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OCCURRENCE AND PHYLOGENETIC SIGNIFICANCE OF LATEX IN THE MALPIGHIACEAE[1]

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Latex and laticifers are reported for the first time in the genera *Galphimia* and *Verrucularia* (Malpighiaceae), with description and illustration of the leaf and stem anatomy of both genera. Those genera and the other two in which latex is known (*Lophanthera* and *Spachea*) constitute a single tribe, Galphimieae, that is at or near the base of the family's phylogeny, which suggests that latex in the Malpighiaceae may indicate an ancestor shared with the Euphorbiaceae.

Key words: *Galphimia brasiliensis*; Galphimieae; latex; laticifers; Malpighiaceae; *Verrucularia glaucophylla*.

The angiosperm family Malpighiaceae includes approximately 65 genera and 1250 species in the world and is distributed in tropical and subtropical regions of both hemispheres (W. R. Anderson, unpublished data). Nearly 950 species are endemic to the New World, northern South America being the major center of diversity (Anderson, 1979). The monophyly of the Malpighiaceae is supported by morphological characters (Anderson, 1979, 1990) and has recently been confirmed with molecular data (Chase et al., 1993; Cameron, Chase, and Anderson, 1995; Wurdack and Chase, 1996). Recent molecular studies of the Malpighiaceae did not provide convincing bases for dividing it into a small number of monophyletic subfamilies or tribes that reflect obvious morphological synapomorphies (Cameron et al., 2001; Davis, Anderson, and Donoghue, 2001), but they did confirm the monophyly of the tribe Galphimieae Nied. This tribe, as circumscribed by Anderson (1978), comprises four genera: *Galphimia* Cav., *Lophanthera* A. Juss., *Spachea* A. Juss., and *Verrucularia* A. Juss. Anderson (1981, 2001) mentioned the presence of milky latex in the stems of *Lophanthera* and *Spachea*, and the present paper documents the occurrence of latex in the other two genera, *Galphimia* and *Verrucularia*.

Galphimia includes approximately ten species of tropical and subtropical America, with a disjunct distribution (Niedenzu, 1928). Most of the species grow in Mexico and Central America and only one, *Galphimia brasiliensis* (L.) A. Juss., is found in South America, in northeastern Argentina, southern Brazil, Bolivia, Paraguay, and Uruguay. This species has leaf and calyx glands, and the structural and ultrastructural anatomy of these glands was studied by Castro, Vega, and Múlgura (2001). The genus *Verrucularia* A. Juss. includes two species, *V. glaucophylla* A. Juss. and *V. piresii* W. R. Anderson. *Verrucularia glaucophylla* has only calyx glands and is endemic to Bahia, Brazil (Niedenzu, 1928).

