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Ontogeny, structure and occurrence of interxylary cambia in Malpighiaceae

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ABSTRACT

Deviations in the regular pattern of secondary growth have occurred multiple times over the history of woody plants and these have been treated under the term cambial variant. Here we present a type of cambial variant that has been studied in the 19th century, but that has never been named or had its ontogeny studied in detail. This cambial variant generates a vascular cylinder with a conspicuous crenate pattern and it is found in two lianescent lineages of Malpighiaceae; *Stigmaphyllon* and a clade informally called the *Banisteriopsis nummifera* group. Twenty species of *Stigmaphyllon* and two species of the *Banisteriopsis nummifera* group were sampled from naturally growing population to investigate ontogenic history starting from shoot apex to the thickest portion of stem by using traditional anatomical methods All species in these two clades have a regular cambium that produces bands of non-lignified axial parenchyma in the xylem by the onset of secondary growth. These xylem parenchyma cells, after a period of secondary growth, acquire meristematic activity and give rise to new, variant cambia referred here as '***interxylary cambia***'. These new cambia give rise to new vascular tissues within the secondary xylem. In *Stigmaphyllon* the variant cambia give rise to a large quantity of variant phloem, and not much variant xylem, while in the members of the *Banisteriospsis nummifera* group a large quantity of both secondary xylem and phloem is formed by every new interxylary cambia. Xylem and phloem originated from both cambial types have features similar to those of other Malpighiaceae lianas, except for the absence of septate fibers and perforated ray cells, the presence of stratified phloem and prismatic crystals in the phloem of the *Banisteriopsis nummifera* group and a concentric ring of cortical secretory cells characteristic of *Stigmaphyllon*. The cambial variant ***interxylary cambia*** is here named for the first time, and this new term is proposed to unmistakably denote its origin from within the secondary xylem, which is likely a synapomorphy for these two Malpighiaceae lineages.

1. Introduction

Regular secondary growth involves the activity of a single vascular cambium that produces secondary xylem to the inside and secondary phloem to the outside; the ancestral condition for all lignophytes (Evert, 2006; Simpson, 2010). This basic model, however, has independently diverged into different patterns in a number of plant lineages, within which the cambium exhibits various modes of secondary growth which differ from the regular pattern. These alternative types of secondary growth, derived from cambial variants, resulted in outstanding stem architectures and are notably common in lianas. This phenomenon has been documented in at least 22 orders (Angyalossy et al., 2012, 2015) and includes groups as diverse as Gymnosperms (Cycadaceae and Gnetaceae), Angiosperm magnoliids (e.g., Piperales), and numerous

families in the eudicots Rosids and Asterids. These anatomical architectures have been surveyed since the turn of the 19th century (Van Tieghem, 1884; Schenck, 1893; Pfeiffer, 1926; Obaton, 1960; Bhambie, 1972; Carlquist, 1991, 2001; Angyalossy et al., 2012, 2015) by authors who have shown how the unusual activity of a single cambium or multiple cambia can produce a wide range of different stem anatomical architectures, such as 1) asymmetrical stems; 2) interxylary phloem; 3) intraxylary phloem; 4) axial elements in plates, i.e, extremely wide and tall xylem and phloem rays alternating with axial elements; 5) phloem arcs and wedges (interrupted xylem); 6) fissured stems; 7) successive cambia; and 8) compound stems (see Angyalossy et al., 2015 for a thorough explanation of each type).

Malpighiaceae is a plant family that contains several lineages of lianas where at least four different types of cambial variants have been

documented so far: 1) interxylary phloem; 2) asymmetrical stems; 3) phloem wedges interrupting the xylem, and 4) fissured stems (Chodat 1892; Schenck, 1893; Solereder, 1908; Chodat et Vischer 1917; Obaton, 1960; Angyalossy et al., 2015). However, here we describe a cambial variant that although mentioned in the 19th century French and German literature, was later overlooked by posterior revisions of cambial variants and has never been properly named, ontogenetically described, or surveyed. This cambial variant is found in the stems of at least two lineages of Malpighiaceae, i.e., the genus *Stigmaphyllon* A.Juss. and the *Banisteriopsis nummifera* group (*sensu* Gates, 1982); both of which are monophyletic lineages within the Stigmaphylloid clade (*sensu* Davis and Anderson, 2010). *Stigmaphyllon* and *Banisteriospsis* are large genera with samaroid fruits (winged mericarps) and are widely distributed in the Neotropics (Anderson, 1997; Davis and Anderson, 2010). *Banisteriopsis* is strictly Neotropical, while *Stigmaphyllon* is also represented in Asia and Oceania by the members of *Stigmaphyllon* subg. *Ryssopterys* (C. Anderson, 2011). *Banisteriopsis* are either shrubs or lianas, while *Stigmaphyllon* are typically lianas, or less often subshrubs. The *Banisteriopsis nummifera* group, however, only contains robust lianas, and is represented by five species, *B. nummifera* (A.Juss.) B.Gates of wide distribution and *B. anisandra* (A.Juss.) B.Gates, *B. gardneriana* (A.Juss.) W.R.Anderson & B.Gates, *B. lyrata* B.Gates, and *B. sellowiana* (A.Juss.) B.Gates, with more restricted distributions (Gates, 1982). This group, initially recognized by Gates (1982) based on morphological features, has emerged as monophyletic in the most recent phylogeny of Malpighiaceae (Davis and Anderson, 2010).

