Central America and the other in the Amazonian region of South America. The phylogenetic position of Goupiaceae has for long been poorly understood. *Goupia* was included in Celastraceae by Bentham and Hooker (1862) as a subfamily, Goupioideae. However, as first suggested by Miers (1862), the placement of the genus *Goupia* was not satisfactory; it had already been placed in many different families, “Willdenow considered it to belong to Araliaceae. Jussieu placed it in Rhamnaceae”, while Endlicher “classed it among the dubious genera of Celastraceae” (Miers 1862, p 289). Miers (1862) thought that *Goupia* did not belong to any of these families, and therefore it should be placed in a separate family, Goupiaceae. More recently, Cronquist (1981) included it in Celastraceae again, and although Takhtajan (2009) placed Goupiaceae in the order Celastrales, near Celastraceae, he mentioned that it was different in many aspects, including petiole anatomy and morphology of the anthers.

Recently, Simmons et al. (2001) found that Goupiaceae belong to Malpighiales, and Feng (2005, 98 BP) and Wurdack and Davis 2009 (100 BP, 100 PP, see above) placed Goupiaceae sister to Violaceae. Goupiaceae are part of a clade that includes Achariaceae, Lacistemataceae, Passifloraceae, and Salicaceae, and it is the only member that has axile placentation, all the others being parietal (Wurdack and Davis 2009).

Few comprehensive empirical studies have ever been published on Violaceae, and the basal part of the family, particularly *Fusispermum* spp. and *Rinorea apiculata* group, has been left “untouched” (Wahlert and Ballard in press). Even fewer studies focused on general floral evolution or development

have been published (but cf. Feng 2005, Arnal, 1945). Most studies of the studies that have been published focus on the widely available genus *Viola*, atypical in the family in being largely herbaceous and temperate (Metcalf and Chalk 1972, Corner 1976, Feng 2005). The majority of the family is woody and tropical in distribution, so extrapolation from the advanced and more recently evolved *Viola* to the other more basal genera in the family is dubious.

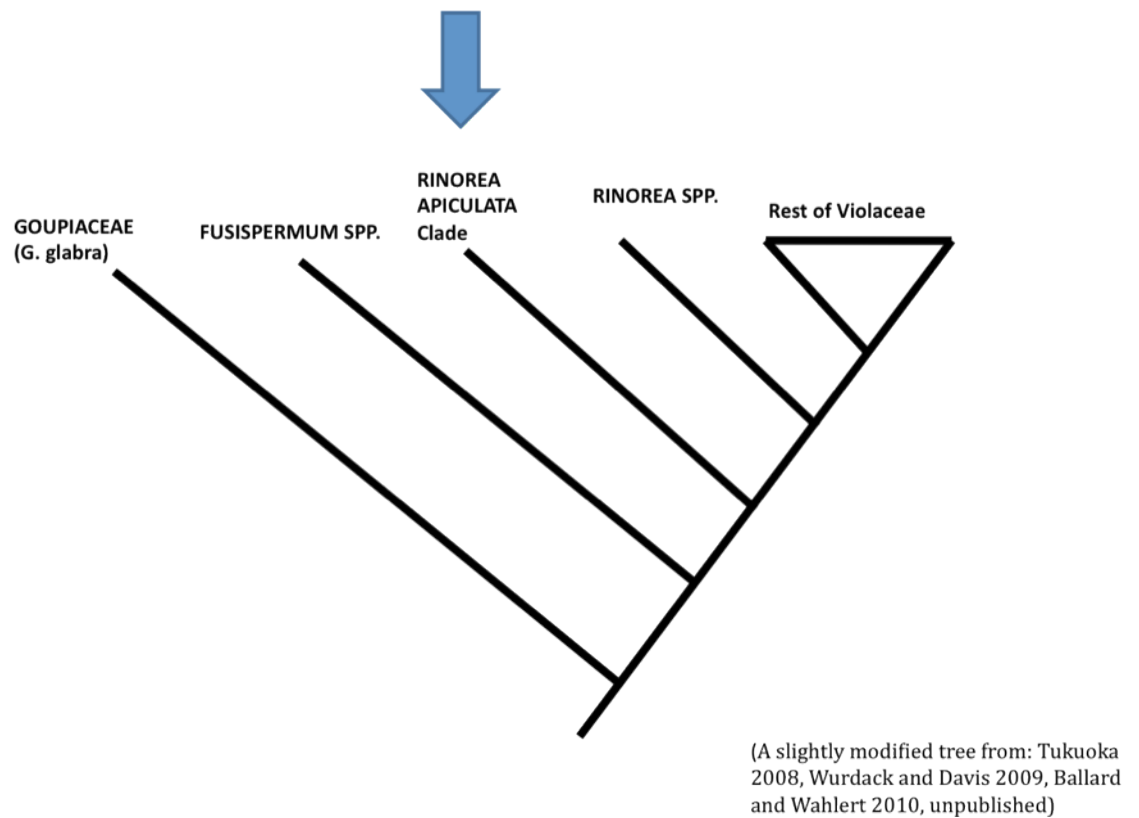


Fig 1. Phylogenetic relationship of Violaceae. (Redrawn from Wurdack & Davis 2009, Wahlert and Ballard 2010 in press)

Details of what is known of the vegetative anatomy of the focal taxa of this study, *Goupia*, *Fusispermum*, and the *Rinorea apiculata* group, are to be found in Metcalfe and Chalk (1972); little is known about Goupiaceae and Violaceae in general and *Fusispermum* and the *R. apiculata* group have not been studied. For seeds, Hekking (1984) briefly described the external morphology of the seeds of *Fusispermum* spp., Corner (1976) investigated the seed anatomy of *Viola* spp., *Hybanthus* spp., and two species of Malesian *Rinorea* (Plisko 1992). The broadest survey of seed anatomy of Violaceae is that by Plisko (1992), but even there only fourteen species are studied; the seed anatomy of *Goupia glabra* is described by Melikiana & Sarinov (2000) (but see below). The seed anatomy of *Fusispermum* spp. and the *R. apiculata* group is unknown.

In an attempt to develop a better understanding of the evolutionary history of Violaceae, here I examine the stem, leaf, flower, and seed of: *Fusispermum laxiflorum* Hekking, *F. minutiflorum* Cuatrec., *F. rubrolignosum* Cuatrec., the *Rinorea apiculata* clade (here comprised of *R. apiculata* Hekking, *R. crenata* S.F. Blake), *Rinorea* s. str. (exemplified by *R. lindeniana* (Tul.) Kuntze, *R. squamata* S.F. Blake, *R. paniculata* (Mart.) Kuntze, *R. dasyadena* A. Robyns, *R. viridifolia* Rusby), and *Goupia glabra* Aubl (Goupiaceae). In particular, I describe anatomical and morphological characters of the stem, node, leaf, androecium (stamens, connective scales, pollen), gynoecium (ovary, style and stigma), and seed anatomy and morphology using scanning electron microscopy (SEM) and light microscopy (LM). In most cases, the species had

not been studied before. The information discovered was placed in the context of the proposed phylogenetic relationships in the Goupiaceae-Violaceae area.

MATERIAL AND METHODS

Field Work

During the summer of 2010 several field trips were made to Central and South America to obtain material; Bolivia (Departamento de la Paz, Provincia Franz Tamayo, Parque Nacional Madidi, Laguna Chalalan. Coordinates 14°25'39.49" S; 67°54'57.68"W; 350 m); Colombia (Antioquia, Municipio de San Luis, Cañon de Rio Claro, Reserva Natural. 05°53'N 074°39'W; 350 m); Costa Rica (Puntarenas, Osa, Sierpe, Reserva Forestal Golfo Dulce, Estación Biológica Los Charcos de Osa. 08°40'18"N 83°30'17"W; 70 m); and Perú (Distrito de Loreto, Provincia de Maynas, carretera Bella Vista-Mazan. 3°31'.419 S. 73°05'.458 W; 86 m.).

Collections

Voucher specimens of the newly collected material were deposited in Costa Rica (INBio), La Paz (LPB), Peru (USM), Colombia (JAUM), and United States (MO). When additional material was needed, I used collections from the Missouri Botanical Garden (MO). No material of *Rinorea oraria* (the third known member of the *R. apiculata* group) was available. For a list of the species and specimens examined, see Table 2.

Anatomical Studies

Stem, leaves, flowers, and fruits from field collections were preserved in 70% ethanol. Leaves from herbarium specimens were used to complete the sampling. Anatomical and morphological characters studied are listed in Table 1.

Leaves from herbarium material were rehydrated. Cross sections of leaves and petioles were made using a razor blade. Microtome sections of seeds were also made. Cresyl violet acetate (CVA) was used for tissue staining and calcium chloride solution (CaCl_2 20%, Ogburn et al. 2009) used as a mountant (Keating 1996, 2000). A Canon Power shot A640 camera coupled to an Olympus BX40 microscope was used to record all microscopic images.

For the Scanning Electron Microscopy (SEM), the material was dehydrated in a series of ethanol concentrations, and subsequently submitted to critical-point drying (EMITECH-K 850). The samples were affixed to supports using carbon adhesive tape and gold-coated (Denton Vacuum Sputter Coater) and examined in a SEM (JEOL Neoscope JCM 5000). The SEM was used to study cuticle and stomata, pollen, fruit, and flower ultrastructure. For the study of pollen the protocol of Halbritter (1997) was used.

Terminology follows Metcalfe (1979) and Mentink and Baas (1992) for leaf anatomy, Wilkinson (1979), and Dilcher (1974) for cuticle, Howard (1979 a,b) for petiole and nodal anatomy, and Baranova (1992) and Dilcher (1974) for stomata.

Table 1. Anatomical and Morphological characters studied

Anatomical	Stem	Arrangement of tissues; nodal anatomy
	Leaf	Epidermis and mesophyll Arrangement of vascular bundles in petiole Stomatal morphology and ornamentation Arrangement of contact cells
	Flower	Petal development and arrangement Stamens Connective scales Pollen morphology Nectary Ovary type and indumentum Style and stigma
	Seed	Anatomy of seed coat Embryo morphology

Table 2. Collections studied under light microscopy (LM) and scanning electron Microscope (SEM) for stem, node, and leaf (SN), floral variation (F), and seed anatomy (S).

Species	Collector Number
<i>Fusispermum laxiflorum</i> Hekking	Hoyos 1119 (SN; F); Harsthorn 2929 (FV);
<i>F. minutiflorum</i> Cuatrec.	K. S. Edwards 754 (SN, F); Gentry 17659 (F.,S); Espina 1330 (S).
<i>F. rubrolignosum</i> Cuatrec.	R. Vasquez 21585 (SN, F); Vasquez 24273 (S);
<i>Rinorea apiculata</i> Hekking	R. Vasquez 11977 (SN); D.N. Smith 13776 (SN); G. Tipaz 706 (SN, F); P. Nunez 6026 (SN); Aulestia 6 (SN); Aulestia 444 (F); Aulestia 382 (F); Espinoza 336 (S); Ceron 5478 (S).
<i>R. crenata</i> S.F. Blake	R. Aguilar 2991 (SN, F); Grayum 10069 (SN, F); B. Hammel 18113 (SN, S); Hoyos 1115 (SN); Burger 10477 (F); Marten 833 (F).
<i>R. lindeniana</i> (Tul.) Kuntze	Hoyos 999 (SN, F); Hoyos 1042 (SN); Hoyos 1009 (SN); Hoyos 1135 (SN); Hoyos 1127 (F); Hoyos 1075 (S); Hoyos 1001(S).
<i>R. viridifolia</i> Rusby	Hoyos 1006 (SN, F); Hoyos 1003 (SN, FV); Hoyos 1004 (SN, S); Hoyos 1090 (S).
<i>R. squamata</i> S.F. Blake	Hoyos 1114 (SN, S); D. Neil 1824 (F); Moreno 26689 (F); Aker 671 (S).
<i>R. dasyadena</i> A. Robyns	Hoyos 1112 (SN, F); Hoyos 1120 (SN, F, S);
<i>R. paniculata</i> (Mart.) Kuntze	Gentry 78660 (SN); B. Hammel 16875 (SN). R. Callejas 3591 (SN, F); Hoyos 1110 (SN, F, S); Renteria 1507 (S).
<i>Goupia glabra</i> Aubl.	Duke 8900 (SN, F); Nervers 7230 (SN); Overbeek 5554 (SN, S); Granville 14257 (SN); Steyermark 113971 (SN); Thomas 3992 (SN); Hammel 21278 (F); Mori 21019 (F); Kawasaki 342 (S); Thomas 3992 (S).

RESULTS

INDUMENTUM

The indumentum on the stem of *F. laxiflorum* and all *Rinorea* species (*R. apiculata*, *R. crenata*, *R. lindeniana*, *R. viridifolia*, *R. squamata*, *R. dasyadena*, and *R. paniculata*) consists of verrucate multicellular hairs (see Fig 22); the stem of *G. glabra* is glabrous. Uniseriate hairs were present on the petiole of *G. glabra*.

YOUNG STEM

All the species studied had lenticels on the stem, the epidermal cork cambium develops early, and is superficial in position in all species.

All species have a band of collenchyma tissue ca. five to ten cells across in the outer part of the cortex. The outgroup *G. glabra* lacked crystals and druses. However, all Violaceae studied had rhombic crystals and druses of calcium oxalate in the collenchyma. No sclereids or other idioblastic cells were observed in the cortex.

The stem vasculature of all the species studied is eustelic. The central vascular cylinder is more or less circular in transverse section, except in *G. glabra*, where it is fluted (Fig. 3-5). The outermost part of the phloem is surrounded completely by a band of pericyclic fibers. In *G. glabra*, *F. laxiflorum*, *R. apiculata*, and *R. crenata* the band of fibers was two to three cells wide (Fig. 4C-D), while in *R. lindeniana*, *R. viridifolia*, *R. squamata*, *R. dasyadena*, and *R. paniculata* (Fig. 5A-D) it was one to two cells wide. Xylem rays were one to two cells wide in all species except in *F. laxiflorum* where they were up to three cells wide.

The pith was well developed in all species, and was usually made up of small, unligified cells more or less similar in size. In *G. glabra* pith cells were ca. 20 - 50 μm across (Fig. 3A); in all the species of *Rinorea* the cells were 15 - 40 μm across; in all species the walls of the pith cells were fairly thin, being ca. 4 - 5 μm across (Fig. 3C-F). On the other hand, in *F. laxiflorum* the pith cells were larger and variable in size, being 50 - 200 μm across (this pith is called "heterogeneous" below); the cell walls were thinner, being ca. 2 - 3 μm across (Fig. 3B).

NODE

Goupia glabra and all species of *Rinorea* had three-trace three-gap nodes (Fig. 6A, 6C-D). However, *Fusispermum laxiflorum* had pentalacunar nodes, traces arising widely spaced from the central cylinder (Fig. 6B). In all the species the stipules were vascularized from the lateral traces.

LEAF

In transverse sections taken at the midpoint of the petiole, there was collenchyma tissue in the periphery of all species. The cells were tanniferous and contained rhombic crystals and druses, except in *G. glabra*, which lacked rhombic crystals, but did have druses.

The main part of the vascular tissue of all the species except *R. apiculata* formed a more or less closed and somewhat dorsiventral-flattened cylinder with phloem on the outside, xylem on the inside and surrounded by fibers. Only in *R. apiculata* was the main petiole bundle arcuate, and even there the edges of the arc were strongly incurved (Fig. 7D). The pith was

usually unligified, but there were dispersed fibers in the pith in *G. glabra* (Fig 7A). All the species had druses in the pith cells, except *G. glabra*. As in the stem, *F. laxiflorum* had much larger and thinner-walled pith cells (Fig. 7B) than those of the other species.

There were rib traces on either side of the main petiole bundle, usually in adaxial-lateral position; the traces consisted of an arcuate band of vascular tissue accompanied by a few fibers, especially adjacent to the phloem. There were six rib traces, three on each side of the main bundle in *G. glabra* (Fig 7A); four rib traces, two on each side of the main bundle in *R. apiculata* (Fig. 7D), *R. crenata* (Fig. 7C), *R. paniculata* (Fig. 7F), and *F. laxiflorum* (these “rib traces” are the large lateral traces joining with the other vascular tissue in the petiole in a complex fashion in the latter. See below). In *R. viridifolia*, *R. dasyadena* (Fig. 7H), *R. squamata* (Fig. 7G) two rib traces were present, one on each side. Only in *R. lindeniana* (Fig. 7E) there were three rib traces, two on one side and one on the other; this odd arrangement was observed in all four specimens examined.

Fusispermum laxiflorum has a rather complex petiole vasculature (Fig. 7B). At the very base of the petiole, immediately after traces had departed to the stipules, the vasculature consisted of a larger central vascular cylinder and two lateral cylinders on either side, the latter representing the lateral traces. The lateral and central bundles merged and reorganized, becoming broadly incurved-arcuate with a pair of adaxial strands of vascular tissue; the incurved margins of the bundle were more or less S-shaped. Finally, towards the top of the petiole, the vasculature assumed the form of a more or less complete

cylinder surrounding a smaller elliptical cylinder of xylem with phloem on the inside; in addition, there were two small lateral wing bundles.

In all the species studied the leaves were dorsiventral, with scattered multicellular hairs on both sides, but principally on the abaxial side; *G. glabra* had scarce uniseriate hairs on the abaxial side only (Fig 22A).

The cuticle was variable. A few wax flakes and granules were present in all species on both abaxial and adaxial sides. In *G. glabra* the cuticle, both adaxial and abaxial, was otherwise smooth while in species of *Fusispermum* there were striations on both surfaces (Fig. 11B). In all species of *Rinorea* examined the cuticle on the abaxial side had striations surrounding the stomata (Fig 11C-D, 12A-D, 13A-B), while the cuticle on the adaxial surface was smooth, lacking any striae.

The epidermis of all species was unligified. In *Goupia glabra* the cells were ca. 25 μm tall; in *F. laxiflorum* ca. 45 μm tall; while in all species of *Rinorea* the epidermis was ca. 25 - 30 μm tall. However, in *R. apiculata* and *R. crenata* there were periclinal divisions of the epidermal cells, the result being a two cell thick epidermis ca. 60 and ca. 45 μm tall respectively (Fig 8C-D). A well defined hypodermis is present only in *F. laxiflorum* where it formed a continuous layer ca. 25 μm tall; the hypodermis was two cells thick near the midrib (Fig. 8B).