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MATERIALS AND METHODS

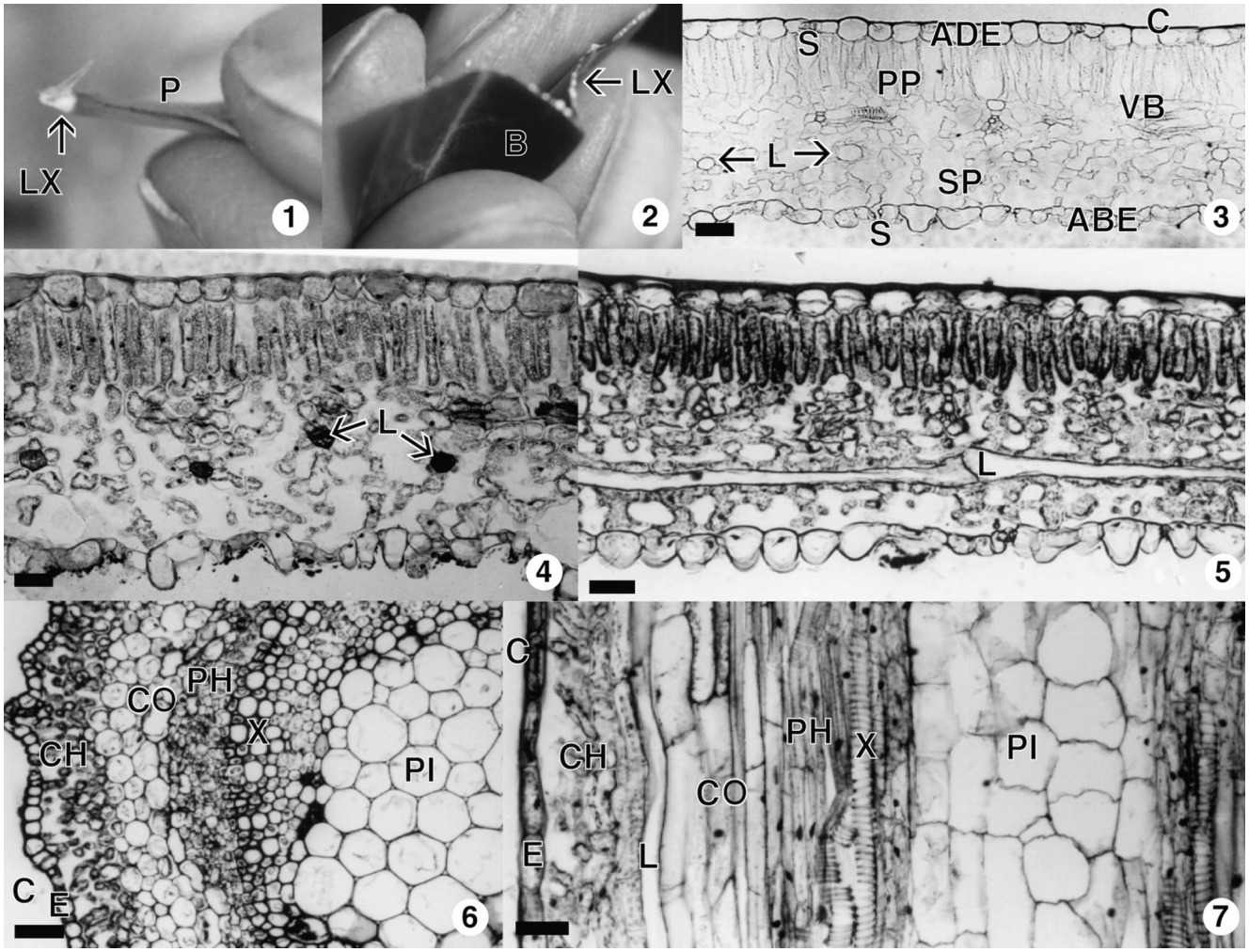
Plant materials—Fresh and fixed materials of *Galphimia brasiliensis* (L.) A. Juss. and *Verrucularia glaucophylla* A. Juss. were studied. Herbarium specimens were deposited in the herbarium of the Instituto de Botánica Darwinion (SI). Studied materials: *Galphimia brasiliensis*: ARGENTINA. *Prov. Entre Ríos. Dpto. Colón*: Parque Nacional El Palmar, Los Loros, 14/III/1998, Múlgura & Vega 1991; 4/XII/1998, Múlgura & Vega 1998. *Dpto. Concordia*: Parque Rivadavia, near José Hernández monument, 15/III/1998, Múlgura & Vega 1996. *Verrucularia glaucophylla*: BRAZIL. Bahia. Municipio de Morro do Chapéu, Cachoeira do Ferro Doido, ca. 1000 m, 4/V/2001, Amorim et al. 3662 (CEPEC, SI).

Anatomical studies—Leaves and stems of *G. brasiliensis* and *V. glaucophylla* were fixed in FAA (formalin : ethanol : acetic acid) and then stored in 70% ethanol. Part of each sample had been previously treated with sodium hypochlorite to eliminate contents. All materials were dehydrated in an ethanol series and embedded in paraffin after infiltration in a vacuum oven. Transverse and longitudinal sections 10 μm thick were cut on a rotary microtome. Sections were double stained with safranin-fast green (D'Ambrogio de Argüeso, 1986). Light microscope studies were made using a ZEISS Phomi III microscope (Zeiss, Oberkochen, Germany) and black and white Kodak Tmax 25 ASA film.

RESULTS

Field work—During field work in Entre Ríos province (Argentina), the presence of latex in *G. brasiliensis* was observed. The latex is milky and was detected in stems and blades (Figs. 1–2). During the preparation of the present paper, Ch. Anderson (personal communication) told us about one collection of *G. brasiliensis* from Bolivia (Ortiz S. 7, MICH) whose label reported it to produce latex. Collectors of herbarium specimens of *V. glaucophylla* have never reported observing latex in the field, but laticifers were detected by anatomical techniques in stems and blades of that species.