The novel cambial variant described here is marked by the presence of multiple cambia in the same stem, and shows intermediate characters between successive cambia and fissured stems (as can be seen in Figs. 1 & 2 D). Successive cambia is the presence of multiple cambia, where every new cambium is typically formed outside of the original vascular cylinder, concentrically (Angyalossy et al., 2015). Fissured stems are derived from a combination of phloem wedges and parenchyma proliferation, dissecting the entire vascular cylinders (Obaton, 1960; Cabanillas et al., 2017). Successive cambia have never been described in Malpighiaceae, although its presence was indicated for *Banisteriopsis nummifera* (Angyalossy et al., 2015), while fissured stems have been described for several Malpighiaceae genera, such as *Alicia* W. R. Anderson (Chodat and Vischer, 1917), *Callaeum* Small (Cabanillas et al., 2017), *Diplopterys* A.Juss. (Chodat and Vischer, 1917), and *Flabellaria* Cav. (Obaton, 1960). In order to understand the ontogeny and uniqueness of the interxylary cambia in Malpighiaceae and its relationship to other cambial variants known for the family, we carried out an anatomical survey of the stems in several species of *Stigmaphyllon* and the *Banisteriopsis nummifera* group. Our survey also allowed us to generate full descriptions of the secondary xylem, phloem, and periderm of the studied species.

2. Material and methods

Altogether we sampled 22 liana species, of these 20 of *Stigmaphyllon*, 19 from the Neotropics (subgenus *Stigmaphyllon*) and one of the Old World [Micronesia, Caroline Islands](subgenus *Ryssopterys*), and two out of the five species that belong to the *Banisteriopsis nummifera* group (Appendix A).

2.1. Ontogenetic analysis and anatomical procedures

Most species were collected from plants growing in natural habitat, their vouchers were prepared and stems sampled from the apex to their thickest portions, with internodes fixed in FAA 50 (Johansen, 1940) to preserve all soft tissues (non-lignified parenchyma, phloem and
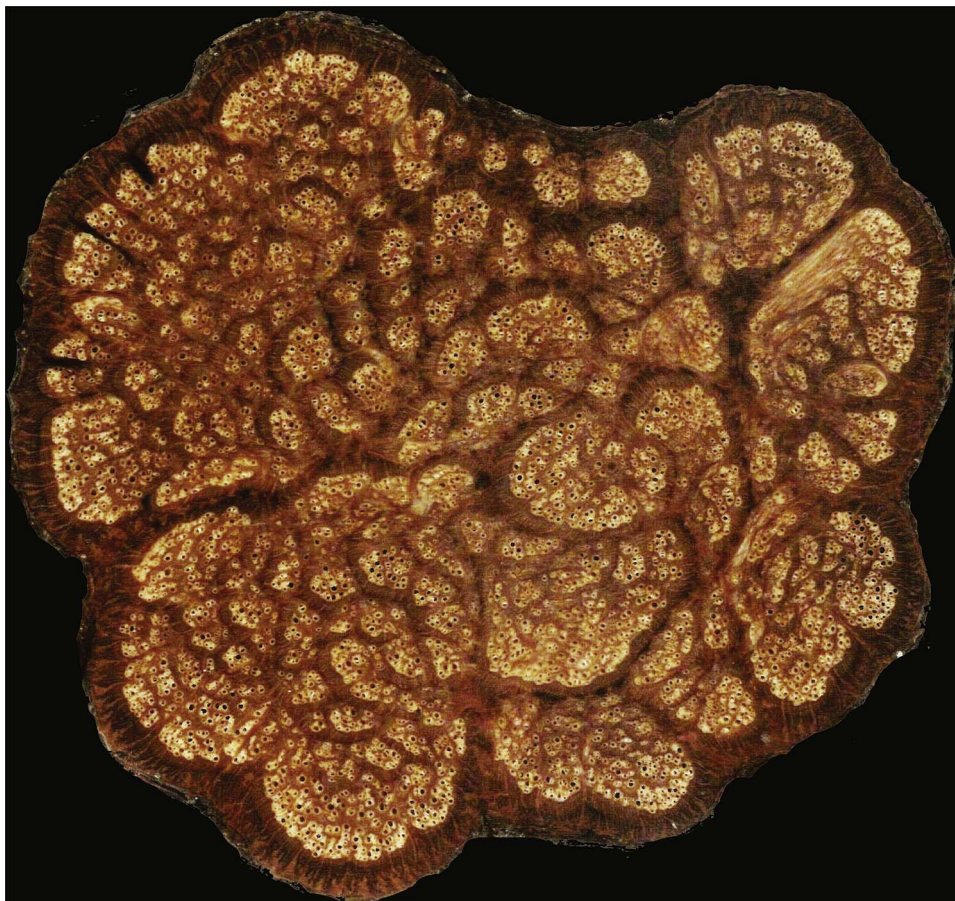