In *G. glabra* (Fig. 10A), *R. crenata* (Fig. 10C), and *R. squamata* the anticlinal walls of the abaxial and adaxial epidermis were slightly undulate (Fig. 9A), while in *F. laxiflorum* (Fig. 10B), *R. apiculata* (Fig. 10D) and *R. viridifolia* they were straight (Fig. 10). In *R. lindeniana* (Fig. 10E) and *R. dasyadena* the

anticlinal walls were rounded. The only species with strongly sinuous anticlinal walls was *R. paniculata* (Fig. 10F).

All species studied had hypostomatic leaves and anomocytic stomata, the stomata being ca. 16 - 20 μm long. In all species of *Rinorea* there were usually three epidermal cells in contact with the guard cells, although the contact cells were not otherwise distinguishable from the other epidermal cells (Fig 10C-F). *Goupia glabra* had four to five contact cells, and *F. laxiflorum* three to five contact cells.

Additionally, in all the species a raised cuticular rim forms the outer part of the stomata cavity surrounding the stoma; there were sometimes wax flakes on the surface of the rim (Fig. 12A, 12C). There are short projections of the poles of the cuticular rim only in *R. apiculata* and *R. crenata* (Fig. 11C-D).

There was variation in leaf thickness. For example, *F. laxiflorum* had the thickest leaf measurement, with ca. 270 μm (Fig. 8B), followed by *G. glabra* with ca. 190 μm (Fig. 8A). In the other species of *Rinorea* the leaf thickness was ca. 150-180 μm (Fig. 8C-H). In both *G. glabra* (Fig 8A) and *F. laxiflorum* (Fig 8B) there was well-defined palisade tissue, two layers of palisade in the former, and two to three layers for the latter. However, in all *Rinorea* species palisade tissue was irregular and not so strongly differentiated from the rest of the mesophyll; it was two to four layers across (Fig 8C-H). Spongy mesophyll occurred in all species; there were no sclereids or other idioblastic cells. There was some chlorophyllous tissue on the adaxial side of the midrib in all the species of *Rinorea*, but *F. laxiflorum* and in *G. glabra* lacked it, but otherwise the midrib was transcurrent; smaller veins were embedded.

The vascular tissue in the midrib of all species studied consisted of two arcuate bands of fibers surrounding the vascular tissue. In *G. glabra* (Fig. 9A) and *F. laxiflorum* (Fig. 9B) there were also fibers in the medullary tissue of the bundle, and in *F. laxiflorum*, this tissue was clearly made up of thin wall cells like those of the stem pith. In *F. laxiflorum* there were two additional bands of vascular tissue in the midrib that were continuous with the elliptical central cylinder in the petiole. The medullary tissue of all *Rinorea* species consisted of parenchymatous cells and lacked any lignified tissue.

ANDROECIUM

In all the species studied the filament was glabrous and varied in length. In *G. glabra* had filaments ca. 200 μm and anther thecae ca. 260 μm long, while in *F. laxiflorum* the filaments were ca. 500 μm long and anther thecae ca. 240 μm long.

In the *Rinorea* species examined the filaments ranged from ca. 300 - 950 μm long, while the anther thecae were rather longer than on *G. glabra* and *F. laxiflorum*, ranging from ca. 530 - 1100 μm long. *G. glabra* was the only species to have trapezoid anthers (Fig. 14A); all the other species had ellipsoid to ovoid anthers. In all species dehiscence was introrse via longitudinal slits in each thecae. Only *R. apiculata* (Fig. 14C) and *R. crenata* (Fig. 14D) had flattened hairs on both the adaxial and abaxial surfaces of the thecae; in *R. squamata* (Fig. 15D) there were scarce adaxial cylindrical hairs on thecae. The other species had glabrous anthers.

In *G. glabra*, the connective forms a thick blunt, prolongation beyond the thecae ca. 90 μm long with cylindrical hairs approximately 230 μm long scattered both on the connective and thecae. After anthesis the hairs point to the center of the flowers as the apical connective bends inwards (Fig. 14A), at least in herbarium specimens. In *F. laxiflorum* the prolongation of the connective is less massive, ca. 80 μm long, and irregularly fringed apically (Fig. 14B). On the other hand, in *R. apiculata* (Fig. 14C) and *R. crenata* (Fig. 14D) the connective forms a broad apical prolongation with a fimbriate margin and exceeding the thecae in length, being ca. 930-1100 μm long. In the other species of *Rinorea* the thecae are not even evident from the dorsal side, the connective forms a slightly fringed acute to triangular projection ca. 1300-2000 μm long (Fig. 15A-D).

In *G. glabra* and *F. laxiflorum* a disc-like structure surrounds the stamens and is quite separate from the anthers; it bears stomata. In *G. glabra*, the disc completely surrounds the flower, and is lobed (Fig 18A). The lobes alternating with the staminal radii. In *F. laxiflorum* the massive disc consists of five lobes alternating with the stamens (Fig. 18C). All species of *Rinorea* have an abaxial gland-like structure at the base of the filaments; these often have hairs in *R. lindeniana*, *R. viridifolia*, and *R. paniculata*.

In both *R. apiculata* (Fig. 19A) and *R. crenata* (Fig. 18D) the gland is massive being ca. 190 μm long, hemispherical and joined at the very base with the adjacent glands. In the other species of *Rinorea* glands are completely separated from each other and can be interpreted as being more or less adnate to the filament. In all the species studied there were stomata on the

disc and glands consistent with their being nectariferous. The gland had different measurements depending on the species.

All the species studied, *G. glabra* (Fig. 16A), *F. laxiflorum* (Fig. 16B), *R. apiculata* (Fig. 16C), *R. crenata* (Fig. 17A), *R. paniculata* (Fig. 17B), *R. viridifolia* (Fig. 17D), and *R. squamata* (Fig. 17C), have tricolporate pollen grains. In *G. glabra* and *F. laxiflorum* the pollen grains are spherical in polar view and ca. 10 x 10 μm (l x w) and ca. 8 x 8 μm respectively (Fig 16A, B). In the species of *Rinorea* they are triangular and rather larger, 10 x 15-20 μm (l x w) (Fig 16C, 17A, C). Only in *G. glabra* is the exine reticulate, all other species have a polyporate exine. All species had pollenkit.

GYNOECIUM

Goupia glabra has a glabrous gynoecium of five carpels with five styles born towards the margin of the carpels. Each style, only ca 200 μm long, has an adaxial furrow and tapers somewhat towards a perhaps hollow but otherwise undistinguished apex. *Goupia glabra* has axillary placentation and many ovules per carpel.

All the species of Violaceae have three fused carpels with parietal placentation. There are many ovules per carpel in *F. laxiflorum*, but only one ovule per carpel in all species of *Rinorea*. In *F. laxiflorum*, *R. apiculata*, and *R. crenata* the ovary was glabrous (Fig. 18, 19), but in the other species of *Rinorea* it was pubescent (Fig. 18, 19). Styles of all species are continuous with the ovary. In *F. laxiflorum*, the straight style is ca 1000 μm long and narrows to the apex, which has an inconspicuous depression (Fig. 18C),

whilen *R. apiculata* (Fig 18A) and *R. crenata* (Fig. 18D) the styles flares conspicuously at the apex. *Rinorea viridifolia* (Fig. 19B) and *R. squamata* (Fig. 19C) have straight styles and stigmas rather like those of *F. laxiflorum* (Fig. 18C). *Rinorea lindeniana* (Fig. 18B) and *R. paniculata* (Fig. 19D) have sigmoid-curved styles, but in *R. lindeniana* (Fig. 18B) subapically swollen, but in both species the stigma apex is like that of the preceding species of *Rinorea*. Different sizes of styles of the species studied are given in table 3.

Table 3. Styles length in Violaceae and Goupiaceae.

Species	Style length (µm)
<i>Goupia glabra</i>	200
<i>Fusispermum laxiflorum</i>	1000
<i>Rinorea apiculata</i>	2100
<i>Rinorea crenata</i>	1600
<i>Rinorea lindeniana</i>	1100
<i>Rinorea viridifolia</i>	2300
<i>Rinorea squamata</i>	1500
<i>Rinorea paniculata</i>	1000

FLOWER DEVELOPMENT

Floral development was studied for *F. laxiflorum*, *R. lindeniana*, and *R. dasyadena*. Suitable material for remaining species was lacking.

In *F. laxiflorum* (Fig. 20A), *R. lindeniana* (Fig. 21A), and *R. dasyadena* (Fig. 20B) floral development was oblique with zygomorphic symmetry in early development. Sepal initiation in *F. laxiflorum* and *R. lindeniana* begins with the

first abaxial and adaxial sepals, which are larger, followed by the next three smaller sepals, with an oblique development. In *R. dasyadena* initiation and develop of sepals was not observed. In all the species, hairs occur on the sepal apex. Petals develop simultaneously after the sepals, with oblique orientation. Stamens develop later, followed by the gynoecium.

SEED

Goupia glabra has a dark brown ovate seeds ca. 2 - 3 mm long, with the anticlinal walls of the exotesta forming a reticulum (Fig. 24F). In transverse section the exotesta is two, perhaps three layers across ca. 60 - 80 μm tall; the outer periclinal walls of the exotesta are slightly thickened. There is also a single layer of exotegmic sclereids, the cells being ca. 60 x 100 μm long and across, massively thickened on the anticlinal and inner periclinal walls, and pitted. The cells seem to be isodiametric. The embryo occupies half the length of the seed, and copious oily endosperm surrounds the embryo (Fig. 24C).

Fusispermum minutiflorum has pale brown elongated seeds ca. 2 - 3 mm long (Fig. 23A). The seed appears to have a wing down one side where the seed coat is much thicker and ca. 11 - 16 cells. The exotesta has elongated cells ca. 55 x 15 x 20 μm (l x w x h), all walls being more or less equally thickened and ca. 2 μm across. There is also a single layer of exotegmic sclereids, cells being ca. 100 x 10 μm long and across, massively thickened on the anticlinal and periclinal walls, and pitted. The straight embryo occupies 3/5 the length of the seed, with oily endosperm surrounding the embryo (Fig. 23B).

Rinorea apiculata (Fig. 25A-F) and *R. crenata* (Fig. 26A-E) have light brown ovate seeds ca. 4 - 5 mm long. In transverse section, there is an outer unlignified layer of more or less isodiametric cells three to four layers thick ca. 40 x 25 μm (l x w). There is a single layer of cells that seems to be a palisade of exotegmen with ca. 70 x 10 μm (h x w). This layer of more or less pitted fibrous cells is equally thickened all the way around. The straight embryo occupies 4/5 the length of the seed and is surrounded by copious oily endosperm surrounding the embryo (Fig. 25B, 26B-C).

Rinorea squamata (Fig. 28A), *R. viridifolia* (Fig. 27C), and *R. dasyadena* (Fig. 28A) have brown globose seeds ca. 6 - 9 mm across. There is an outer layer of more or less eight to ten layers of unlignified flattened cells thick in transverse section with ca. 100 x 20 μm (l x w). The exotegmen layer is about four to six cells thick ca. 70 μm across. It is made up of cells with walls that are more or less pitted and equally thickened walls (ca. 60 – 100 μm across) all the way around; the cells themselves are elongated and ca. 160 x 15 μm long and across. The embryo occupies 4/5 the length of the seed, and the cotyledons have several sinuous foldings (Fig. 27D, 28E). Copious oily endosperm surrounds the embryo.

Goupia glabra lacks any crystals in the seed coat, and only in *R. apiculata* and *R. crenata* rhombic crystals were present in the testa (Fig. 26E). All *Rinorea* species and *F. minutiflorum* have druses in the testa.

DISCUSSION

Table 4. Characters and character states used for understanding relationships between *Goupia glabra*, *Fusispermum spp*, *Rinorea apiculata*, *R. crenata*, *R. lindeniana*, *R. viridifolia*, *R. dasyadena*, *R. squamata*, *R. paniculata* and the rest of Violaceae.

	CHARACTERS	STATES
1	Node anatomy	Trilacunar (0)
		Pentalacunar (1)
2	Epidermal thickness	One-celled (0)
		Two-celled (1)
3	Stomata rim	With poles (0)
		Without poles (1)
4	Flowers	Actinomorphic (0)
		Zygomorphic (1)
5	Placentation	Axile (0)
		Parietal (1)
6	Disc-like structure	Present (0)
		Absent (1)
7	Disc-like structure lobes	Alternating with anthers (0)
		Opposite to the anthers (1)
8	Hairs on thecae	Present (0)
		Absent (1)
9	Type of hairs on thecae	Flat (0)
		Cylindrical (1)

10	Connective scale	Apical (0)
		Basal (1)
11	Abaxial gland	Present (0)
		Absent (1)
12	Pollen grains	Round (0)
		Triangular (1)
13	Carpel + Styles	Five + five (0)
		Three + one (1)
14	Ovary ornamentation	Glabrous (0)
		Pubescent (1)
15	Styles	Straight (0)
		Curved (1)
16	Stigma	Acute (0)
		Truncate (1)
17	Seed	Ovate (0)
		Filiform (1)
		Globose (2)
18	Exotegmen	Fibers (0)
		Sclereids (1)
19	Embryo-cotyledons	Straight (0)
		Folded (1)

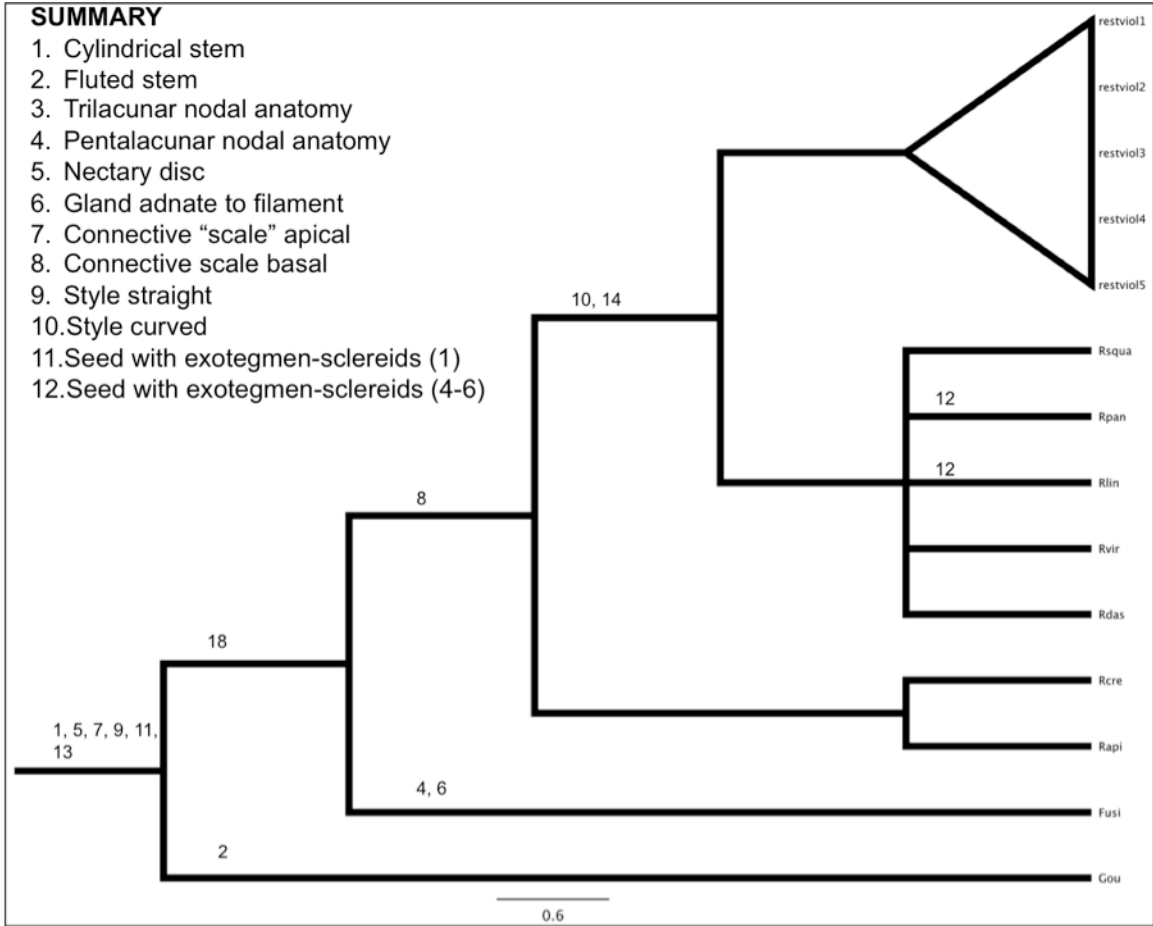


Fig. 2 Summary tree of *Violaceae* and *Goupiaceae* displaying characters useful for understanding relationships between taxa.

The results of this study suggest that morphological and anatomical characters are useful for understanding the relationships among neotropical Goupiaceae and the basalmost genera of Violaceae (i.e. *Fusispermum* and the *Rinorea apiculata* and R. groups). Some characters are useful in understanding relationships between these taxa (see also Table 4) and by displaying their distribution on a summary tree of Violaceae and Goupiaceae (Fig. 2), derived from that of Walhert and Ballard (2010 in press). I integrate my observations with the sparse previous literature on the group (see especially Metcalfe & Chalk 1972 for anatomy; Corner 1976 for seeds) and provide evidence of relationship between taxa.

Indeed, in general there has been little study on the morphology and anatomy of these groups, and my findings will be useful for understanding the evolution of Violaceae. Understanding the variation in stem, node, petiole, leaf, flower, and seeds will contribute in the interpretation of the relationships between them.

INDUMENTUM

Metcalfe and Chalk (1972) noted that the hairs of Violaceae were simple and uniseriate. However my results show that for *F. laxiflorum* and *Rinorea* species studied hairs are multicellular. I also found that that the surface of these hairs is verrucate, and that this is also true for Goupiaceae. Although, *Goupia glabra* has only a few hairs on the lamina and petiole, while in the other species studied there were hairs on the stem too.

YOUNG STEM

Although there is variation among anatomical characters in the stem for the species studied, all *Rinorea* species are quite similar. The pith of the species of *Fusispermum* and *Rinorea* examined is circular in transverse section, although that of *G. glabra* is fluted, an apparently trivial difference which is nevertheless constant (see also Metcalfe & Chalk 1972). Although little is known about pith shape in Achariaceae, it is apparently circular there, too (Metcalfe & Chalk 1972), so the pith of *Goupia* may be an apomorphy. There is variation in the cells that make up the pith. In *F. laxiflorum* the pith is heterogeneous, the cells being of differing sizes and having thin walls (such cells are also found in the medullary position in both the petiole and midrib). However, all *Rinorea* species and *G. glabra* have an homogeneous pith, with all cells being of similar size and having thickened walls. The pith of *Fusispermum* is unique, and potentially an apomorphy for the genus. Metcalfe & Chalk (1972) mentioned for Violaceae, “the pith is solid in woody species, but frequently becomes hollow in herbs” (p. 103), however, neither they nor other authors have mentioned anything about heterogeneous pith in the family. The pith of *Fusispermum* is potentially an apomorphy for the genus.