Anatomical studies—*Galphimia brasiliensis*—The blade in transverse and longitudinal sections is shown in Figs. 3–5. The adaxial epidermis is one-layered, the cells quadrangular to rectangular, with a thick outer tangential cell wall and cuticle. The papillate abaxial epidermis has a thin outer tangential cell wall. Trichomes are absent. Stomata are present in both surfaces and are more abundant in the abaxial epidermis. The

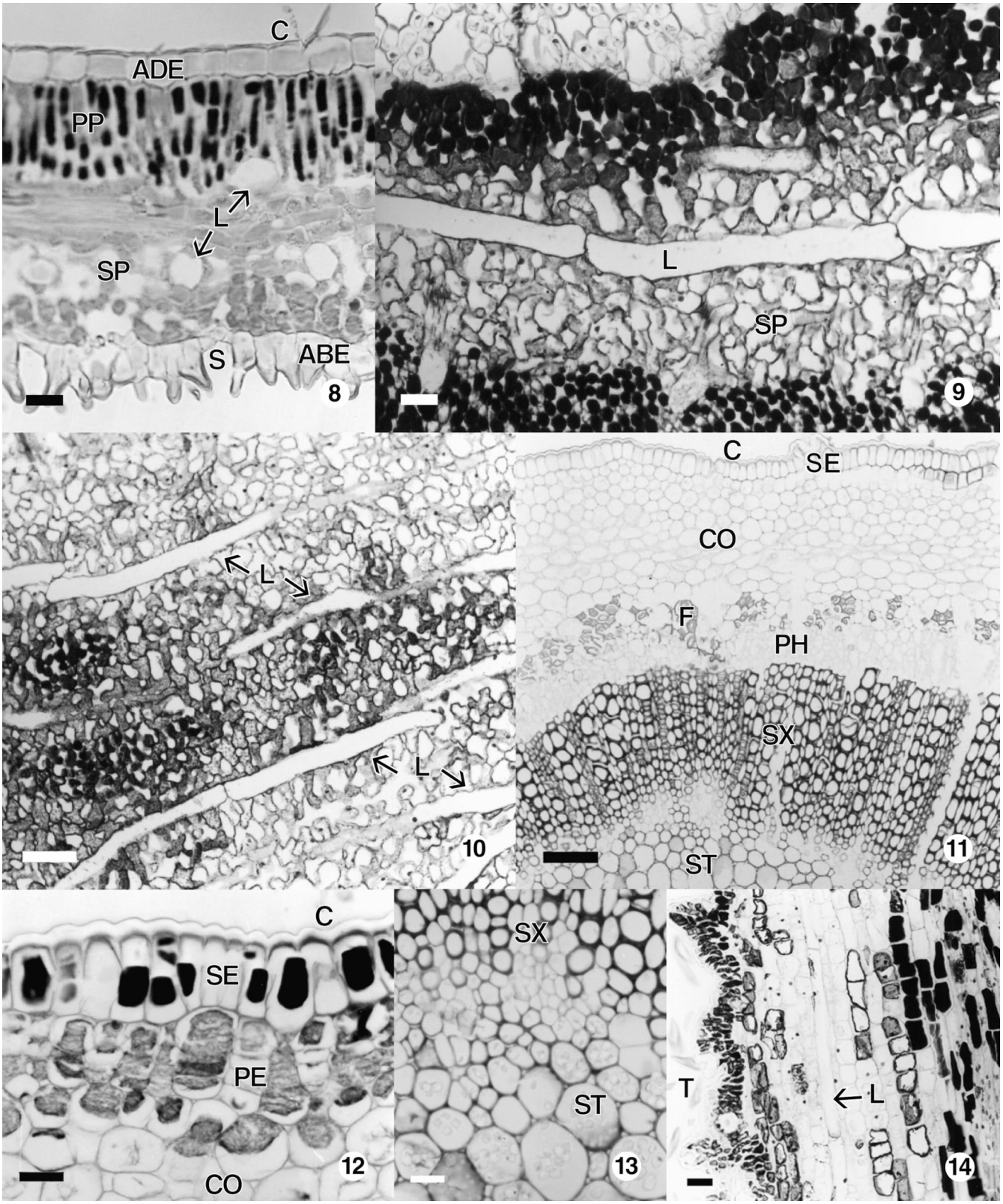


Figs. 1–7. *Galphimia brasiliensis* (L.) A. Juss. **1–2.** Field photographs of latex in petiole and blade, respectively. Figs. 3–4. Blade in transverse section, with laticifers immersed in spongy parenchyma. **3.** Cleared section. Bar = 50 μm. **4.** Uncleared section. Bar = 50 μm. **5.** Blade in longitudinal section showing an articulated and unbranched laticifer. Bar = 50 μm. Figs. 6–7. Stem. **6.** Transverse section. Bar = 50 μm. **7.** Longitudinal section. Bar = 50 μm. ABE, abaxial epidermis; ADE, adaxial epidermis; B, blade; C, cuticle; CH, chlorenchyma; CO, cortex; L, laticifer; LX, latex; P, petiole; PH, phloem; PI, pith; PP, palisade parenchyma; S, stomata; SP, spongy parenchyma; VB, vascular bundles; X, xylem.