Fig. 1. *Stigmaphyllon acuminatum* transverse section. Stem diameter = 5 cm. Conspicuous cambial variant resulting in a crenate pattern. Photo by C.L. Bastos.
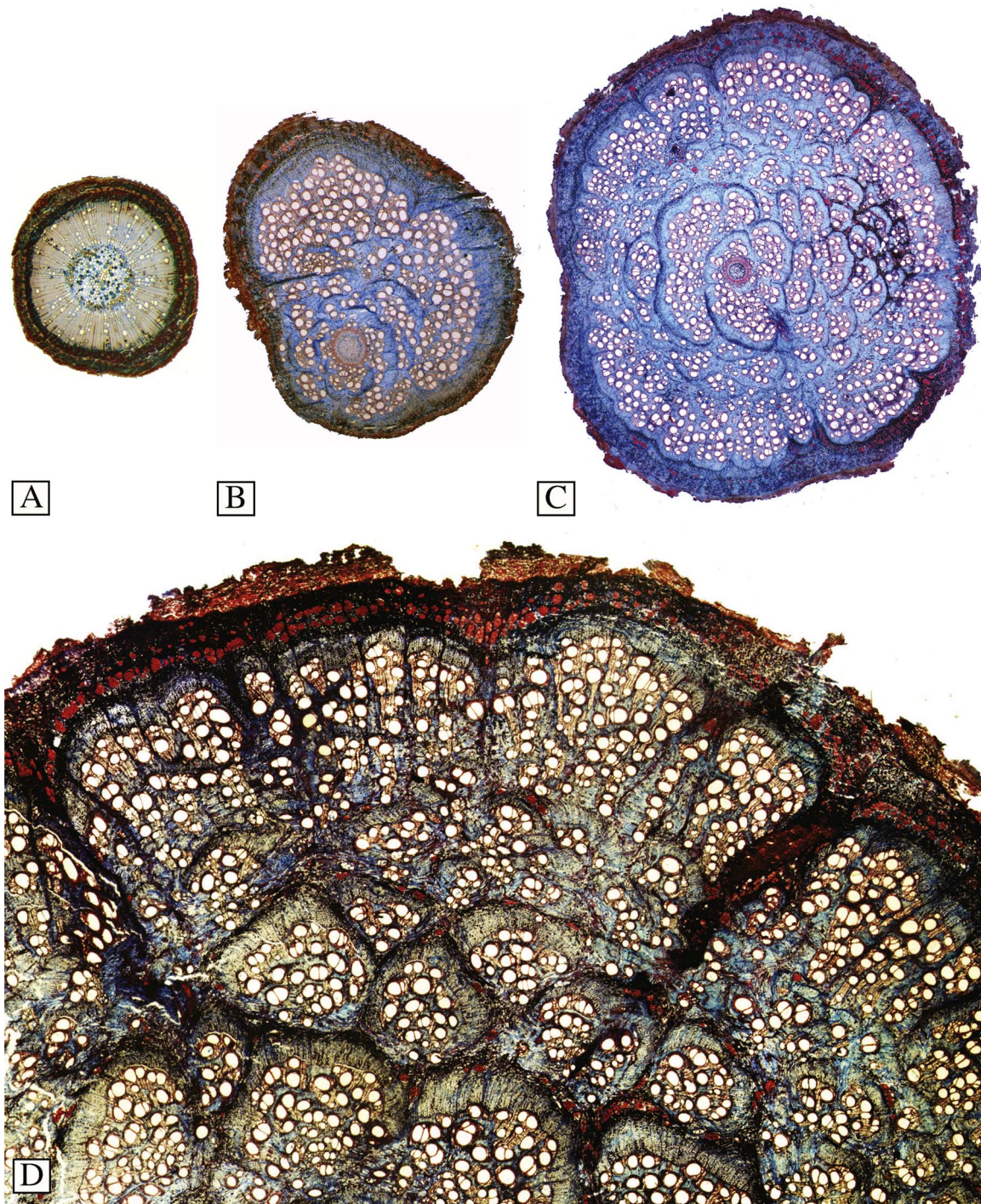
Fig. 2. *Stigmaphyllon acuminatum* development, transverse sections. A. Regular secondary growth in young stem. B. Interxylary cambia appear first on the innermost non-lignified parenchyma bands. C–D. Large stems, showing a crenate anatomical pattern with several arcs of interxylary cambia. Diameter of stems: A = 0.3 cm, B = 1.1 cm, C = 2.8 cm, D = 5 cm.

meristematic cells). These samples were transferred to a solution of 70% ethanol after a week of fixation. The samples of the Old World member, *Stigmaphyllon* (=*Ryssopterys*) *abutilifolium* (A.Juss.) C.E.Anderson was gathered from a dried voucher from the US herbarium and rehydrated following the IAWA Bark Committee procedures (Angyalossy et al., 2016).