The width of the xylem rays showed little variation. *Goupia glabra* had rays of one and occasionally two cells wide; note that Metcalfe and Chalk (1972) found that the rays there were two to five cells across. In the other species examined here the rays were 2-3 cells across. *Gloeospermum sprucei* and *Hymenanchera dentata* var. *alpina* have uniseriate or only partially

biseriate, while rays are absent for *Viola* species (Metcalfe & Chalk 1972), perhaps correlated with their herbaceous habit.

Metcalfe and Chalk (1972) and Solereder (1908) reported that a band of pericyclic sclerenchymatous fibers is frequently present in Violaceae (they recorded it in *Rinorea*, *Amphirox*, *Hybanthus*, *Hymenanchera*, *Melicytus*, and *Paypayrola*), and this was found in all species studied here. Although some species of *Viola* lack this band (Metcalfe & Chalk 1972), this may be another feature connected with the habit of the genus; thus Howard (1974) noted that in general sclerenchyma tissue was lacking in herbaceous plants. In the plants studies here, the fibrous sheath varied between one and three cells thick, but the thickness could well have been affected by the vigor of the shoots studied. In all Violaceae species studied, there were rhombic crystals and druses in cells surrounding the pericyclic fibers, however, these were absent in *G. glabra*.

NODE

Sinnott (1914) early reported a three-trace, three-gap nodal anatomy for Violaceae, Passifloraceae, and Flacourtiaceae, and this is found throughout Malpighiales (the main exception is the Bonnetiaceae-Clusiaceae clade – see Stevens 2001). All species studied here had trilacunar nodes, with the exception of *Fusispermum*, which had pentalacunar nodes. In the context of Malpighiales, this is likely to be an apomorphy.

Goupia glabra was considered to belong to Celastraceae by some authors based on gross morphology (Cronquist 1981). However, using molecular markers, Simmons et al. (2001) demonstrated that this taxon was a

member of Malpighiales. The nodes of Celastraceae are overwhelmingly unilacunar (Sinnott 1914, Cronquist 1981). The three-trace, three gap nodes I found for *G. glabra* are more consistent with its new position, the more so since other Celastraceae with other than unilacunar nodes (e.g. *Perrottetia*, *Bhesa*) have also been removed from Celastraceae to clades where these nodes are common (Stevens 2001).

LEAF

The petiole anatomy of *G. glabra*, *F. laxiflorum*, and *Rinorea* species showed considerable differences that may be useful in distinguishing between genera, and in some cases between species, and also may be of evolutionary interest. Note that the vascular anatomy of stem, petiole and midrib cannot be considered in isolation, a point emphasized by Howard (1974, 1979a, 1979b).

The most conspicuous gross variation in the species studied in midrib anatomy is in the number of adaxial-lateral bundles and the configuration of the vascular tissue in main central bundle; this can best be understood by making sections along the length of the petiole. Although *Goupia glabra* and *F. laxiflorum* have medullary plates of vascular tissue in the central bundle, they are different in composition – adaxial xylem and abaxial phloem in the former (see Metcalfe & Chalk 1972), xylem entirely surrounding phloem in the latter - and develop in different ways along the length of the petiole. *Rinorea apiculata* has an open central bundle, while that in the other species examined is annular. Variation in the number of rib bundles is also interesting, in

particular the asymmetry of those of *R. lindeniana* (two on one side, one on the other).

Unfortunately, this extensive variation cannot be optimized on the summary tree used here. However, the structure of the petiole bundle (and associated nodal anatomy) throughout the family needs examination. In the herbaceous *Viola* Metcalfe and Chalk (1972) reported an arcuate central vascular strand accompanied by one or two rib strands on each side. Petiole anatomy may well be of systematic interest, and there are possible correlations with leaf insertion. Thus *F. laxiflorum*, *Rinorea apiculata*, *R. crenata*, and *R. paniculata*, all with distichous leaves, have two pairs of rib bundles. It will be interesting to see if other *Rinorea* s. str. species with alternate leaves, i.e. Subgroup II a of Hekking (1988) are similar. *Rinorea lindeniana*, with its two rib bundles on one side and one on the other, has a very strongly asymmetric lamina base, perhaps connected with this odd number of vascular bundles. *Rinorea viridifolia*, *R. dasyadena*, and *R. squamata*, all with opposite leaves and symmetric lamina bases, have a single rib bundle on either side. Note that the rib bundles of *F. laxiflorum* are not in the same position as those of *R. apiculata*, *R. crenata*, and *R. paniculata*, perhaps connected with their origin.

All Violaceae species studied here had calcium oxalate crystals (i.e. rhombic crystals and druses) in the collenchyma, (see also Metcalfe & Chalk 1972). Only *G. glabra* lacked rhombic crystals in the collenchyma tissue.

The thickness of the lamina of the species studied varies: the thickest leaves, ca. 270 μm across, occur in *F. laxiflorum*, the thinnest, ca. 140 μm , in

R. viridifolia with. The thickness of the mesophyll and the anatomy has been correlated with the environment (Dickinson & Weitzman 1996, Alvarenga and Lombardi 2010). Interestingly, *F. laxiflorum* can reach the canopy being as tall as 20 m or more, while the *Rinorea* species studied are understory species from 2 to 10 m tall and, never reaching the canopy. The cuticles on the abaxial side of the lamina of all the Violaceae species studied were striate. (see also Metcalfe & Chalk 1972); the striations were normally restricted to near the stomata on the abaxial surface, only in *Fusipermum* was the whole adaxial surface also striate. The presence of wax plates and granules in all species studied here agrees with the findings of Metcalfe and Chalk (1972) and is phylogenetically uninformative.

Variation in many epidermal features are connected with environment. Thus epidermal cells with straight anticlinal walls are common in xeromorphic plants, those with undulate anticlinal walls are more common in mesomorphic plants or plants from the understory (Stace 1965; Fahn 1990). Such a correlation is difficult to see in the few species examined here; the extensive variation in epidermal cells shape examined may, however, be of systematic significance at lower levels and also of ecological interest.

Metcalfe and Chalk (1972) recorded two types of stomata, anisocytic and paracytic, from Violaceae. The anomocytic stomata found for the species studied here are likely to represent the plesiomorphic condition for Malpighiales; the anisocytic and paracytic stomata are likely to be derived. The stomata rim projections at the poles of *R. apiculata* and *R. crenata* is tentatively considered to represent a derived condition.

A hypodermis has not been recorded in Violaceae before (Metcalf & Chalk 1972); its distribution tends to be fairly sporadic within higher groups. However, it is interesting that both species of the *R. apiculata* group studied had a divided epidermis and *Fusispermum laxiflorum* had a hypodermis. These are tentatively considered to be apomorphies.

The presence of palisade parenchyma differentiates *G. glabra* and *F. laxiflorum*, which both have well-developed palisade tissue. The *Rinorea* species studied, have a poorly organized palisade tissue. But this variation may also be connected with ecology and the environment – *Goupia* and *Fusispermum* are trees, while the *Rinorea* I collected were smaller plants (however, *R. apiculata* can grow to 30 m tall – Hekking 1988), and palisade tissue tends to be better developed in well insolated conditions (Dickinson 1996, Alvarenga & Lombardi 2010). Metcalfe and Chalk (1972) mentioned that some species of *Viola* have no clear defined palisade tissue, but the genus is herbaceous, and a number of its species grow in somewhat shaded conditions. For all the species studied, spongy mesophyll is present on the abaxial side of the lamina.

The apparent glandular tissue found in *Fusispermum* species on the adaxial epidermis was not found in the other species studied. Solereder (1908) noted that many Violaceae had a mucilaginous epidermis, this being true of their inner periclinal epidermal walls in particular. Unfortunately, the material at hand did not allow the nature of these cells in *F. laxiflorum* to be clearly understood.

The structure of the midrib largely mirrored that of the petiole. However, in *Rinorea apiculata*, with its incurved-arcuate main petiole bundle, had a fully bi-layered midrib, like the other species of *Rinorea s. str.* examined, while in *Goupia glabra* the midrib was also bi-layered, most evidence of the medullary plate being lost except for the presence of a few fibers.

ANDROECIUM

The stamens of many Violaceae are notable for the nectar glands abaxially placed on some filaments and the complex, flattened connective (Arnal 1945). *Rinorea* species have glands adnate to the base of the filament that can be fused or free; variation is taxonomically important. Additionally, the anthers have an appendage or connective scale. Results presented here are consistent with previous studies (Hekking 1988).

The anther connective is stout and rounded in *Goupia glabra*, while flattened and relatively thin in the rest. *Fusispermum laxiflorum* has a very short, erose connective ca. 80 μm long, while the longest connective occurs in *R. viridifolia* (ca. 2 mm long). In the *Rinorea apiculata* group the anther thecae are visible in an adaxial view, in the other species they are invisible. Assuming that Goupiaceae are sister to Violaceae, the observations suggest an increasing elaboration of the connective in basal Violaceae, even if polarization of the variation is difficult.

Particular flattened hairs are only present in the anthers of *R. apiculata* and *R. crenata*; on the other hand, *R. squamata* and *G. glabra* anthers have cylindrical hairs.

Both *Goupia glabra* and *Fusispermum laxiflorum* have rather massive disc surrounding the staminal whorl and rather similar to the nectaries of other members of the parietal-placentation group. The nectary lobes alternate with the filaments. In the species of *Rinorea* examined (and commonly in the rest of the family) the nectary is more specifically associated with the abaxial part of the filament. The fat, hemispherical disc of the *Rinorea apiculata* group is morphologically similar to the thick disc of *Fusispermum* and *Goupia*, while in the other species of *Rinorea s. str.* the nectariferous tissue is more closely associated with the filament and not a disc-like structure.

Two characters, nectary lobes alternating with/opposite to filaments, and “nectary disc-like/part of filament” are included on the tree (see Fig. 2). However, more detailed comparative studies on the stamens/nectaries in Violaceae are likely to disclose more phylogenetically interesting information. Details of anther indumentum (for instance, the long hairs on the connective of *Goupia*) and connective margin and apex are all of interest here.

Pollen grains of *G. glabra* and *F. laxiflorum* are spherical, as seen from a polar view, however, all the species of *Rinorea* are triangular. The pollen of those few Violaceae examined is tricolporate (Erdtman 1952; Perveen & Qaiser 2009); as perhaps might be expected, my observations suggest that this is the condition for the whole family. Pollen grains of Goupiceae are also reported as being tricolporate (Lobreau-Callen 1977), this is confirmed.

GYNOECIUM

Variation in the gynoecium is of considerable interest. The axile placentation and basal ovules of *Goupia* (see e.g. Miers 1862) are unique in the parietal-placentation group and are likely to be apomorphies, as are the 5-carpellate gynoecium with marginal styles. However, details of the stigmatic area of *Goupia* and Violaceae are similar: the stigmatic area is not expanded and there is a central depression/pore in all. Such a stigma is also found in the *Rinorea* s. str studied, however, in the *Rinorea apiculata* group the apex of the style flares outwards – a likely apomorphy. Most of the Violaceae studied had straight styles, but in *R. lindeniana* the style is curved at the base, and in *R. paniculata* the style is sigmoid at the base. For some other genera the styles are also straight (i.e. *Hybanthus*, *Amphirox*, *Paypayrola*, *Gloeospermum*) (Steyermark *et al.* 2005), in *Rinoreocarpus* style is straight to slightly curved (Hekking 1988).

Of the species studied, only *Goupia* and *Fusispermum* have more than a single ovule per carpel, probably the plesiomorphic condition for Violaceae as a whole, the *Rinorea* examined having but one ovule per carpel. However, there is considerable variation in ovule number in the rest of Violaceae.

FLOWER DEVELOPMENT

This is the first time flower development of *Fusispermum* and *Rinorea* species is mentioned. Feng (2005) studied floral development on the genera *Paypayrola*, *Hekkingia*, *Amphirox*, *Leonia*, *Gloeospermum*, *Agatea*, *Corynostylis*, and *Anchietea*. *Gloeospermum* has “approximately

actinomorphic to very weakly zygomorphic flowers” (Feng 2005, pag. 210). In *Leonia*, the flowers are actinomorphic in early stages of development (Feng 2005). However, nothing about early zygomorphy has been mentioned before for *Fusispermum* and *Rinorea* species.

Based on early work by Arnal (1945) and Hekking (1988), it has been thought that the flowers of *Rinorea* and *Fusispermum* species were actinomorphic. Although, recent studies of floral development have revealed zygomorphic corollas in mid development in *Rinorea*, with “actinomorphy” achieved late in the ontogeny (Feng 2005), it was thought that the family may have a zygomorphic, rather than actinomorphic, most recent common ancestor (Feng and Ballard 2005). In this study, using early developing flower buds of *F. laxiflorum*, the basal most genus of Violaceae and two species of *Rinorea* (i.e. *R. lindeniana* and *R. dasyadena*), I confirm the zygomorphic flowers with oblique orientation as being an synapomorphy for Violaceae.

SEED

There is little comparative information on the seed morphology and anatomy of the Violaceae, even though Corner (1976) described important details of the seed anatomy of a few species, and despite the often repeated statements of the value of seed characters in systematics (e.g. Bartlott 1981, 1984; Tobe et al. 1988; Heiss et al. 2011). When studying seed coat anatomy, ideally one should look at developmental stages so that one can follow the development of particular parts of the integuments into particular parts of the seed coat (e.g. Corner 1976); unfortunately, such developmental series could not be carried out in this study. For instance, Corner (1976) reports for

Violaceae seeds with multilayer exotesta, having crystals in the endotesta and with multiple layers of exotegmic fibers. I confirm these observations, and provide information for *F. minutiflorum*, *R. apiculata*, *R. crenata*, *R. dasyadena*, *R. squamata* and *R. viridifolia*.

Although seed morphology has recently been used in Violaceae to distinguish genera. For instance, Souza & Souza (2003) described a new genus, *Hybanthiopsis* based on gross seed morphology, unfortunately, they did not look at seed anatomy. This new genus identical to *Hybanthus* in floral structure.

The size and shape of the seeds varies for the species studied. For instance, *G. glabra*, *R. apiculata* and *R. crenata* had ovate seeds, while the other species of *Rinorea* had globose seeds; the embryo and the endosperm completely occupied the seed. However, *F. minutiflorum* has filiform seeds with rather defined longitudinal ridges or wings, the embryo occupying about half the length of the seed. This very different seed type can be considered an apomorphy; it is linked to differences in seed coat anatomy.

In all taxa, there was a more or less well developed layer of lignified cells; all walls of these cells were more or less evenly thickened, and there were very fine plasmodesmata. The seeds of *F. minutiflorum* differed from those of all the other species examined in having a coat with a wing 11-16 cell layers across. There was also a single layer of rather weakly lignified and elongated sclereids. The coat of *G. glabra* was at least two to three cell layers across; the exotesta was well developed, but unthickened, and there was a single layer of massively cubical cells, presumably exotegmic (but see below).

In the *Rinorea apiculata* group the coat was three to four cell layers across, and the sclerified layer consisted of a single layer of obliquely overlapping cells. The other species of *Rinorea s. str.* examined had a coat eight to ten cell layers across, of which 4-6 layers were lignified, the cells being elongated and flat-lying.

Recent work by Plisko (1992), Melikian and Sarinov (2000) helps to put this variation in phylogenetic context. A single layer of sclerified cells occurs in all Violaceae studied, including the berry-fruited *Leonia*. However, this layer is absent in flat seeds with a broad, membranous wing. Corner (1976) found this layer in the species of *Viola* and Malesian *Rinorea* he examined. In the drawing by Melikian and Sarinov (2000) of the seed coat of *Goupia glabra*, the sclereids are clearly shown as being mesotestal. However, although the material examined here was rather limited, there was clearly more than one layer of cells above the sclerified layer suggesting that this layer is exotegmic. This layer was also definitely longitudinally ridged, another feature not noted by Melikian and Sarinov (2000).

Some kind of exotegmen made up of more or less fibrous cells is common in Malpighiales (e.g. Corner 1976; Stevens 2001) – which is why Plisko's (1992) report is particularly remarkable. Details of cell elongation and degree – and plane – of flattening vary, and need to be integrated with phylogeny across the clade. However, despite our still limited knowledge of seed coats, the 4 - 5 layers of fibrous cells found in the species of *Rinorea s. str.* studied provisionally can be considered unique.

Only in *Rinorea apiculata* and *R. crenata* were rhombic crystals in the exotesta, while in *F. minutiflorum*, and all *Rinorea* species, there were druses in the endotesta. *G. glabra* was the only taxa to lack any type of crystals in the seeds. Again, this variation is systematically suggestive and should be extended.

All taxa studied have an embryo about the length of the seed that is embedded in endosperm. The embryo in *G. glabra*, *F. minutiflorum*, and the *R. apiculata* was straight, with flat cotyledons, a condition considered to be normal for the family (e.g. Plisko 1992). However, in the other *Rinorea s. str.* species the cotyledons were folded in a rather complex fashion. Vogel (1980) highlighted that *Rinorea* species had a seedling type I (Macaranga), where cotyledons, in most cases were more or less folded in the seed.

CONCLUSIONS

Overall, my results show the value of anatomical studies when related to phylogenetic work. Nodal anatomy is a good example. The trilacunar nodes of *Goupia glabra* that I found are consistent with the movement of *Goupia* from Celastraceae to Malpighiales – although of course not restricted to Malpighiales. Furthermore, the pentalacunar nodes of *Fusispermum laxiflorum* are uncommon in Malpighiales, this is a potential apomorphy for the genus, and here nodal anatomy is connected with a distinctive petiole anatomy. Variation in the androecium and nectary in the Violaceae and Goupiaceae examined clarified the relationships of the distinctive androecium so common in other Violaceae with more conventional structures found in many other members of the parietal placentation group. And although many features of the gynoecium are quite different from the parietal classification group – which is why before the use of molecular data, the two had never been associated – in stigma, androecium, and basic anatomy they agree quite well. Without such knowledge we cannot hope to understand evolution and diversification, not only of Violaceae, but of the Malpighiales as a whole, such an important component of the small trees that are so abundant in tropical rainforests (Davis et al. 2005).