dorsiventral mesophyll includes one layer of palisade parenchyma, composed of axially elongated cells slightly attached to one another and representing one-third of the total thickness. The spongy parenchyma has conspicuous intercellular spaces in which are immersed abundant laticifers distributed in the proximity of collateral vascular bundles. The laticifers were easily detected in transverse and longitudinal sections of the blades (Figs. 3–5) and stained intensively in uncleared sections (Fig. 4). In transverse section, laticifers are circular, 21.6–28.3 (–35.1) μm in diameter, numerous, with a parallel and regular pattern in relation to the axis of the leaf and (51.3–) 126–139 μm apart (Fig. 3–4). In longitudinal section, laticifers are tubular, articulated, not ramified, and not anastomosing (Fig. 5). The cells of the laticifers are 64.1–87.7 μm long, elongated, with dilated extremes like a trumpet and semi-thick terminal walls.

The stem in transverse and longitudinal sections is illustrated in Figs. 6–7. The stem is circular in shape and is bounded by a one-layered epidermis with stomata. The cortex consists of subepidermal spongy chlorenchyma and parenchyma tissue, then secondary phloem and xylem and a conspicuous pith of parenchyma cells. Laticifers are distributed in the cortex and are scarcely recognizable due to their similarity to parenchyma cells (Fig. 7).

Verrucularia glaucophylla—The blade in transverse and longitudinal sections is shown in Figs. 8–10. The adaxial epidermis is one-layered, the cells quadrangular, with a thick outer tangential cell wall and cuticle. The conspicuously papillate abaxial epidermis exhibits one simple or occasionally bifurcate papilla on each cell, and a thick outer tangential cell wall. Trichomes are absent. Stomata are present in the abaxial epidermis overarched by adjacent papillate epidermal cells. The mesophyll is dorsiventral, including one-layered palisade parenchyma, which represents about one-half of the total thickness. The cells of typical palisade parenchyma are axially elongated and attached to one another. The spongy parenchyma has conspicuous intercellular spaces. Abundant laticifers are immersed between the palisade and spongy parenchyma, in the prox-



Figs. 8–14. *Verrucularia glaucophylla* A. Juss. **8.** Blade in transverse section showing laticifers immersed in the mesophyll. Bar = 50 μm. Figs. 9–10. Blade in paradermal section. **9.** Bar = 50 μm. **10.** Bar = 100 μm. Figs. 11–13. Stem in transverse section. **11.** Bar = 100 μm. **12.** Cuticle, epidermis, incipient periderm, and cortical parenchyma in detail. Bar = 20 μm. **13.** Secondary xylem and central pith of parenchyma showing a peripheral starchy cell zone in detail. Bar = 20 μm. **14.** Stem in longitudinal section showing cortical laticifers (arrow). Bar = 25 μm. ABE, abaxial epidermis; ADE, adaxial epidermis; C, cuticle; CO, cortex; F, phloem fibers; L, laticifers; PH, phloem; PE, periderm; PP, palisade parenchyma; S, stomata; SE, stem epidermis; SP, spongy parenchyma; ST, starchy parenchyma cells; SX, secondary xylem; T, trichomes.

imity of collateral vascular bundles. The laticifers were easily detected in both transverse and longitudinal sections of the blades (Figs. 8–10). In transverse section, the laticifers are circular, (31.2–) 46.8–57.2 (–62.4) μm in diameter, numerous, and (52–) 83.2–156 (–176.8) μm apart, with a parallel and irregular pattern in relation to the axis of the leaf (Fig. 8). In longitudinal section, the laticifers are tubular, articulated, not ramified, and not anastomosing. The cells of the laticifers are (369.2–) 431.6–587.6 (–728) μm long, uniform in diameter throughout their length (Fig. 9). In paradermal section the laticifers show a complex sinuous pattern (Fig. 10).