All internodes from the onset of secondary growth to the base of the stem were hand-sectioned to delimit all ontogenetic stages in the formation of cambial variants. Several internodes were chosen as discrete stages and embedded in polyethylene glycol 1500 (Rupp, 1964) and later sectioned with the aid of a sliding microtome. In order to obtain

entire cross-sections to illustrate the development of stems, we used a Styrofoam resin, produced as indicated by Barbosa et al. (2010). Sectioned materials were double stained in astra blue and safranine (Bukatsch, 1972) or in alcian blue and safranine (Johansen, 1940) and mounted in a synthetic resin to produce permanent slides. Histochemical tests to detect the presence of phenolic substances and their nature were performed using ferric chloride and vanillin-HCL, as indicated by Angyalossy et al. (2016). Details of cell types were obtained after embedding cubes of 3 mm containing cambium, secondary xylem and phloem in glycol methacrylate (Historesin, Leica Microsystems), subsequently sectioned with aid of a rotary microtome and stained in

0.05% toluidine blue in glacial acetic buffer at pH 4.7 (O'Brien et al., 1964).

Anatomical descriptions and quantitative measurements of xylem and phloem followed the recommendations of the IAWA Committees for Hardwoods (IAWA Committee, 1989) and Barks (Angyalossy et al., 2016). We analyzed regular and variant phloem separately; however, since they were qualitatively equal, we only provide here one description, while quantitatively we present the differences in sieve element diameter encountered for both phloem types. In *Stigmaphyllon* not much xylem is produced, so the descriptions corresponds to the regular xylem only. The secondary xylem was similar quantitatively and qualitatively in members of the *Banisteriopsis nummifera* group, except for a few differences that are highlighted in the text. Measurements were obtained through the aid of ImageJ 1.45 s (http://imagej.nih.gov/ij). Mean values obtained from regular and variant phloem were compared with unpaired *t*-tests.

3. Results

3.1. Morphology and macroanatomy of the stems

Stems of most *Stigmaphyllon* are cylindrical or less often lobed (some *S. blanchetii* C.E.Anderson) or flat (some *S. tomentosum* A.Juss.). Out of the 20 species of *Stigmaphyllon* we studied, 14 (70%) of them exhibited stems with cambial variants with a conspicuous crenate pattern, as seen in transverse section (Fig. 1). The remaining six (*Stigmaphyllon abutilifolium*, *S. auriculatum* (Cav.) A.Juss., *S. blanchetii*, *S. ciliatum* (Lam.) A.Juss., *S. cordatum* Rose ex Donn.Sm., and *S. sinuatum* (DC.) A.Juss.) lacked any cambial variant. However, with the exception of *S. sinuatum*, sectioning of these species was based on stems < 1 cm in diameter, and in order to confirm the absence of cambial variants it would be necessary to survey wider stems, as these features sometimes only develop in older stages of development. For example, in 2 cm diameter stems of *S. ellipticum* (Kunth) A.Juss. the presence of cambial variant was evident, while a sample of 0.8 cm diameter of the same species did not show any.

Stems of the *Banisteriopsis nummifera* group are cylindrical and the two sampled species (*B. gardneriana* and *B. nummifera*) had conspicuous cambial variant.

3.2. Ontogeny of the interxylary cambia

Cambial variants in *Stigmaphyllon* and the *Banisteriopsis nummifera* group all belong to the same type, although they look different at first sight. They are marked by the presence of multiple cambial segments, distributed concentrically in discontinuous arcs, forming a coalescing crenate, fish scale-like pattern (Figs. 1, 2B–D, 5B). These multiple cambia always produce both xylem and phloem. The apparent differences observed among the species are due to the production of less secondary phloem by the multiple cambial segments in some species as compared to others. In those species with a more conspicuous production of phloem by the multiple cambial segments, the lignified xylem cells appear as embedded in phloem and non-lignified xylem axial parenchyma (Fig. 3A–B). All species in these two lineages, even those without cambial variants, have abundant non-lignified xylem axial parenchyma in bands (Fig. 4A–D).

At the onset of secondary growth, all species display a regular pattern (Fig. 2A). The vascular cambium, however, soon starts producing non-lignified bands of xylem axial parenchyma (Fig. 4A). At first, the parenchyma bands formed are narrow (Fig. 4A), 1–3 cells wide, but as development progresses, cambium starts producing 7–10 cells wide bands of axial parenchyma (Fig. 4B–C). The first new cambia in *Stigmaphyllon* always differentiate around the innermost, oldest bands of non-lignified parenchyma closest to the pith (Fig. 4D), and newer cambia gradually arise centrifugally (Fig. 2B–D). Not all bands of non-lignified xylem axial parenchyma differentiate into a new cambium,

some remain unchanged.

This cambial variant is derived from non-lignified xylem parenchyma and emerges within the xylem. In order to distinguish this cambial variant from other cambial variant types we propose the term ***interxylary cambia*** to denote the origin of cambial tissue within the xylem. In some species, new interxylary cambia appear in young stems (e.g. *Stigmaphyllon lalandianum* A.Juss.), while in others they appear only in stems wider than 1 cm in width (e.g., *S. bonariense* (Hook. & Arn.) C.E. Anderson, *S. ellipticum, S. sinuatum*).

The new cambia in *Stigmaphyllon* produce abundant secondary phloem (Figs. 2D, 3A–B, 4D–E) and only little amounts of xylem that is mainly composed of fibers. In species where the activity of new cambia is very intense, most of the stem is occupied by phloem, e.g., *S. lalandianum* (Fig. 3A). In others, while axial parenchyma is very abundant, only few parenchyma bands differentiate into new cambia (Fig. 4C; *S. ellipticum*).