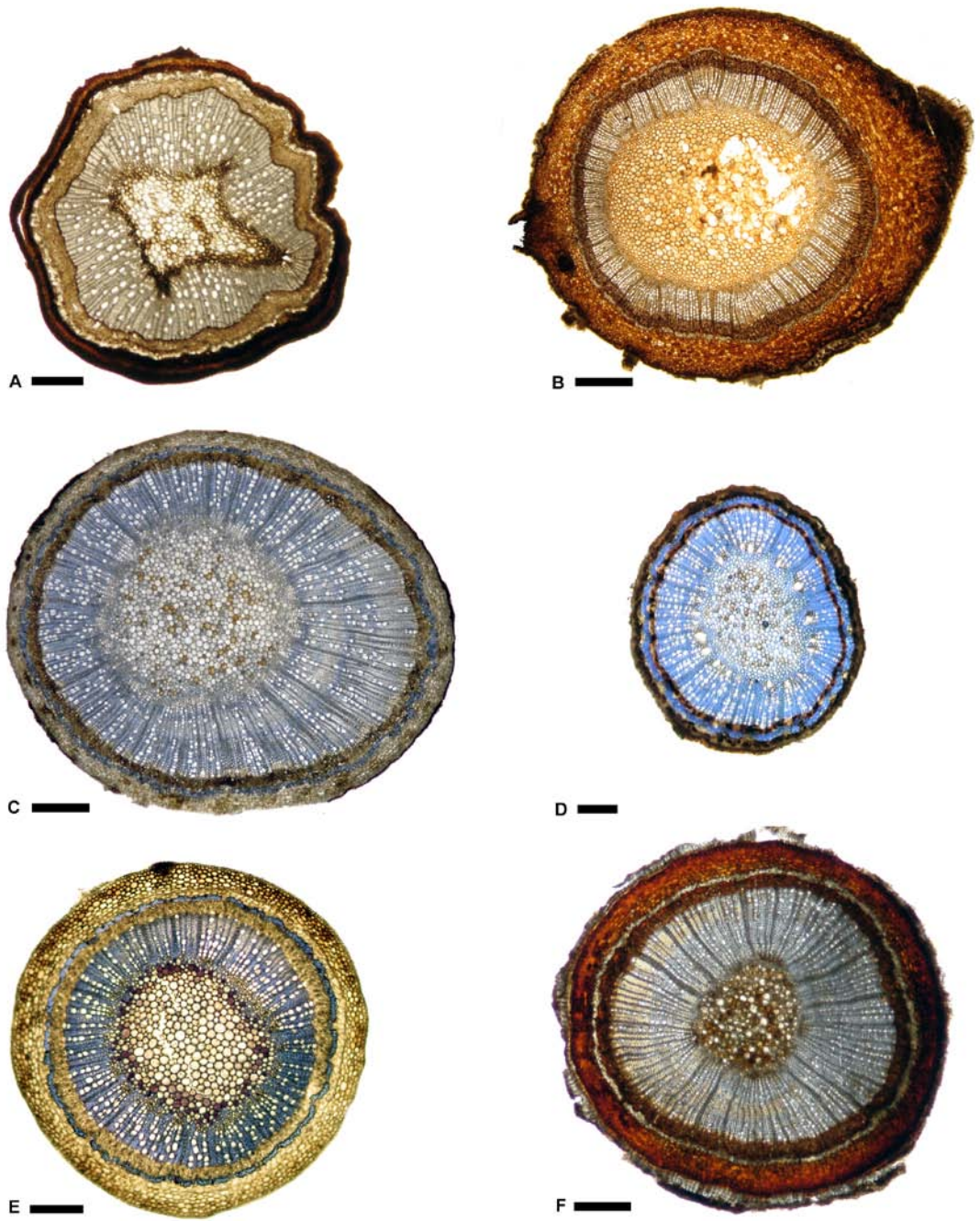


Figure 3. Light microscope images of transverse sections of stem. **A**, *G. glabra* (3992). **B**, *F. laxiflorum* (Hoyos 1119). **C**, *R. crenata* (Hoyos 1115). **D**, *R. apiculata* (Núñez 6026). **E**, *R. paniculata* (Hoyos 1110). **F**, *R. lindeniana* (Hoyos 1135). Scale bars: A,B=500 μ m; C,E,F=350 μ m; D=250 μ m.

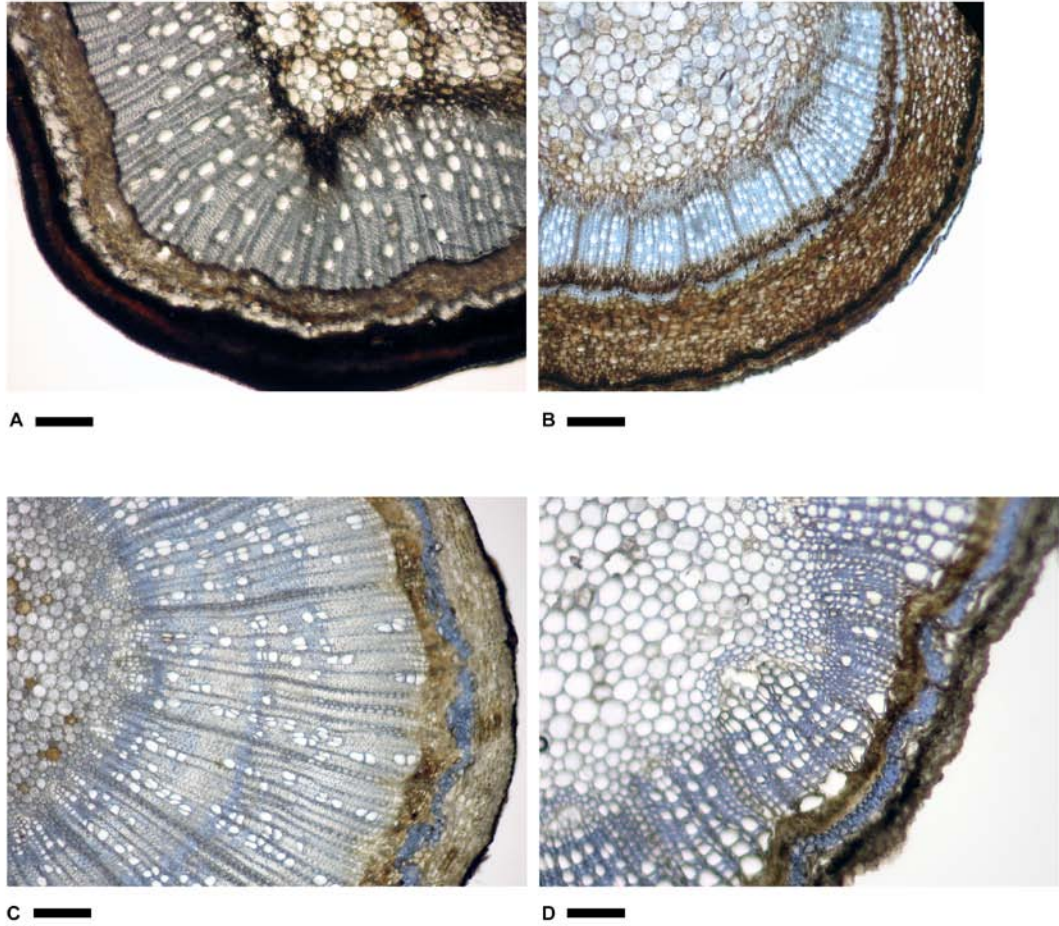


Figure 4. Light microscope images of transverse sections of stem. **A,** *G. glabra* (3992). **B,** *F. laxiflorum* (Hoyos 1119). **C,** *R. crenata* (Hoyos1115). **D,** *R. apiculata* (Núñez 6026). Scale bars = 50 µm.

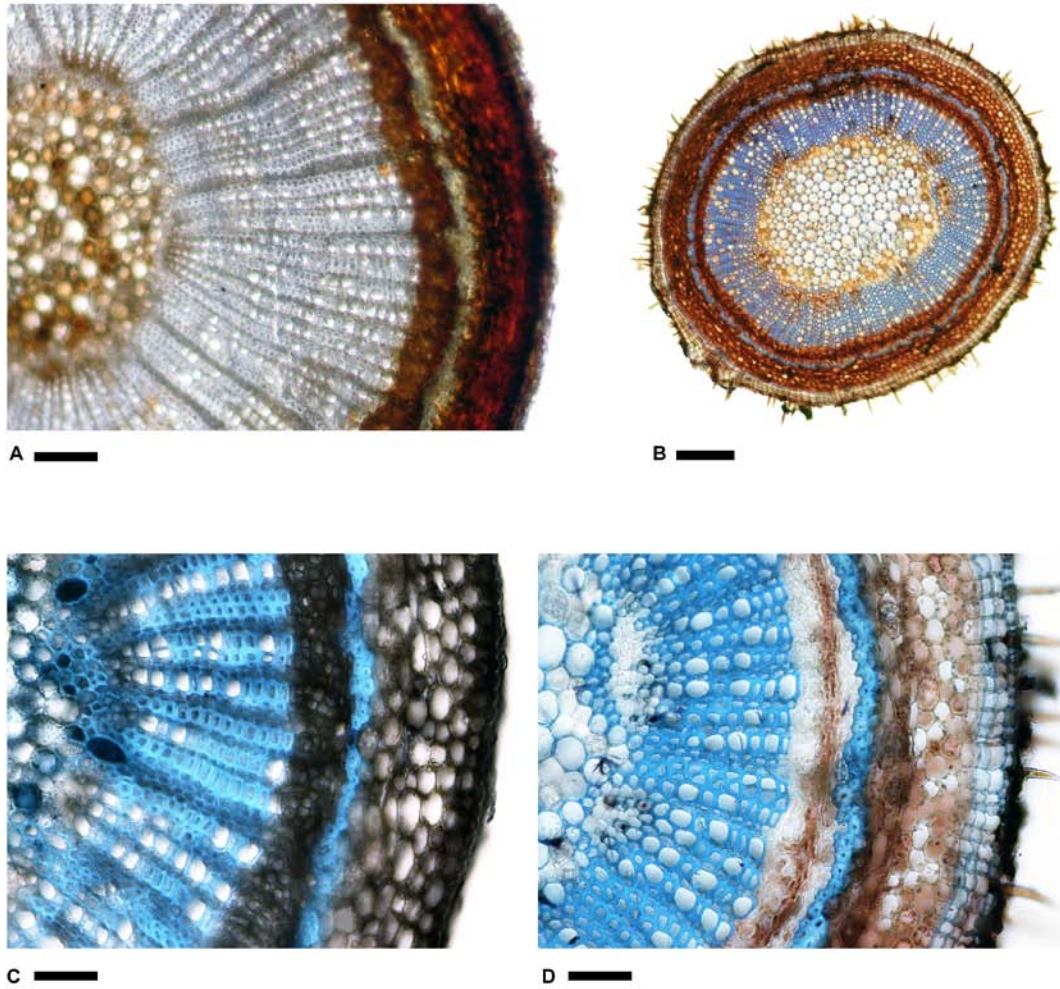


Figure 5. Light microscope images of transverse sections of stem. **A**, *R. lindeniana* (Hoyos 1135). **B**, *R. squamata* (Hoyos 1114). **C**, *R. paniculata* (Hoyos 1110). **D**, *R. squamata* (Hoyos 1114). Scale bars: A,C,D = 50 µm; B = 200 µm.

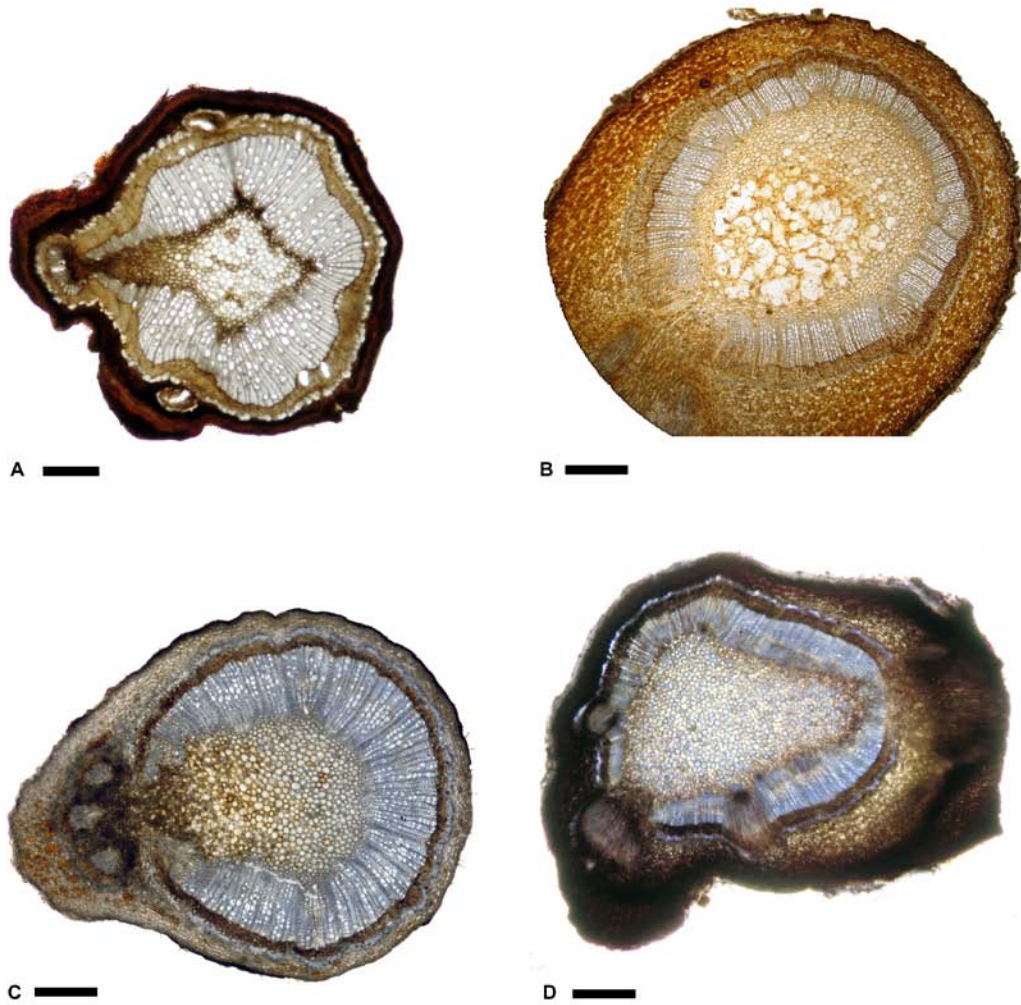


Figure 6. Light microscope images of transverse sections of nodal anatomy. **A**, *G. glabra* (3992). **B**, *F. laxiflorum* (Hoyos 1119). **C**, *R. crenata* (Hoyos 1115). **D**, *R. dasyadena* (Hoyos 1112). Scale bars: A,B,D = 500 μ m; C = 350 μ m.

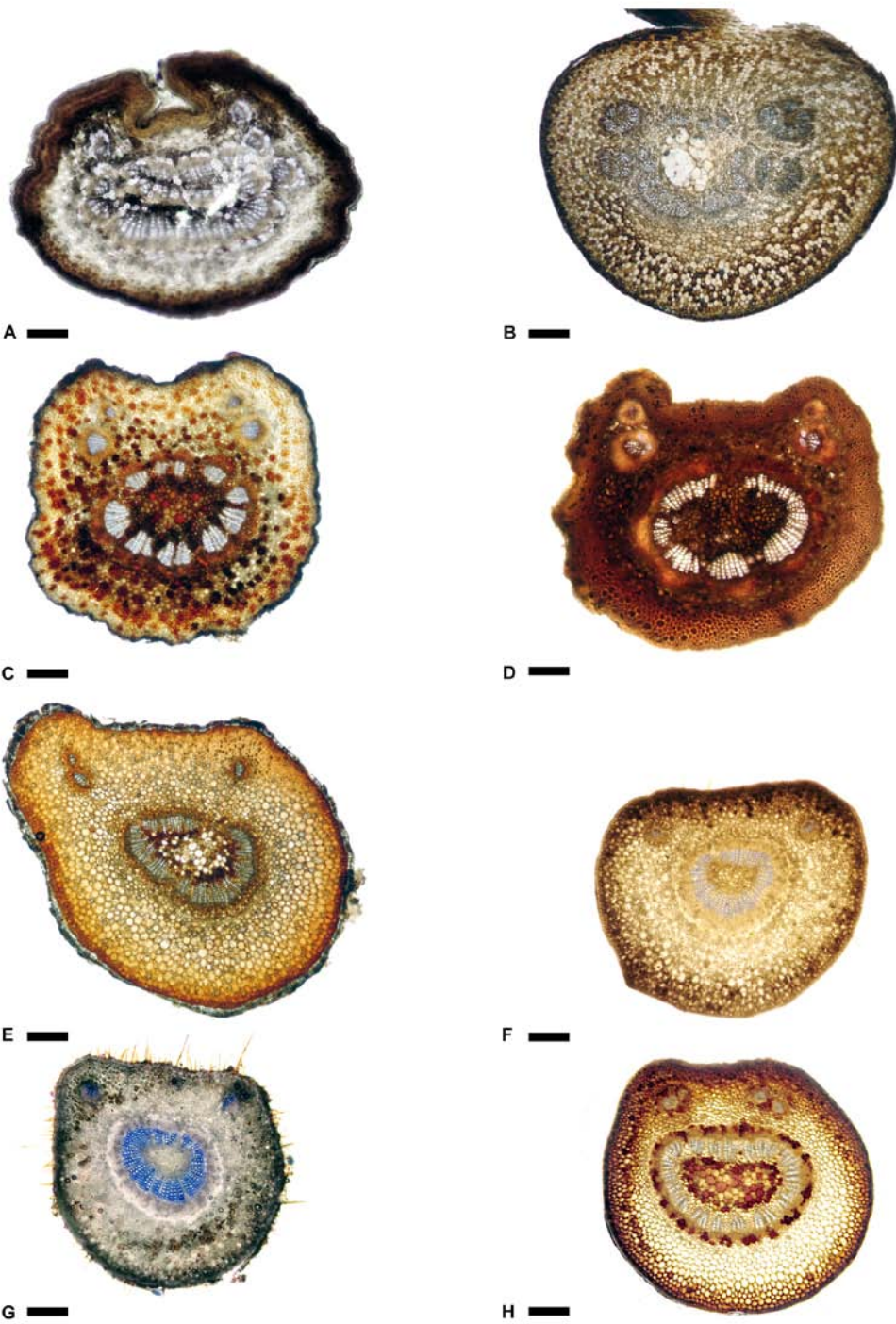
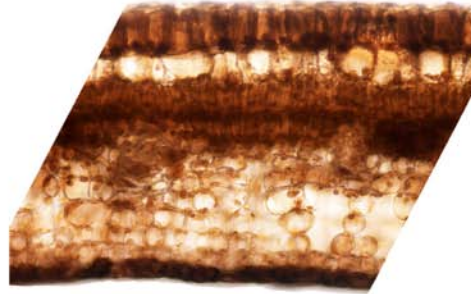


Figure 7. Light microscope images of transverse sections of petiole. **A,** *G. glabra* (3992). **B,** *F. laxiflorum* (Hoyos 1119). **C,** *R. crenata* (Hoyos 1115). **D,** *R. apiculata* (Hurtado 2964). **E,** *R. lindeniiana* (Hoyos 1135). **F,** *R. viridifolia* (Hoyos 1004). **G,** *R. squamata* (Hoyos 1114). **H,** *R. paniculata* (Hoyos 1110) Scale bars: A,F = 150 μ m; B = 300 μ m; C,D,E,G,H = 200 μ m.