The stem in transverse and longitudinal sections is illustrated in Figs. 11–14. The stem in early stages of secondary growth is circular in shape and surrounded by a one-layered epidermis with stomata (Fig. 11). The epidermal cells are radially elongated, with a thick outer tangential wall and cuticle. There is an incipient subepidermal periderm composed of 3–4 layers of cells (Fig. 12). The cortex contains 5–8 layers of parenchyma cells with laticifers and scarce starch and calcium oxalate druses. The vascular cylinder is composed of partially lignified phloem fibers, secondary phloem, and xylem. A central pith of parenchyma shows a peripheral zone of starchy cells (Fig. 13). Laticifers, present in the cortex, are easily recognized in longitudinal section (Fig. 14) but scarcely recognizable in transverse section because of their similarity to the parenchyma cells in which they are immersed.

DISCUSSION

Anderson (1978) restricted the tribe Galphimieae Nied. to four genera (*Galphimia*, *Lophanthera*, *Spachea*, and *Verrucularia*) on the basis of morphological characters. Cameron et al. (2001), using molecular data from the same four genera, found 100% bootstrap support for the tribe Galphimieae as restricted by Anderson, and Davis, Anderson, and Donoghue (2001) reported similar support, although their study lacked material of *Verrucularia*. Latex has previously been reported from only two genera of the Malpighiaceae, *Lophanthera* and *Spachea* (Anderson, 1981, 2001). The present study constitutes the first report of latex in *Galphimia* and *Verrucularia* and the first description to be published of the laticifers in this family. The fact that laticifers and latex are present in all four genera of the tribe Galphimieae provides a synapomorphy giving additional support to the monophyly of the tribe.

There is an alternate, more interesting way to interpret the presence of latex in the tribe Galphimieae. The family Malpighiaceae was included in a large clade recently named Malpighiales (APG, 1998), with Violaceae, Passifloraceae, Linaceae, Clusiaceae, Peridiscaceae, and Euphorbiaceae, among others (Chase et al., 1993; Savolainen et al., 2000; Soltis et al., 2000). Anderson (1990) suggested that *Lophanthera* may be basal in the family, and the trees published recently (Cameron et al., 2001; Davis, Anderson, and Donoghue, 2001) place the tribe Galphimieae at or near the base of the family's phylogeny. It is therefore tempting to speculate that the presence of latex in this one tribe of Malpighiaceae constitutes a character linking the family to the Euphorbiaceae. In that interpretation, latex in the basal genera of the Malpighiaceae may be a symplesiomorphy inherited from a common ancestor shared with the Euphorbiaceae sensu stricto, i.e., the subfamilies Euphorbioideae and Crotonoideae of Webster (1975). Those are the groups of Euphorbiaceae that have laticifers, and they also share with the Malpighiaceae the character of having only one ovule in each locule of the ovary. The Euphorbioideae further resemble the Malpighiaceae in having simple leaves and simple hairs, their biglandular bracts recall the gland-bearing leaves and sepals of the Malpighiaceae, and their pollen is tricolporate, as in the basal clades of Malpighiaceae (Webster, 1975; Davis, Anderson, and Donoghue, 2001). The laticifers in the Euphorbioideae are non-articulated, whereas in the Crotonoideae, which in most characters are less like the Malpighiaceae, the laticifers are articulated like those reported here for the Galphimieae. Rudall (1987) argued that articulated and non-articulated laticifers probably evolved from a common ancestor in the Euphorbiaceae, and the evidence from the Malpighiaceae would seem to support the presence of articulated laticifers in the common ancestor of Euphorbiaceae sensu stricto and Malpighiaceae.

Because the resolution of the phylogeny at the base of the Malpighiaceae is still rather labile, it would be best to regard the above phylogenetic speculations as preliminary, but they do point the way to future studies. It would be worthwhile to seek laticifers in the genera identified in the recent molecular studies (Cameron et al., 2001; Davis, Anderson, and Donoghue, 2001) as most closely related to the Galphimieae: *Acmanthera* Griseb., *Blepharandra* Griseb., *Byrsonima* L. C. Rich. ex Kunth, *Coleostachys* A. Juss., *Diacidia* Griseb., and *Pterandra* A. Juss. The fact that latex is unknown in those genera does not mean they do not have laticifers; latex had not been reported from *Verrucularia*, either, and we discovered its laticifers only because one of us (W. R. Anderson) predicted their existence on the basis of that genus's position in the tribe Galphimieae. If laticifers are basal in the Malpighiaceae, a symplesiomorphy shared with an outgroup like Euphorbiaceae, one would predict that laticifers, even if non-functional, may have been retained in some of the other genera near the base of the family's phylogeny.

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