Most of the new cambia differentiated in *Stigmaphyllon* preserve a regular polarity, producing phloem to the outside and xylem to the inside, successively (Figs. 2B–D, 4D). However, in thicker stems, cambia have been detected as differentiating also perpendicularly to the regular cambium, i.e., parallel to the rays (Fig. 4F). Given that parenchyma bands are arranged in discontinuous bands, the new differentiated tissues result in a crenate, fish scale-like pattern (Figs. 1, 2B–D).

Interxylary cambia in the *Banisteriopsis nummifera* group differs from that of *Stigmaphyllon* by its centripetal differentiation from non-lignified axial parenchyma bands that are next to the first produced cambium (regular cambium) towards the center of the stem (Fig. 5A–B). Curiously, the innermost bands of non-lignified parenchyma do not seem to differentiate into new cambia, since a stem 8 cm in diameter that we sampled did not show signs of cambial differentiation within the inner bands (Fig. 5B). Another marked difference is that in the *B. nummifera* group, the new cambia produce large quantities of both phloem and xylem (Fig. 5C). In contrast, *Stigmaphyllon*, where little quantities of xylem are produced, composed mainly of fibers. The latest produced xylem (variant xylem) can be distinguished from the older xylem (regular xylem) by its axial and radial parenchyma cells that contain less phenolic substances and thinner-walled fibers (Figs. 5C, 6 D), appearing less stained in the anatomical preparations (Figs. 5C, 6D). Since the production of new vascular tissues within the already differentiated regular xylem is intense, it is possible to see parenchyma elongation and division within and adjacent to the regular xylem (Figs. 5D, 6D), which aims to accommodate the new secondary growth within the old stem. This elongation occurs in cells of the radial and axial system (Figs. 5D, 6D). Because the axial parenchyma bands in *Banisteriopsis nummifera* and *B. gardneriana* are much longer tangentially than those of *Stigmaphyllon*, the appearance of the final anatomical architecture of these two species is intermediate between a crenate pattern (as seen in *Stigmaphyllon*) and a successive cambial pattern (Fig. 5A–B).

3.3. Secondary xylem

The secondary xylem of members of *Stigmaphyllon* and the *Banisteriopsis nummifera* group has indistinct growth rings (Figs. 2D, 3A, 6A,D). The vessels have no particular arrangement, but exhibit a conspicuous vessel dimorphism (Fig. 6A–D), with large vessels either solitary, in tangential or radial multiples of 2–3(4) cells (Fig. 6A–D). Narrow vessels are commonly associated with wide vessels and grouped in clusters or in radial chains of usually 5–12 cells (Fig. 6A–D), in *Stigmaphyllon* some with over 30 cells associated in a single radial row. Radial chains of narrow vessels are frequently present side-by-side, forming larger conglomerates (Fig. 6A). Perforation plates are simple; intervessel pits are alternate (Fig. 6G), small (4–7 μm; Fig. 6G, Table 1), and vestured (Fig. 6G); vessel-ray pits with distinct borders, similar to intervessel pits in size and shape; vessel diameter ranging from 94 to 236 μm for wide vessels (Table 1) and 18–28 μm for narrow vessels (Table 1); wide vessel element length 220 ± 20 μm (Table 1); wide
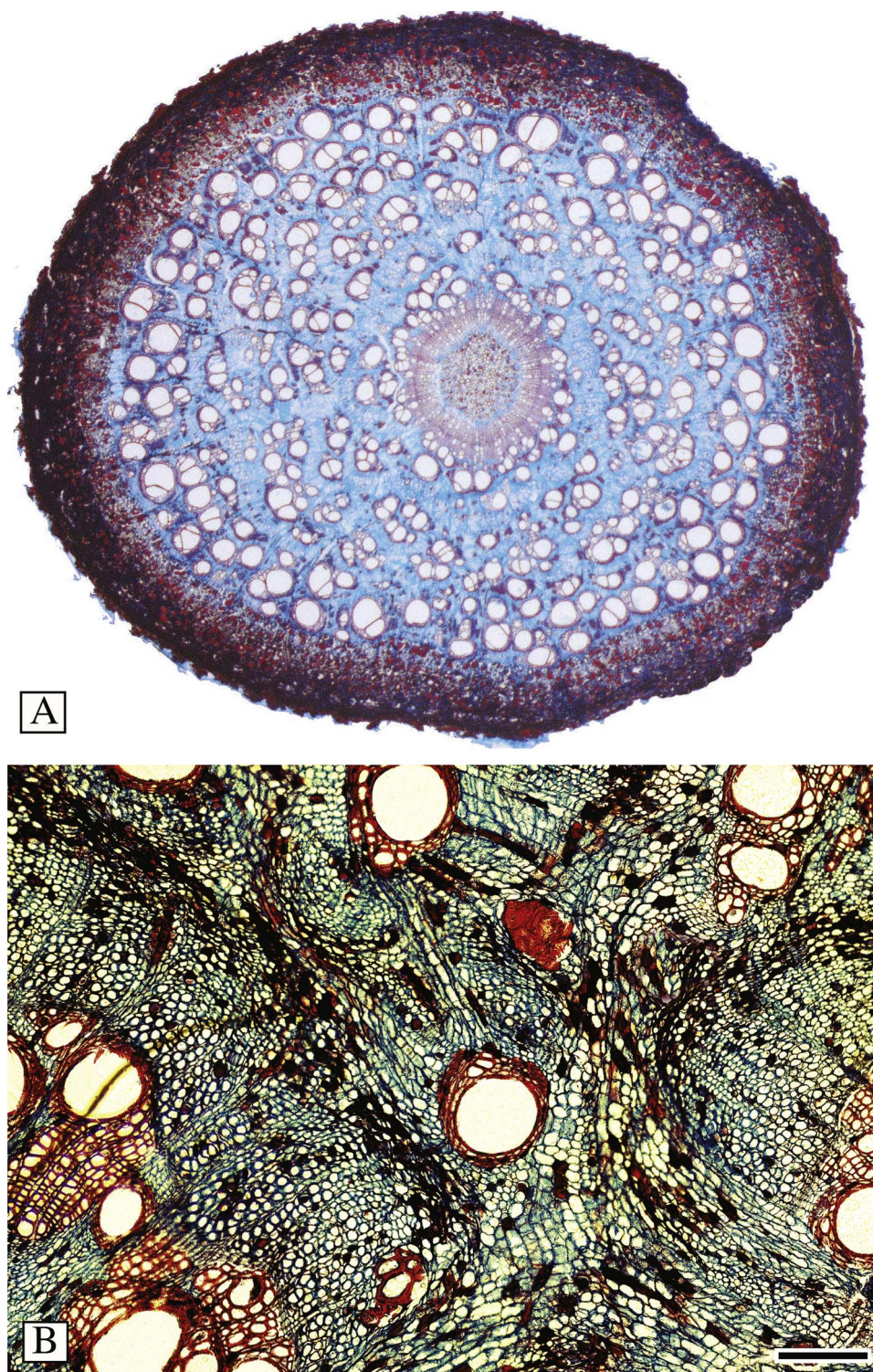
Fig. 3. A. *Stigmaphyllon lalandianum* transverse section (TS), interxylary cambia produce so much secondary phloem that islands of secondary xylem become embedded in non-lignified tissues. B. *Stigmaphyllon acuminatum* TS. Intense activity of the interxylary cambia result in vessels surrounded by fibers immerse in a matrix of non-lignified xylem axial parenchyma of the regular cambium and the variant secondary phloem. Scales: A = stem of 1.1 cm diameter, B = 200 μm.