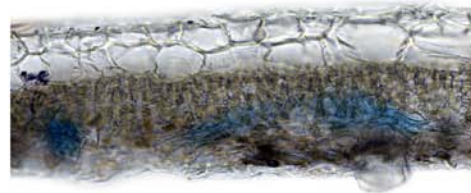
A



B



C



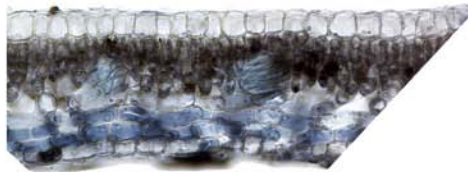
D



E



F



G



H

Figure 8. Light microscope images of transverse sections of leaf. A, *G. glabra* (5554). B, *F. laxiflorum* (Hoyos 1119). C, *R. crenata* (Hoyos 1115). D, *R. apiculata* (Núñez 6026). E, *R. lindeniiana* (Hoyos 1009). F, *R. viridifolia* (Hoyos 1006). G, *R. dasyadena* (Hoyos 1120). H, *R. squamata* (Hoyos 1114) Scale bars = 50 μ m.



Figure 9. Light microscope images of transverse sections midrib. **A**, *G. glabra* (Cremers 14257). **B**, *F. laxiflorum* (Hoyos 1119). **C**, *R. crenata* (Hoyos 1115). **D**, *R. apiculata* (Núñez 6026). **E**, *R. lindeniana* (Hoyos 1009). **F**, *R. viridifolia* (Hoyos 1004). **G**, *R. dasyadena* (Hoyos 1120). **H**, *R. squamata* (Hoyos 1114). **I**, *R. paniculata* (Hoyos 1110). Scale bars: A,C,E,F,G,H,I = 300 µm; B = 700 µm; D = 500 µm.

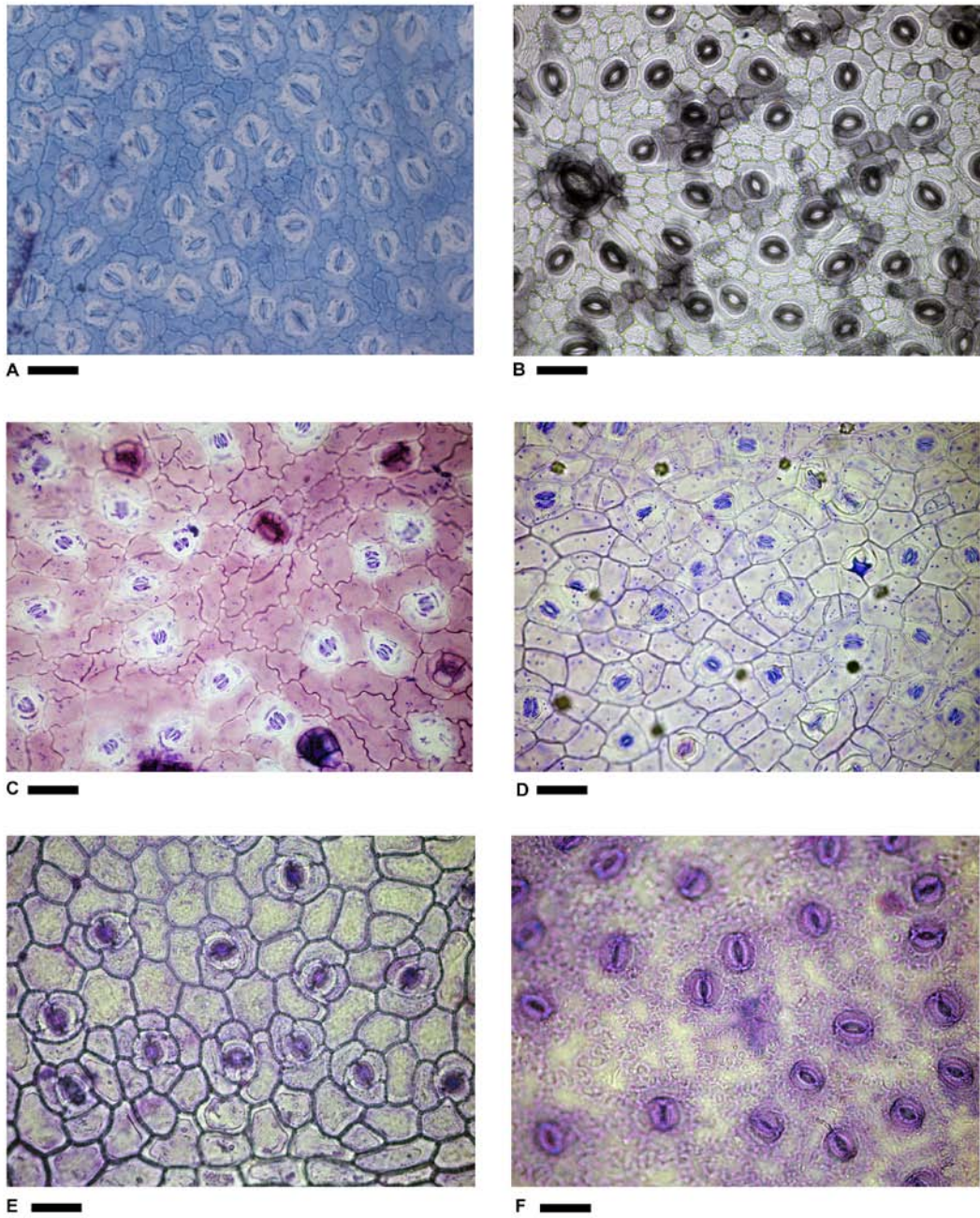


Figure 10. Light microscope images of transverse sections of abaxial leaf cuticle. **A,** *G. glabra* (8900). **B,** *F. laxiflorum* (Hoyos 1119). **C,** *R. crenata* (Grayum 10069). **D,** *R. apiculata* (Núñez 6026). **E,** *R. lindeniiana* (Hoyos 999). **F,** *R. paniculata* (Hoyos 1110). Scale bars = 30 µm.

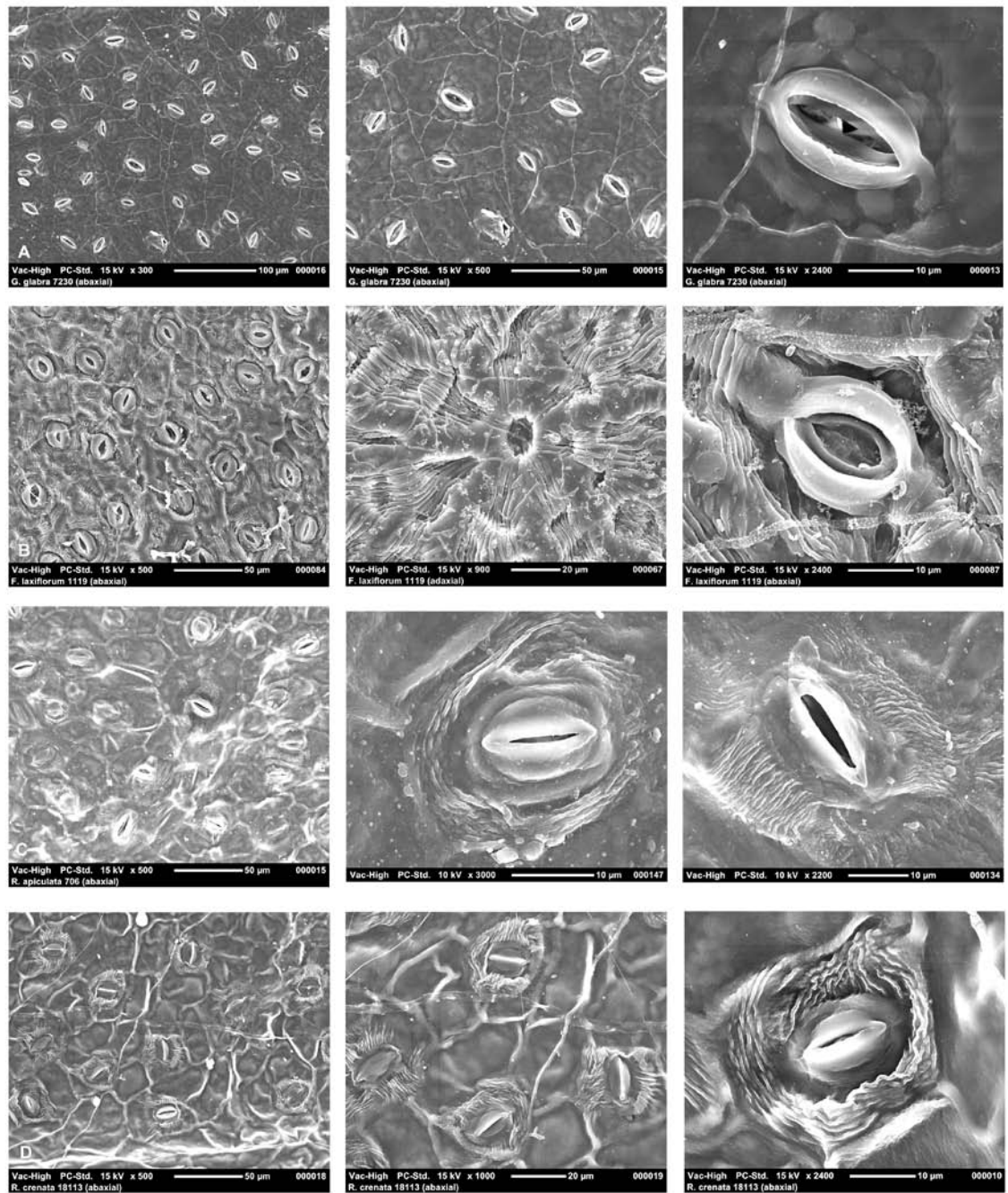


Figure 11. SEM microscopy of stomata. A, *G. glabra* (7230). B, *F. laxiflorum* (Hoyos 1119). C, *R. apiculata* (Núñez 6026). D, *R. crenata* (Grayum 18113).

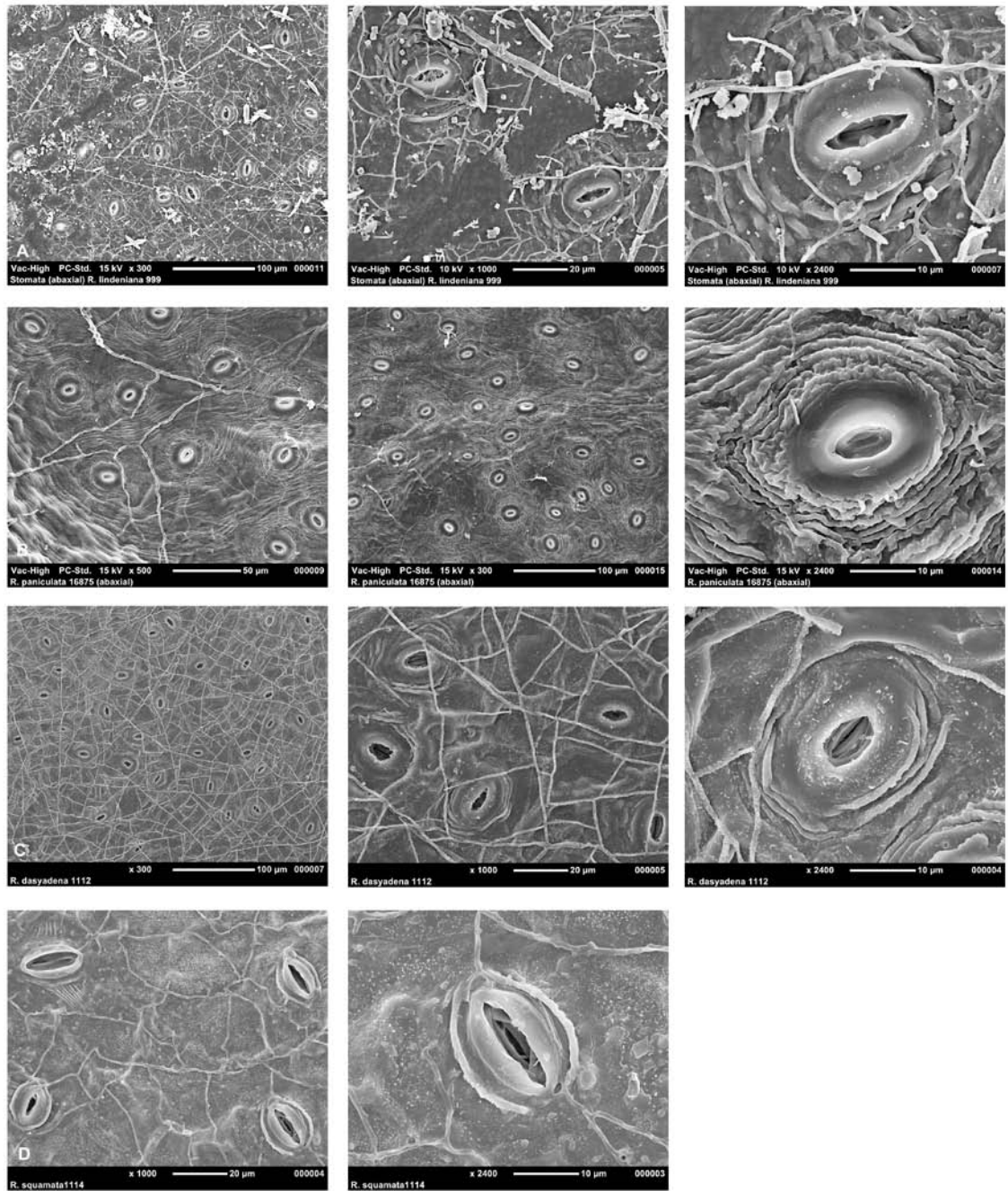


Figure 12. SEM microscopy of stomata. A, *R. lindeniana* (7230). B, *R. paniculata* (Hammel 16875). C, *R. dasyadena* (Hoyos 1112). D, *R. squamata* (Hoyos 1114).

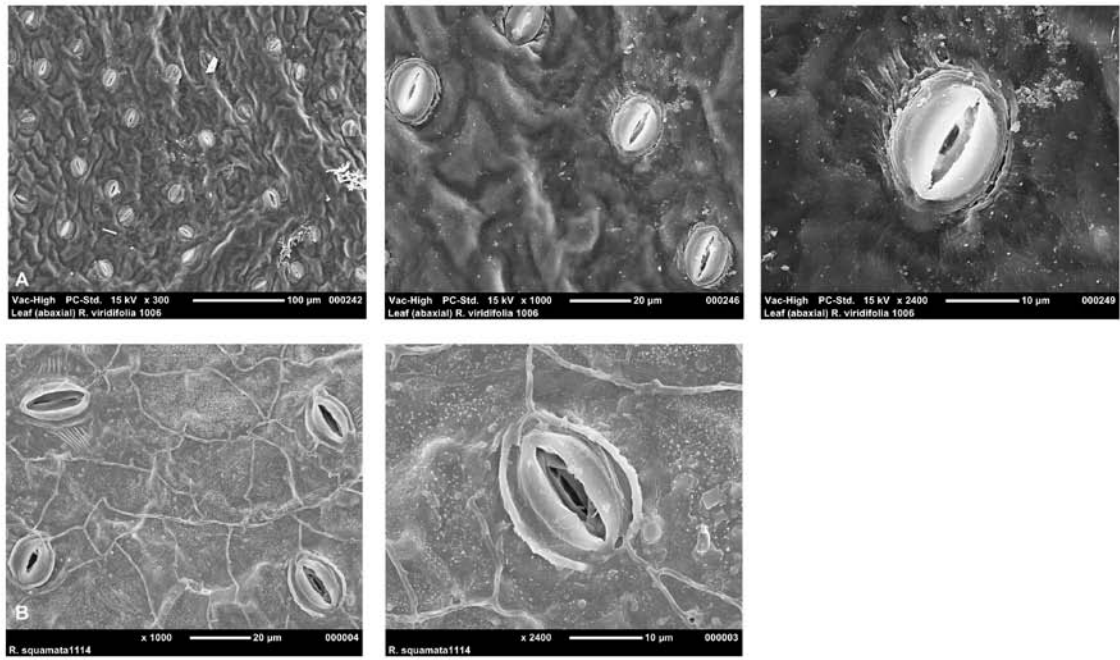


Figure 13. SEM microscopy of stomata. A, *R. viridifolia* (Hoyos 1006). B, *R. squamata* (Hoyos 1114).