vessel frequency about 16 ± 4 vessels per mm^2; when narrow vessels are included it increases to 100 ± 30 vessels per mm^2 (Table 1); tyloses present only in isolated vessels in a few samples, generally around injured areas.

Fibers are thick walled (Figs. 4E, Fig. 55C, 6A–C), with simple to minutely bordered pits. In *Banisteriopsis*, the variant xylem has thinner fiber walls than the regular xylem, making it possible to visually distinguish both xylem types (Figs. 5B, 6D). Fibers are the main tissue

produced by the variant, interxylary cambia of *Stigmaphyllon*.

Axial parenchyma of two types, one vasicentric to scanty paratracheal (Fig. 6A–D), lignified, and the other, much more abundant, non-lignified, in narrow to broad bands distributed in a crenate pattern (Fig. 6A–C) from where interxylary cambia are later developed. Parenchyma strands are composed of four cells in both *Stigmaphyllon* and member of the *Banisteripsis nummifera* group (Fig. 6E; two in *S. abutilifolium*). Production of phloem by the multiple interxylary cambia
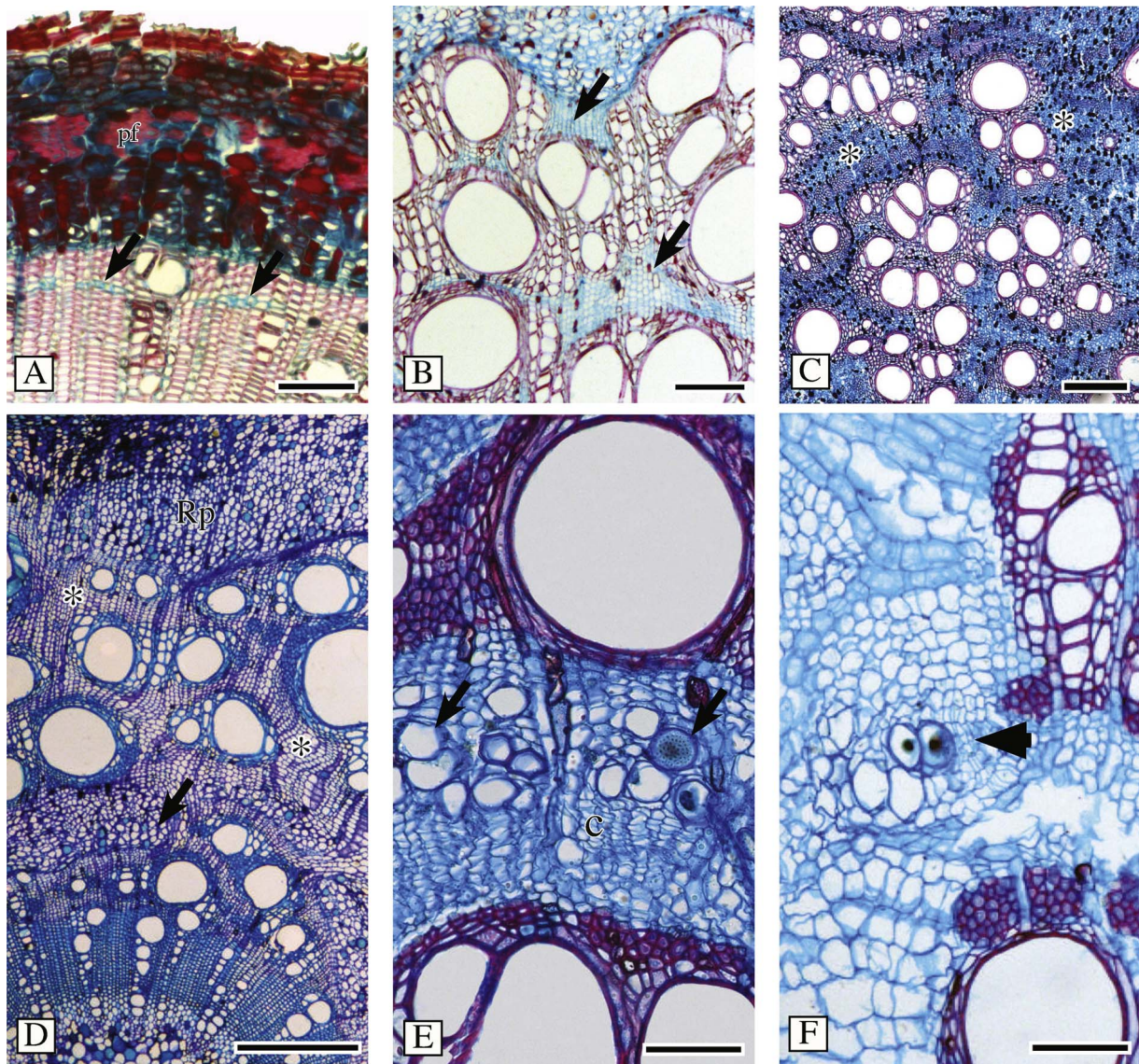
Fig. 4. A–B, D–F, *Stigmaphyllon acuminatum*, C, *Stigmaphyllon ellipticum*, transverse sections. Interxylary cambia development in detail. A. At the onset of secondary growth, lines of non-lignified axial parenchyma of 1–3 cells are formed (arrows). Pf = pericyclic fibers. B. Later on development, instead of lines, bands are formed, usually more than 4 cells wide. Note non-lignified xylem axial parenchyma being produced (arrow). C. Abundant non-lignified axial parenchyma (asterisks) is usually present in the stem of *Stigmaphyllon*. D. New cambia producing phloem start its activity in the innermost non-lignified parenchyma bands (arrows). Asterisks indicate younger non-lignified parenchyma bands where new cambia has not yet been formed. Rp = Regular phloem. E. New cambia produce mainly phloem. Sieve tubes are wide and have simple sieve plates (arrow). The secondary xylem produced is composed mainly of fibers. C = interxylary cambium. F. Some cambia may be formed perpendicularly to the other tissues (arrowhead). Scale bars: A, D = 100 μm, B–C, E–F = 200 μm.

sometimes creates a pattern of vessels surrounded by fibers immersed in non-lignified tissues (Fig. 3B), which correspond to both axial xylem parenchyma and the variant phloem produced within the stem.

Rays are non-storied, 2–6 cells wide (Fig. 6E; 2 cells wide in *Stigmaphyllon abutilifolium*, and up to 6 cells wide in *S. saxicola*), and dimorphic, with high and short rays occurring side by side, high rays taller than 1 mm. Low rays are heterocellular with procumbent body and 3–4 marginal square to upright cells and high rays are heterocellular mixed, with procumbent, square and upright cells mixed throughout the ray (Fig. 6F); abundant prismatic crystals present in xylem chambered axial parenchyma cells (Fig. 6H).

3.4. Bark

3.4.1. Secondary phloem (regular and variant)

Qualitatively, the regular and variant secondary phloem types are equal, and therefore a single description is provided. Quantitative differences in sieve tube element diameter were detected, and details are given in Table 1.

Growth rings are indistinct (Fig. 7A). Conducting phloem occupies a larger area in *Stigmaphyllon* than in the *B. nummifera* group (Fig. 7A–B). Nonconducting phloem is marked by the collapse of sieve tubes, dilatation of axial and ray parenchyma, and sclerosis of parenchyma cells (Fig. 7A–B).

Sieve tubes are diffuse (Fig. 7C), solitary or in radial groups of 2 cells (Fig. 7C), sieve plates are simple, transverse to slightly inclined (Fig. 7E), ranging from 224 to 1899 μm^2 in area (19–49 μm in diameter) in the regular phloem (Table 1), and 328–1146 μm^2 in area (17–38 μm in diameter) in the variant phloem (Table 1) and with variant and regular phloem statistically different between one another (Table 2), but without a distinctive pattern; sieve tubes are sometimes narrower or wider in one of the phloem types (Tables 1–2). Sieve tube elements length 200 ± 50 μm (Table 1), sieve pores ranging from 3 to 6 μm in diameter (Table 1). Slime plug always evident at the sieve plates (Fig. 7D).