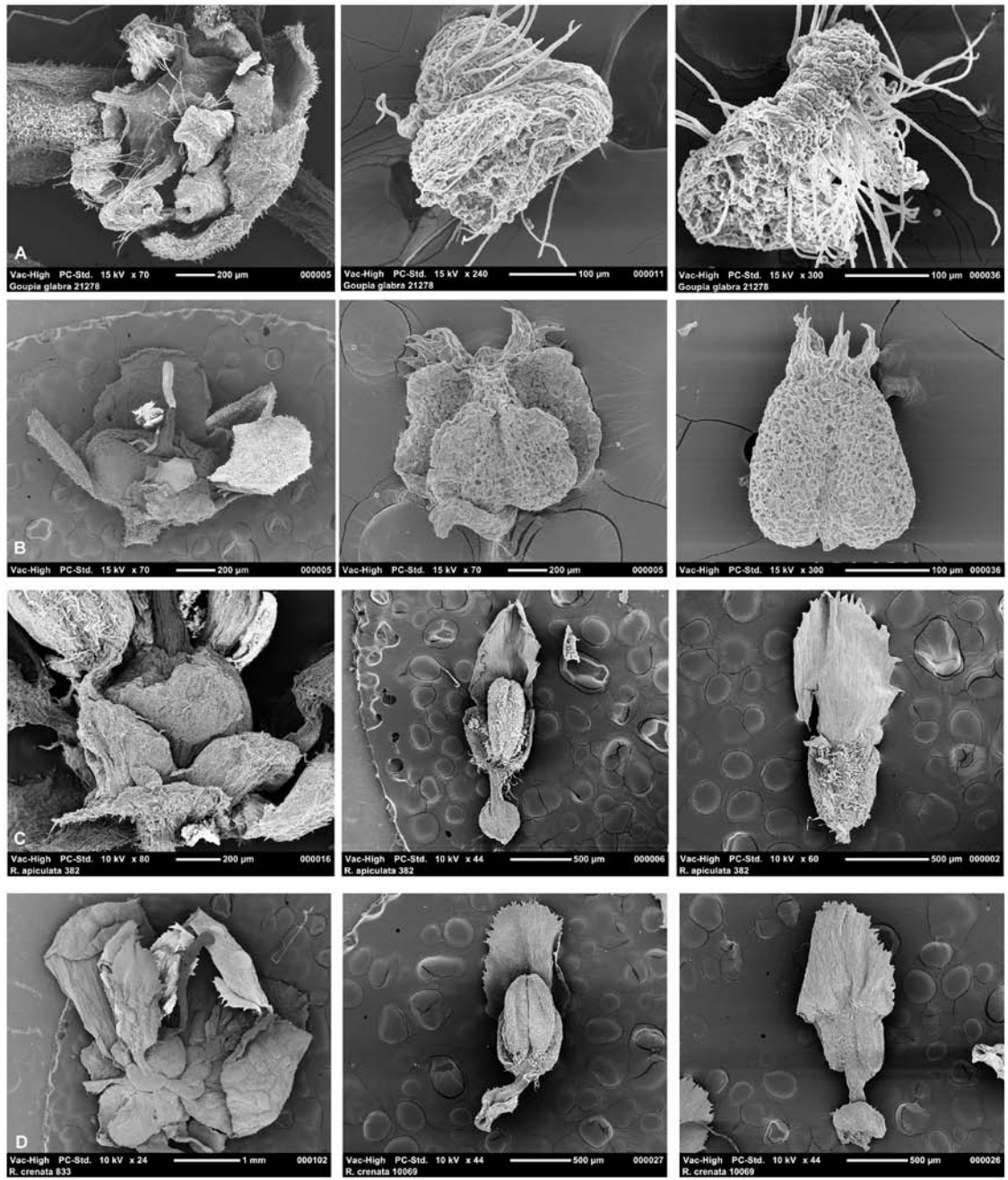


Figure 14. SEM microscopy of androecium. A, *G. glabra* (Hammen 21278). B, *F. laxiflorum* (Hartshorn 2929). C, *R. apiculata* (Kawasaki 382). D, *R. crenata* (Grayun 1069)

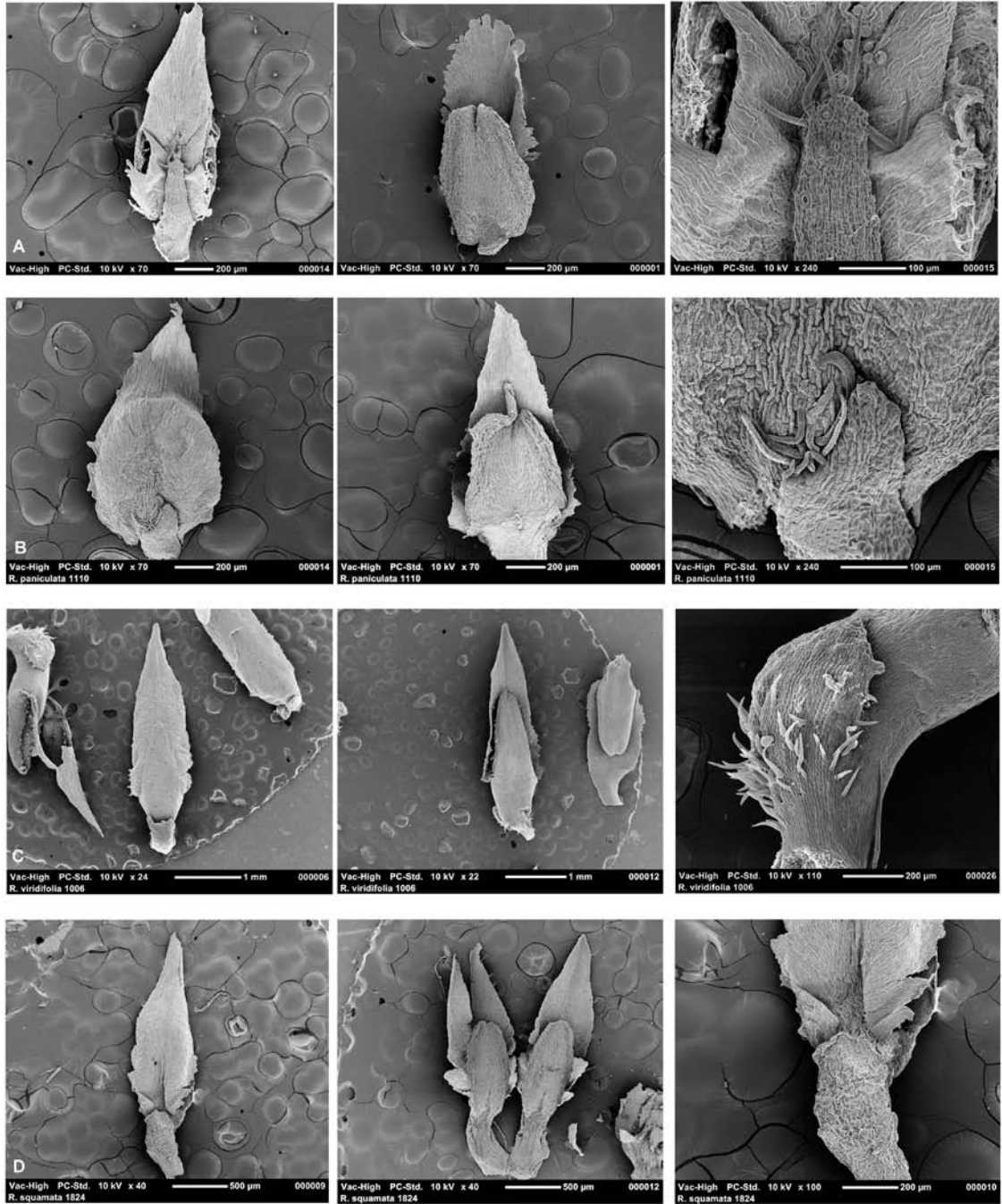


Figure 15. SEM microscopy of androecium. **A,** *R. lindeniana* (Hoyos 999). **B,** *R. paniculata* (Hoyos 1110). **C,** *R. viridifolia* (Hoyos 1006). **D,** *R. squamata* (Neill 1824).

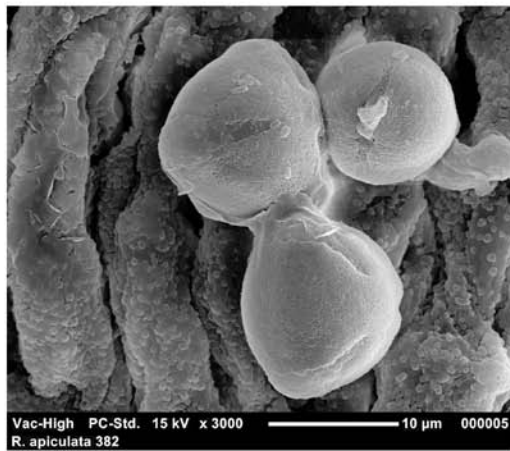
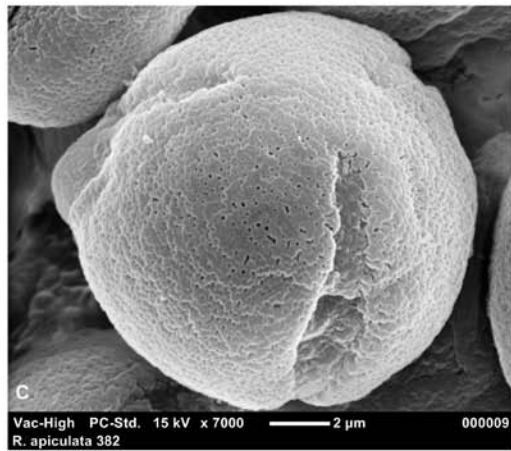
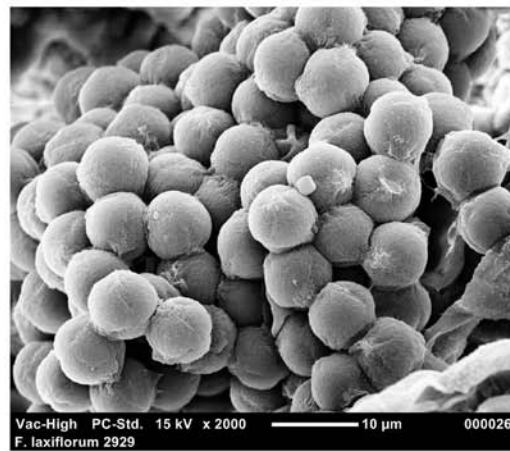
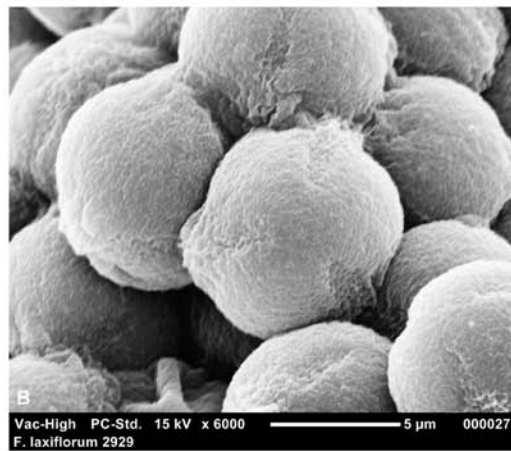
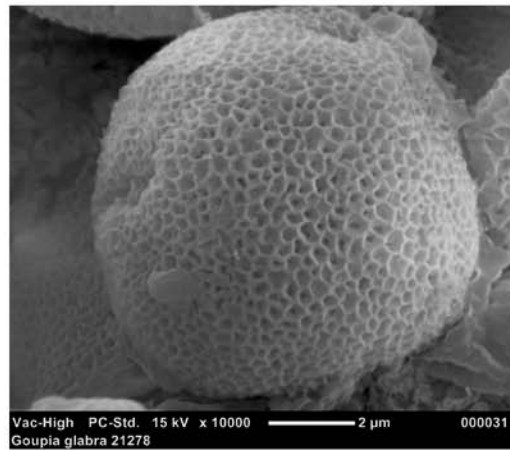


Figure 16. SEM microscopy of pollen. A, *G. glabra* (Hammel 21278). B, *F. laxiflorum* (Hartshorn 2929). C, *R. apiculata* (Kawazaki 382). D, *R. paniculata* (Hoyos 1110).

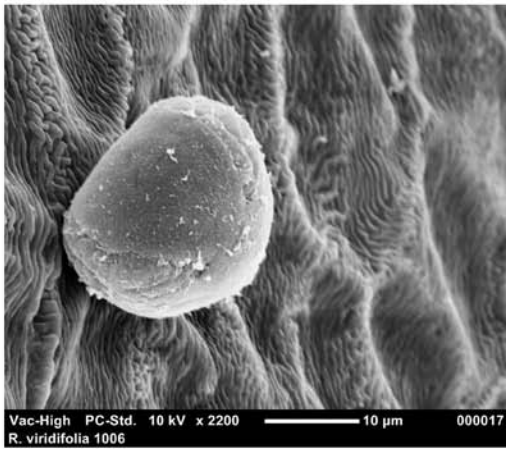
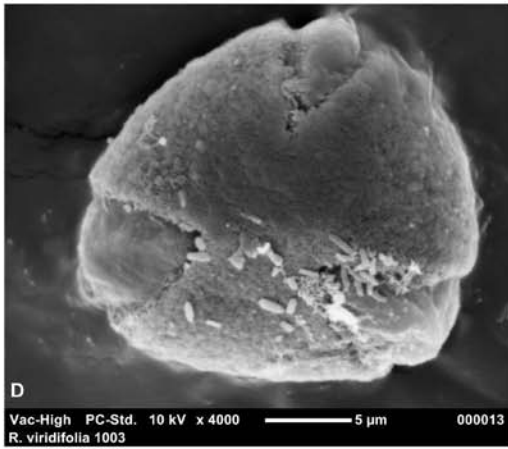
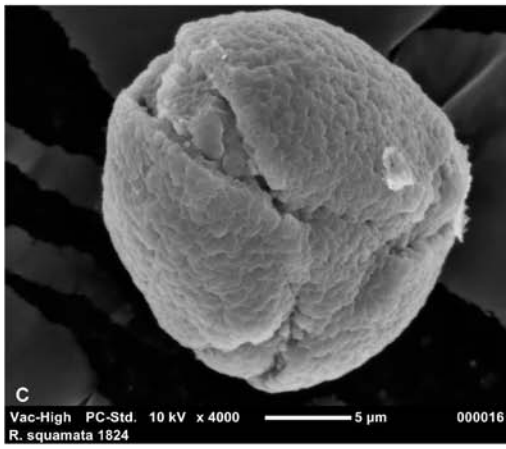
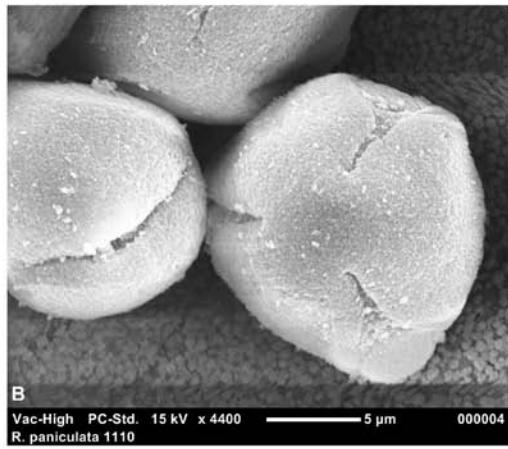
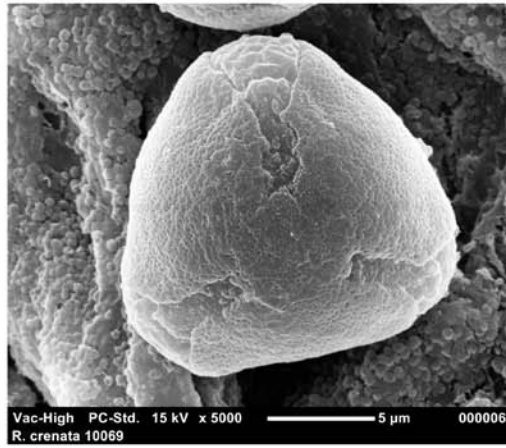
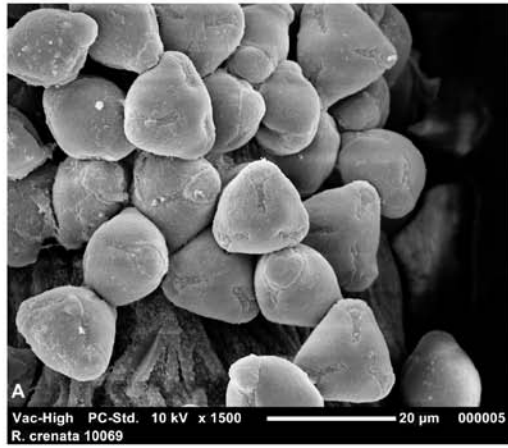


Figure 17. SEM microscopy of pollen. A, *R. crenata* (Grayum 10069). B, *R. paniculata* (Hoyos 1110). C, *R. squamata* (Neill 1824). D, *R. viridifolia* (Hoyos 1003).

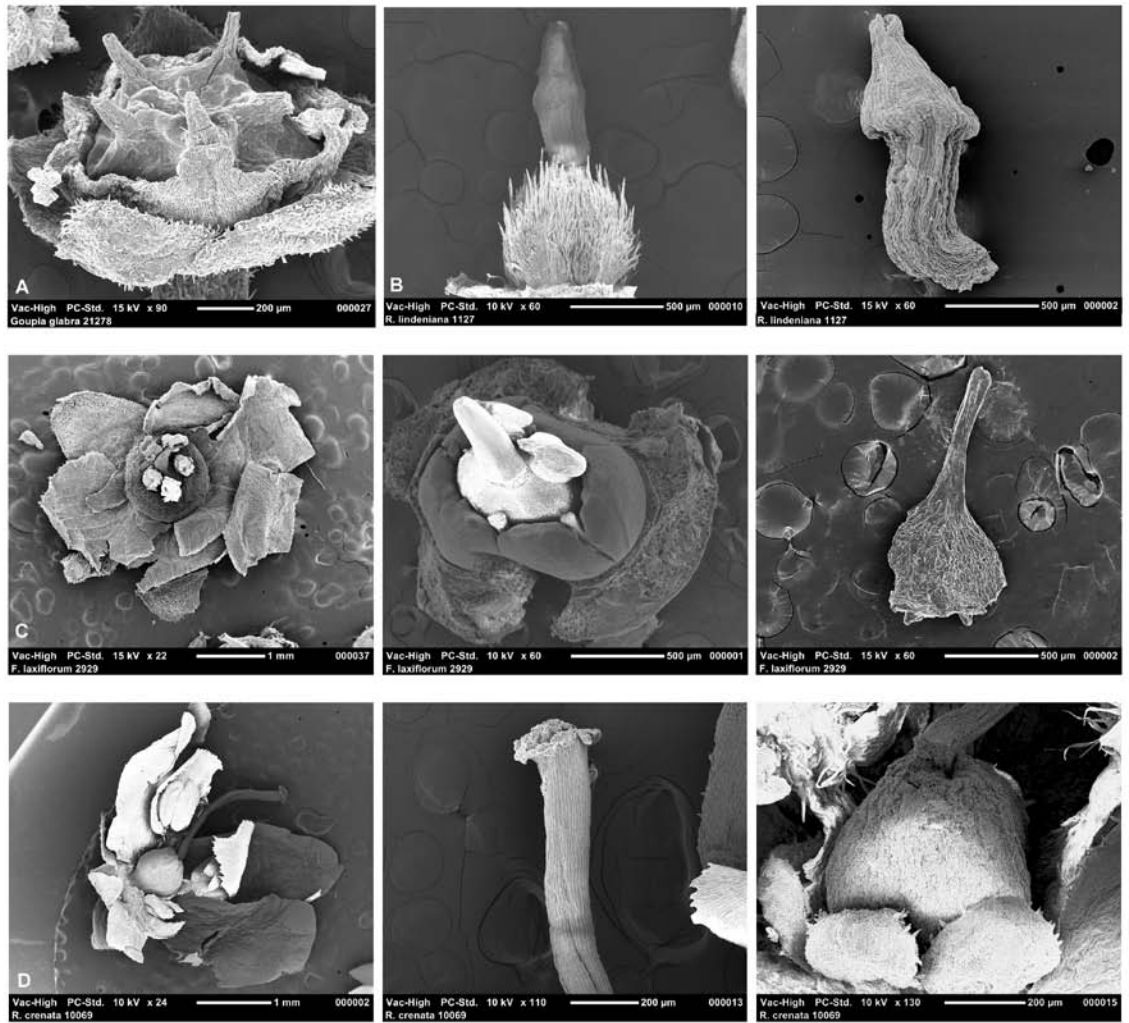


Figure 18. SEM microscopy of gynoecium. A, *R. crenata* (Grayum 10069). B, *R. paniculata* (Hoyos 1110). C, *R. squamata* (Neill 1824). D, *R. crenata* (Grayum 10069).

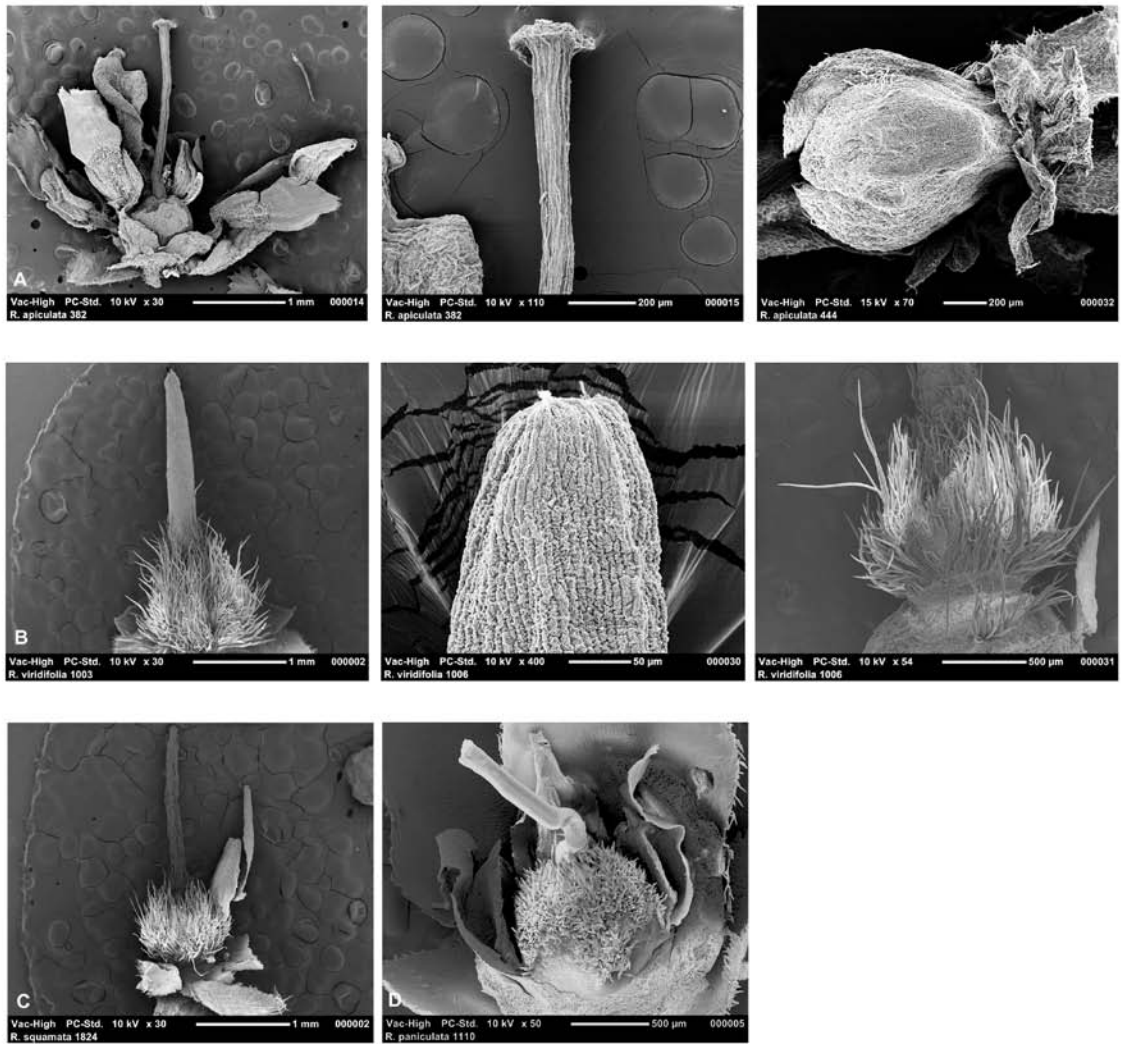


Figure 19. SEM microscopy of gynoecium. **A,** *R. apiculata* (Kawazaki 382). **B,** *R. viridifolia* (Hoyos 1003). **C,** *R. squamata* (Neill 1824). **D,** *R. paniculata* (Hoyos 1110).

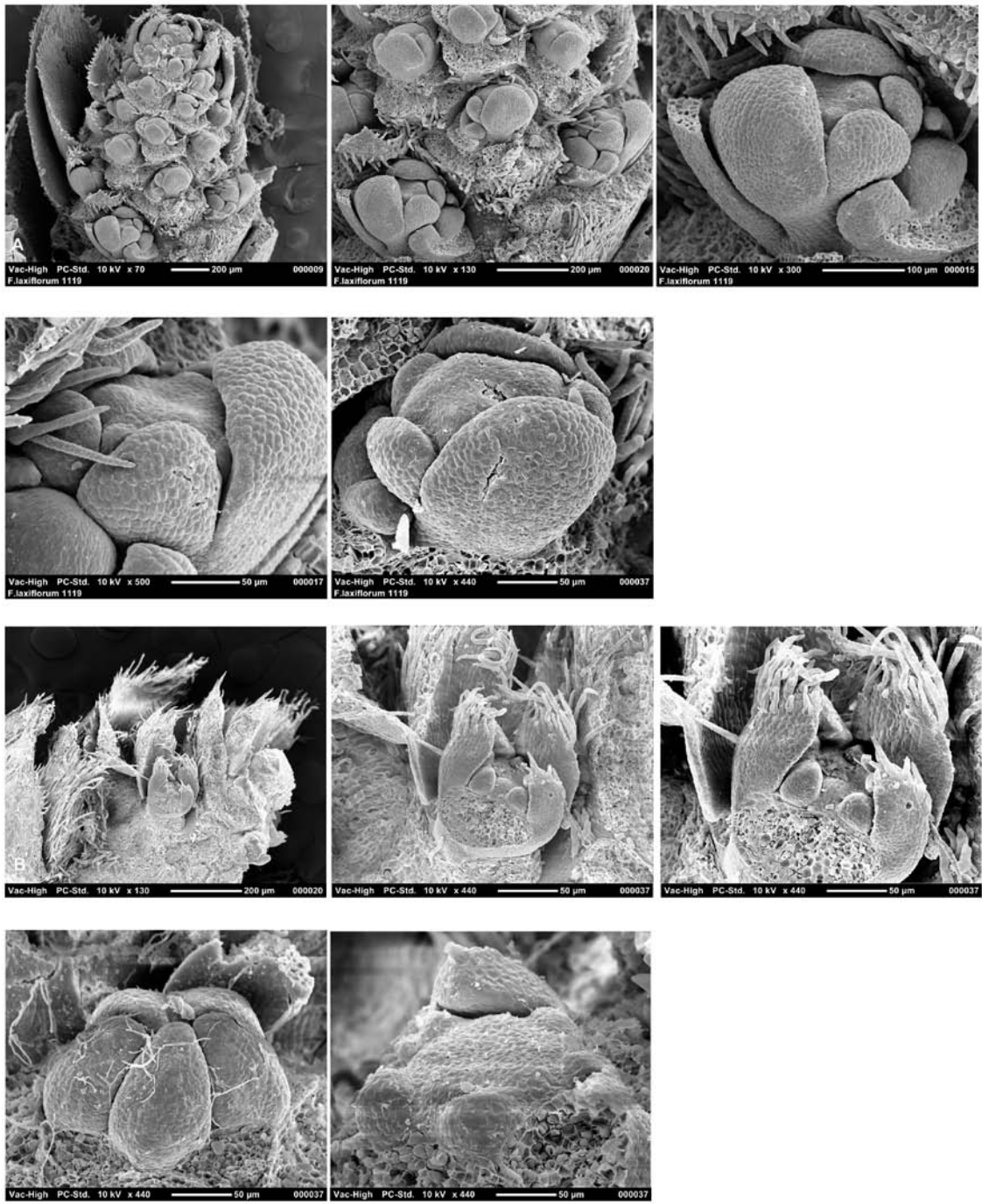


Figure 20. SEM microscopy of Floral development. A, *F. laxiflorum* (Hoyos 1119). B, *R. dasyadena* (Hoyos 1120).

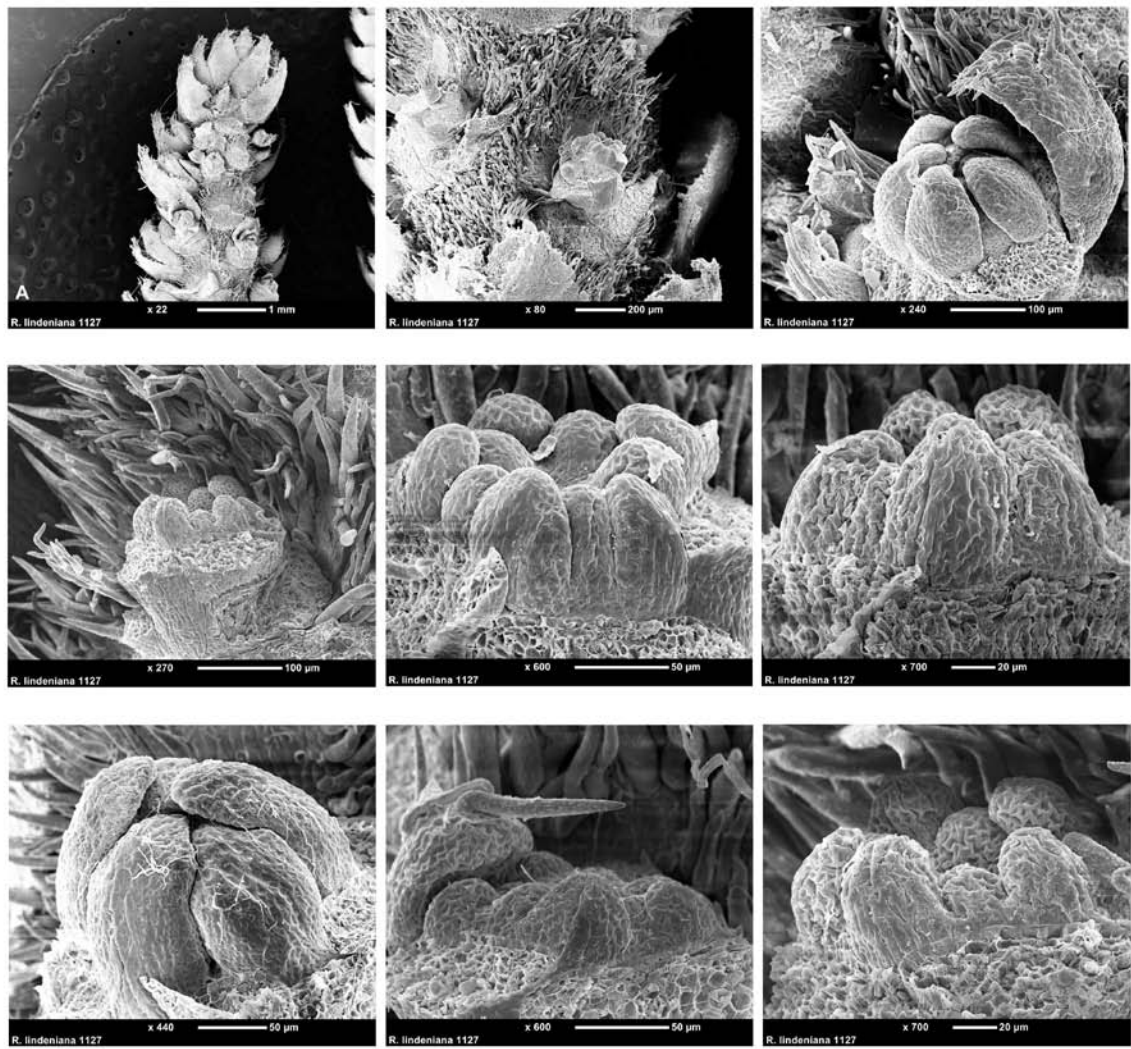


Figure 21. SEM microscopy of Floral development. A, *R. lindeniana* (Hoyos 1127).

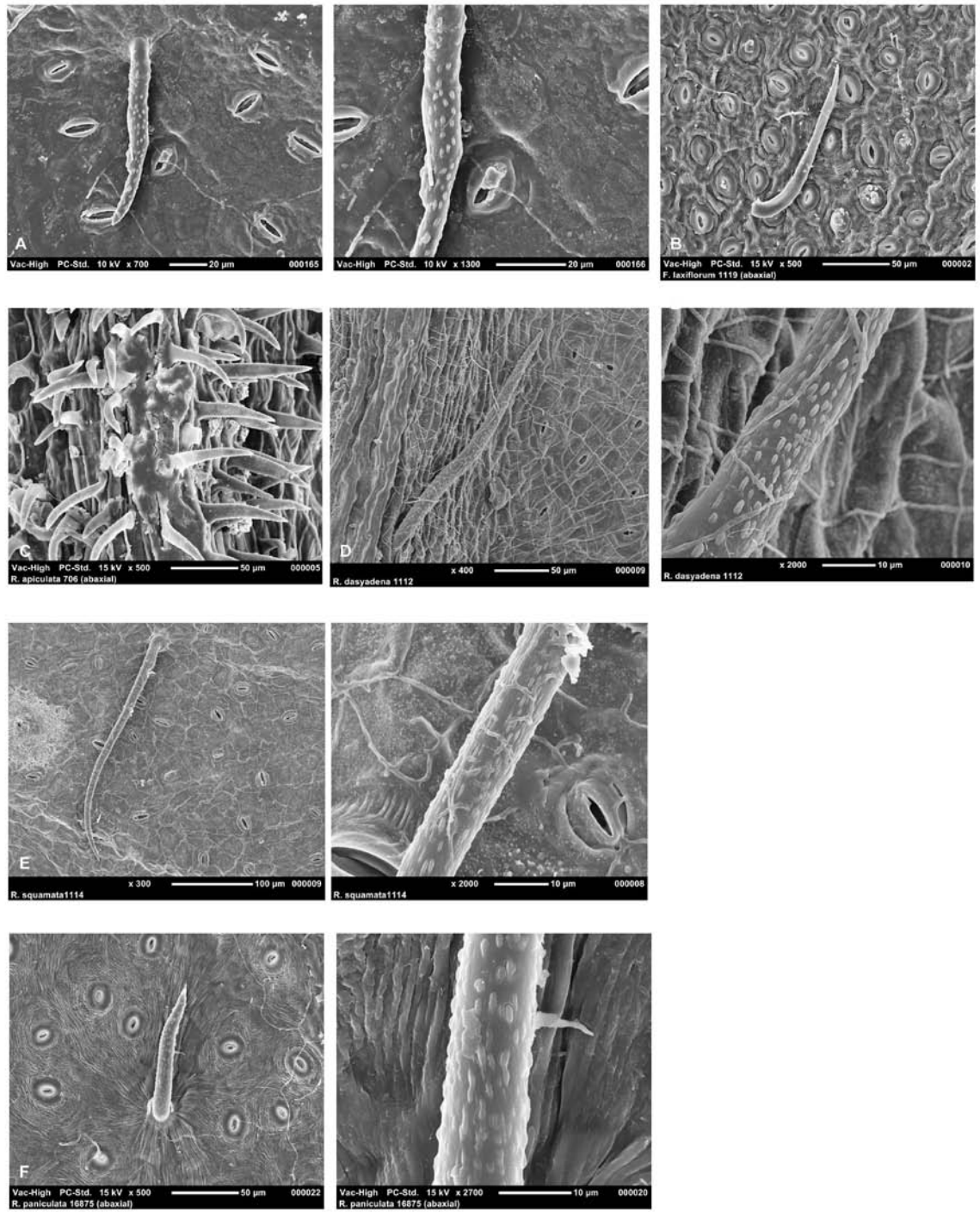


Figure 22. SEM microscopy of trichomes. **A**, *G. glabra* (Nevers 7230). **B**, *F. laxiflorum* (Hoyos 1119). **C**, *R. apiculata* (Tipaz 706). **D**, *R. dasycadena* (Hoyos 1112). **E**, *R. squamata* (Hoyos 1114). **F**, *R. paniculata* (Hammel 16785).

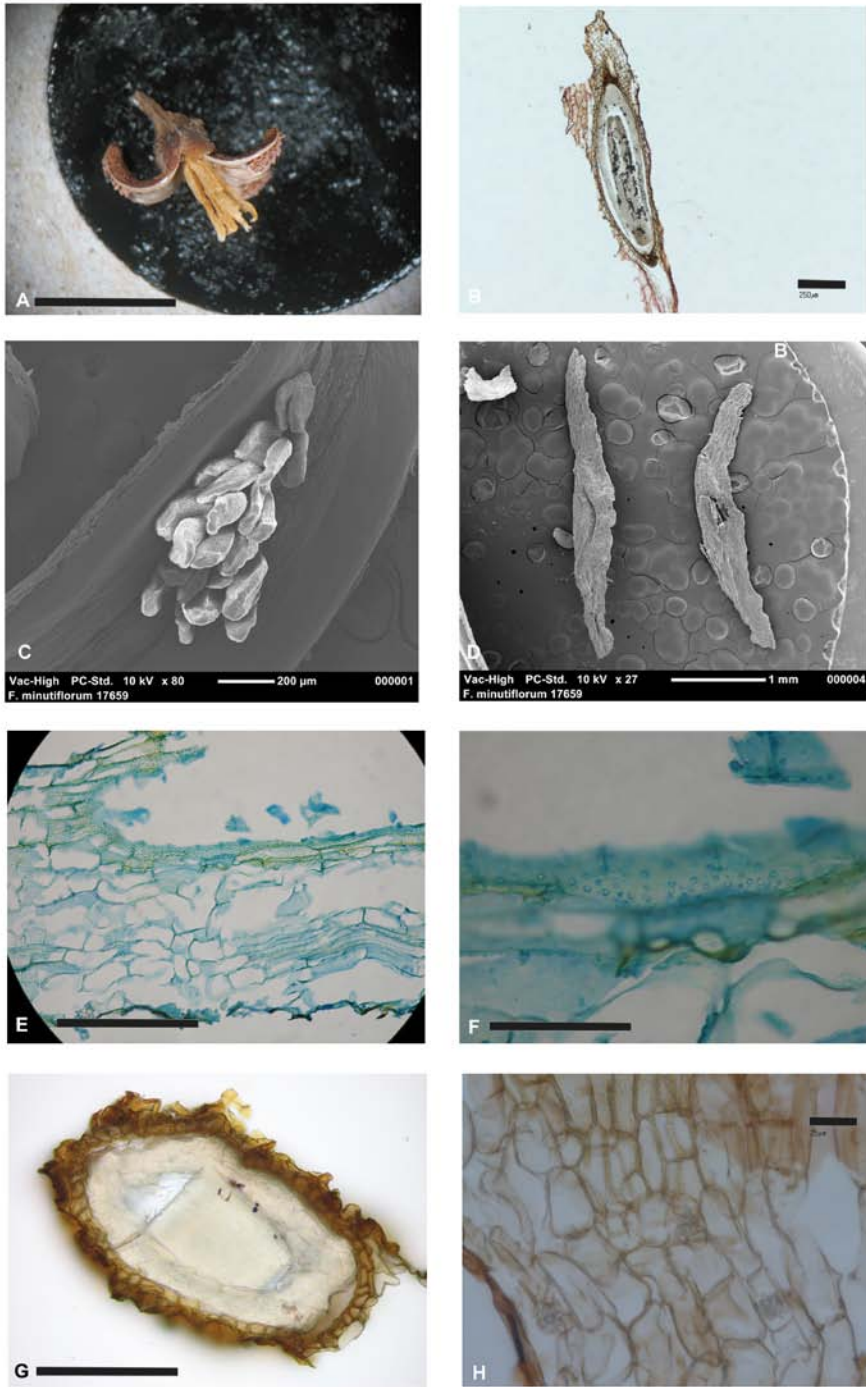


Figure 23. Light and SEM images of seeds. A, B (LS), E (LS), F (LS), G (TS), H (LS), (LM) *F. minutiflorum* (Espina 1330). C, D *F. minutiflorum* (Gentry 17659). Scale bars: A = 3 mm, B,E,G = 250 µm, C = 200 µm, D = 1 mm, H = 25 µm.

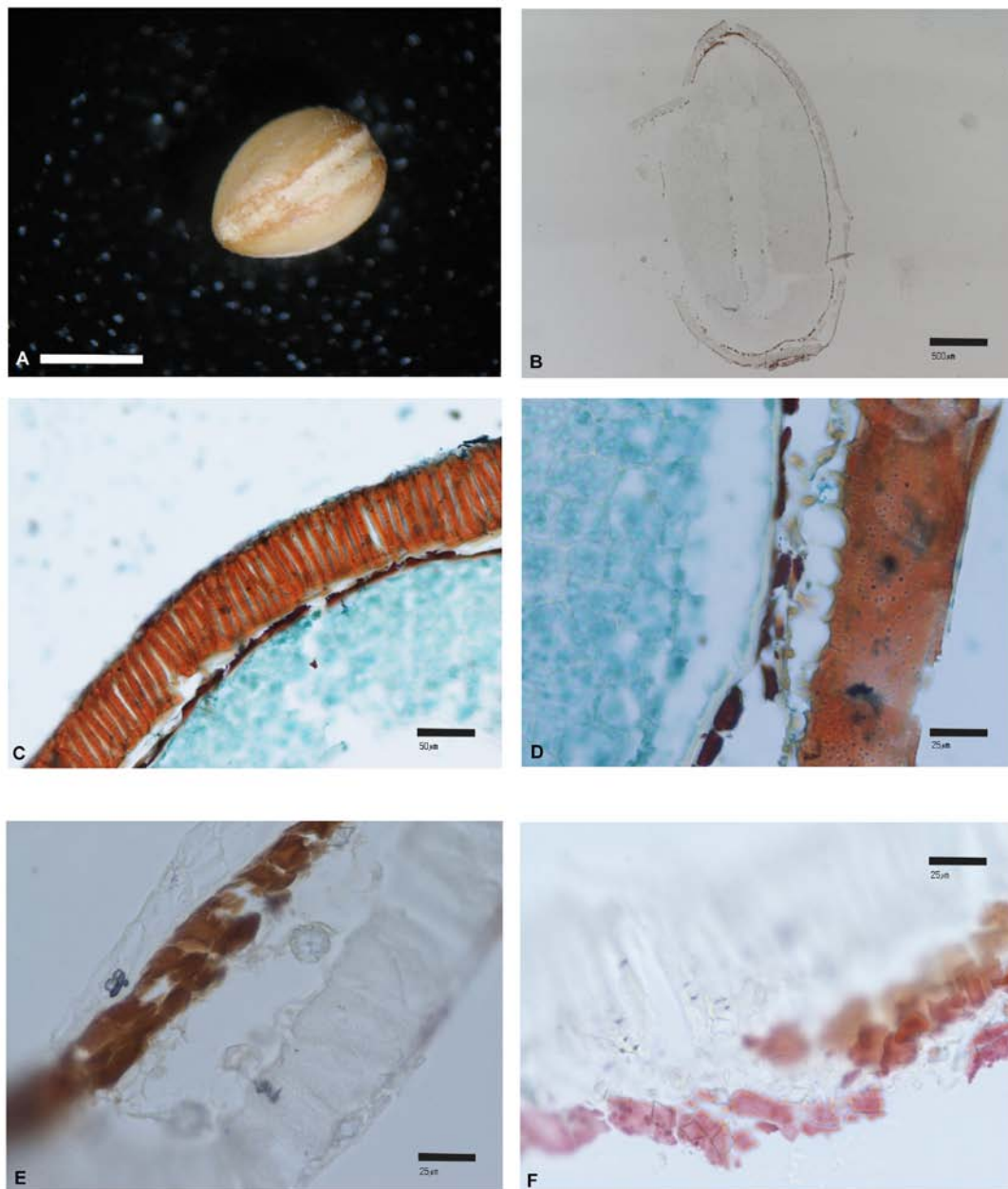


Figure 25. Light Microscope (LM) images of seeds. **A**, *R. apiculata* (Espinoza 336). **B** (LS), **C** (LS), **D** (TS), **E** (LS), **F** (LS) *R. apiculata* (Ceron 5478) **A**= scale bar 2 mm. **B**=scale bar 500 µm. **C**= scale bar 50 µm, **D**, **E**, **F**=scale bar 25 µm.



Figure 26. Light Microscope (LM) images of seeds. **A (LS), B (LS), C (LS), D (LS), E (LS)** *R. crenata* (Hammel 18113). **A=** scale bar 2 mm. **B=**scale bar 500 μ m. **C=** scale bar 250 μ m, **D=**scale bar 50 μ m, **E=**scale bar 50 μ m.

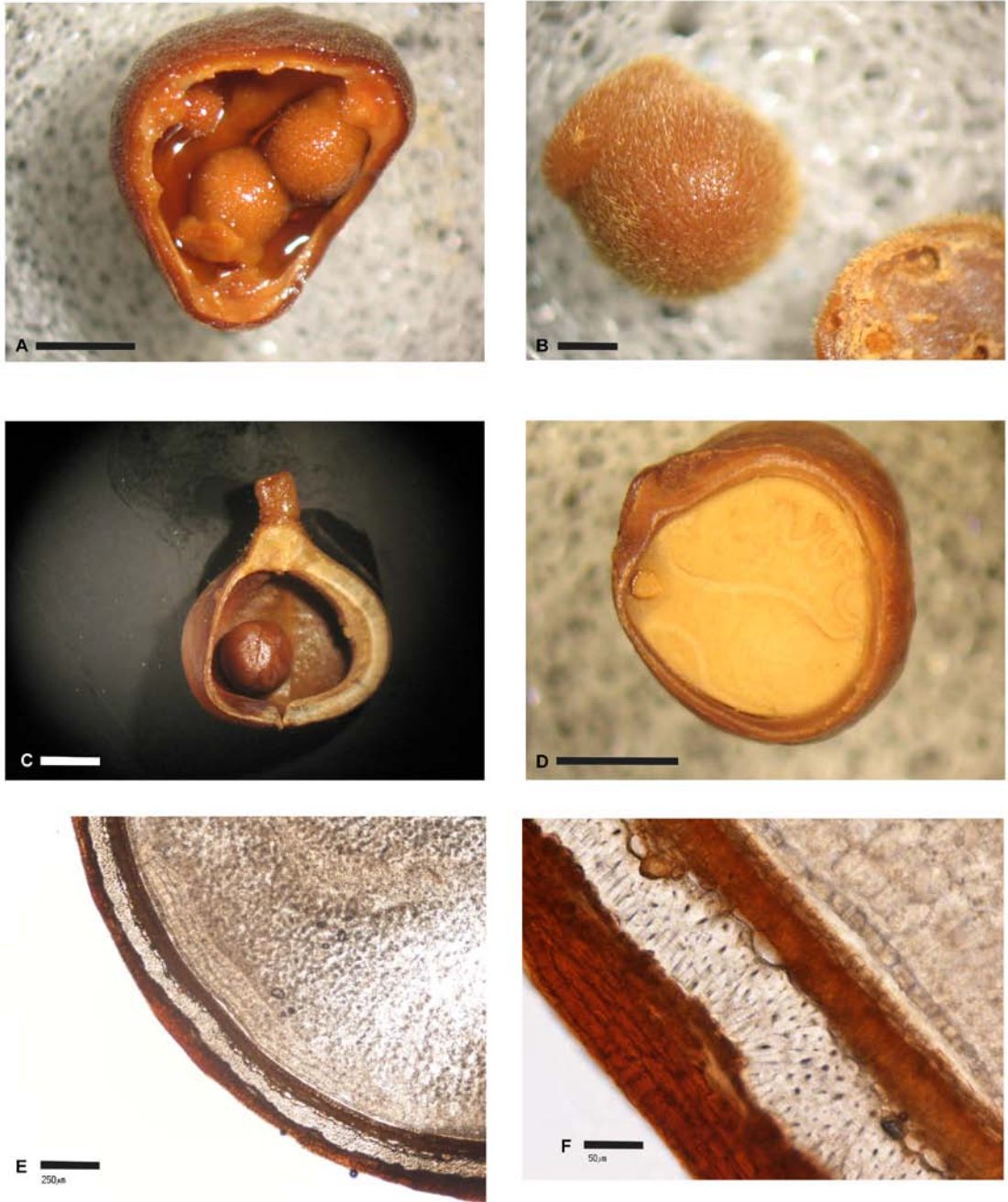


Figure 27. Light Microscope (LM) images of seeds. **A, B,** *R. lindeniana* (Hoyos 1001). **C, D (LS), E (TS), F (TS)** *R. viridifolia* (Hoyos 1004). **A, B=** scale bar 2 mm. **C, D=** scale bar 4 mm, **E=**scale bar 250 μm, **F=**scale bar 50 μm.

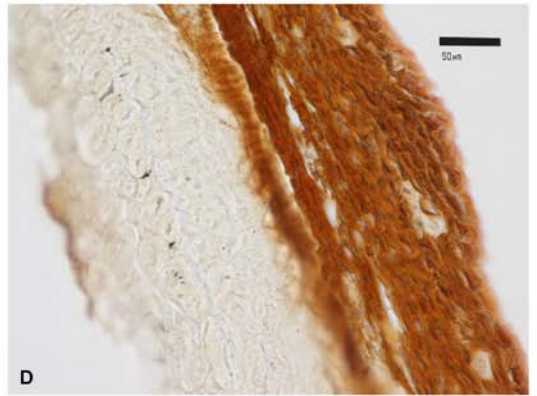
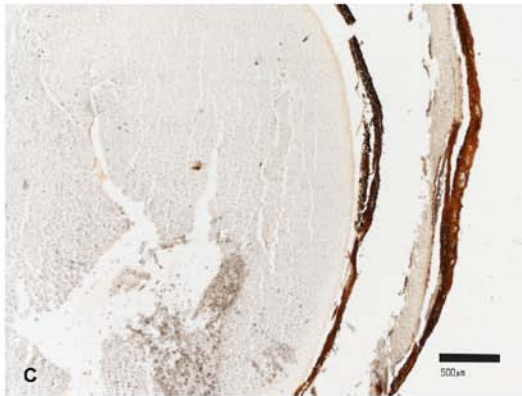
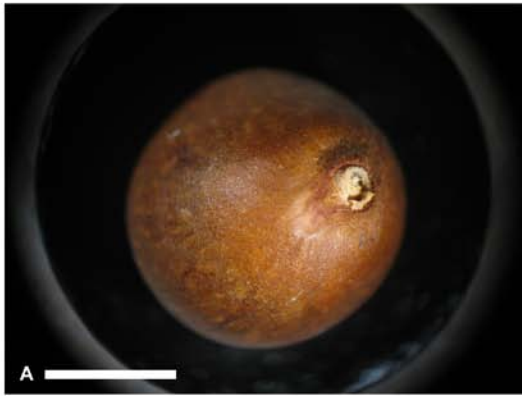


Figure 28. Light Microscope (LM) images of seeds. **A, B (LS), C (TS), D (TS)** *R. dasyadena* (Hoyos 1120). **E (TS)** *R. squamata* (Hoyos 1114). Scale bars: **A, B = 3 mm. C = 500 µm.**

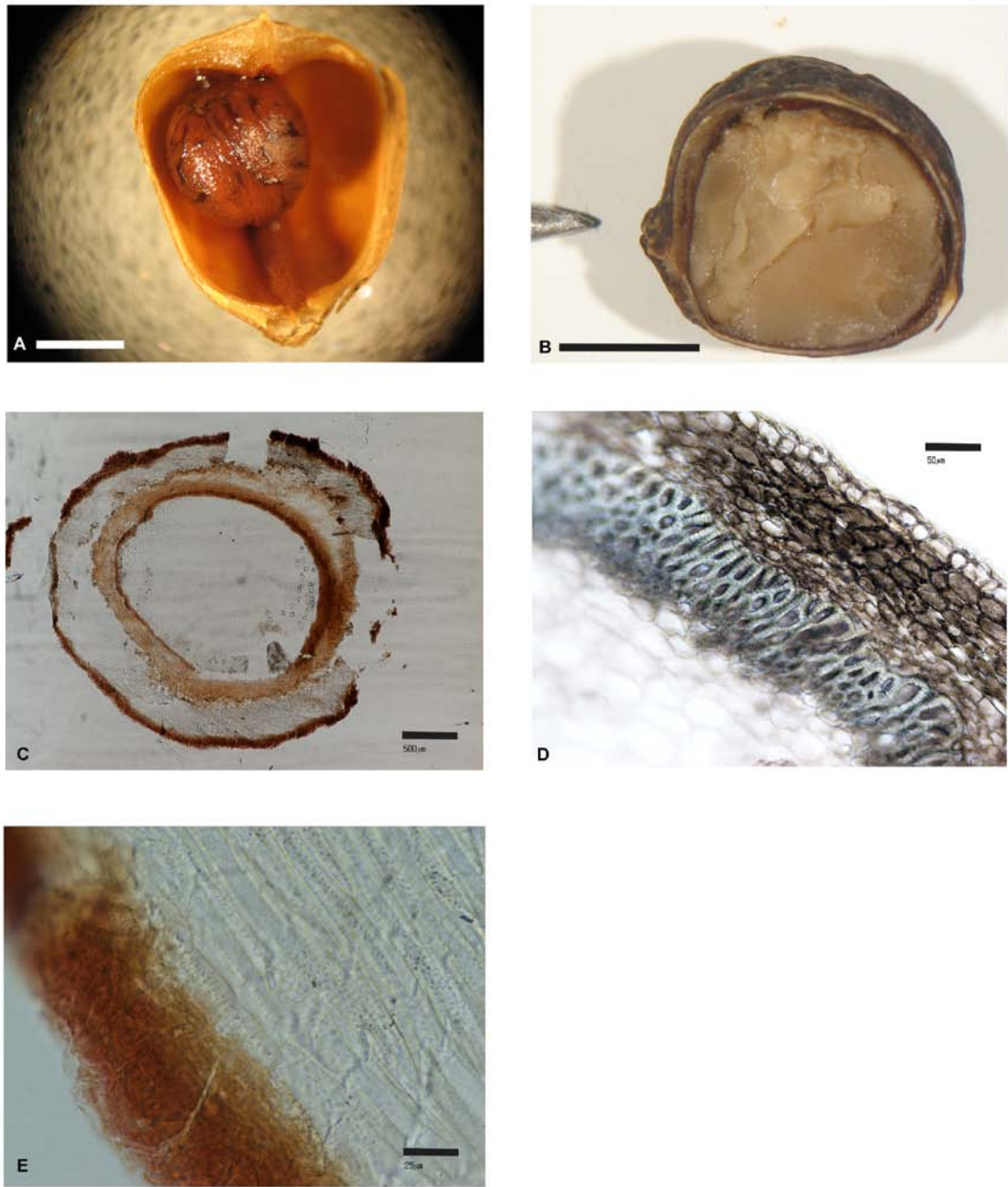


Figure 29. Light Microscope (LM) images of seeds. A, B (LS), C (LS), D (TS), E (LS) *R. squamata* (Hoyos 1114). A, B= scale bar 4 mm. C=scale bar 500 μm . D= scale bar 50 μm . E=scale bar 25 μm .

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