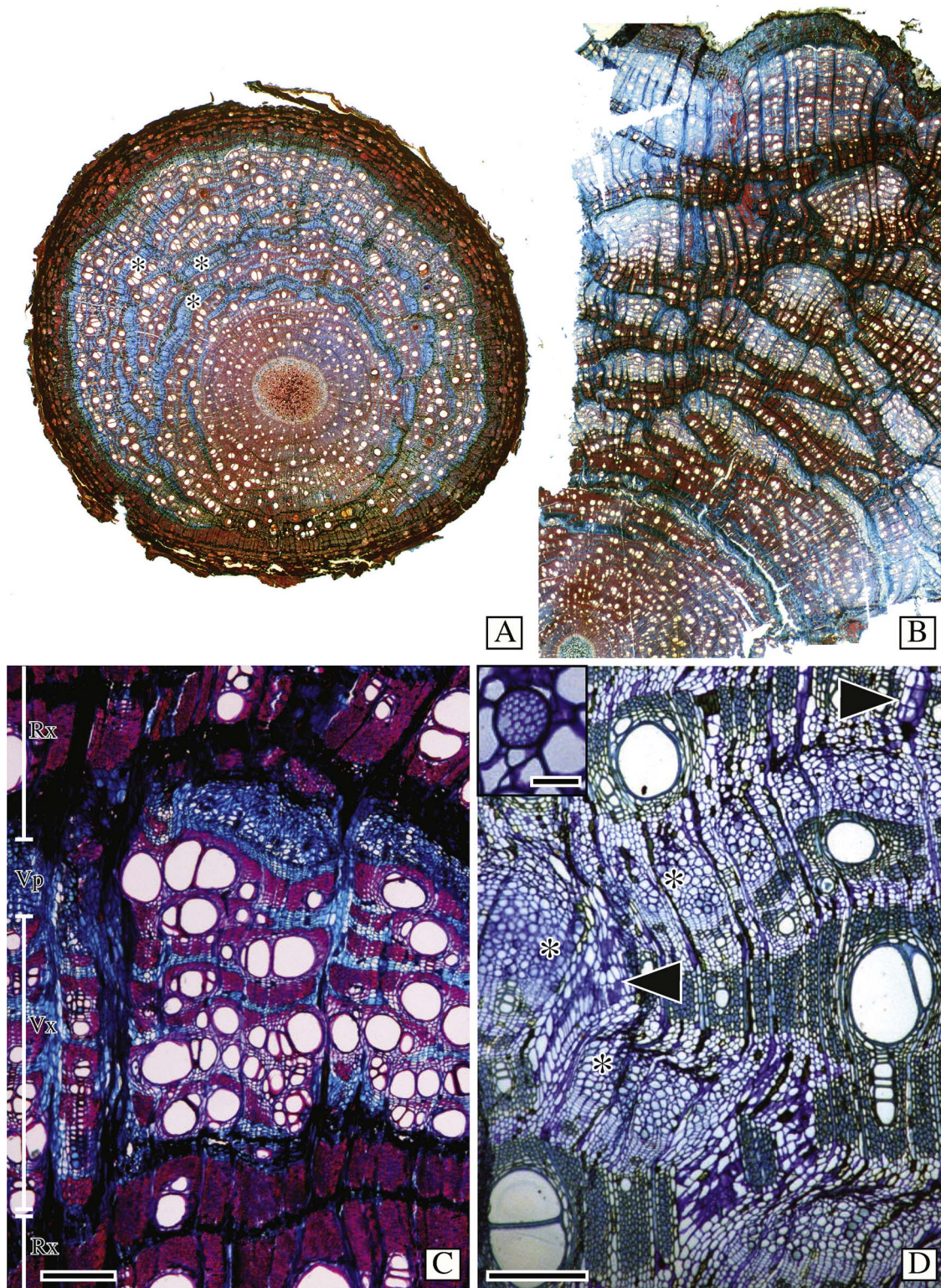One to two companion cells per sieve tube element (Fig. 7C), as seen

Fig. 5. Interxylary cambia development in *Banisteriopsis nummifera*, transverse sections. A. Adult stem, interxylary cambia differentiate in non-lignified axial parenchyma bands far from the pith (asterisks). B. Large stem, with multiple interxylary cambia and their products (secondary xylem and phloem). Note that the new xylem, produced by the interxylary cambia, appear less stained than the previously formed xylem, since it has less phenolic substances and thinner walled fibers. C, Detail of the variant xylem derived from the interxylary cambia (Vx), which has less phenolic substances and fibers with thinner walls than the previously formed, regular xylem (Rx), derived from the regular cambium. D. Bands of interxylary cambia and its derived phloem (asterisks) frequently coalesce. Note cell elongation to accommodate secondary growth (arrowheads). Detail showing sieve tube bearing simple sieve plates. Rx = regular xylem, Vp = Variant phloem, Diameter of stems: A = 1.7 cm, B = 8 cm. Scale bars: C = 200 μm, D = 400 μm.
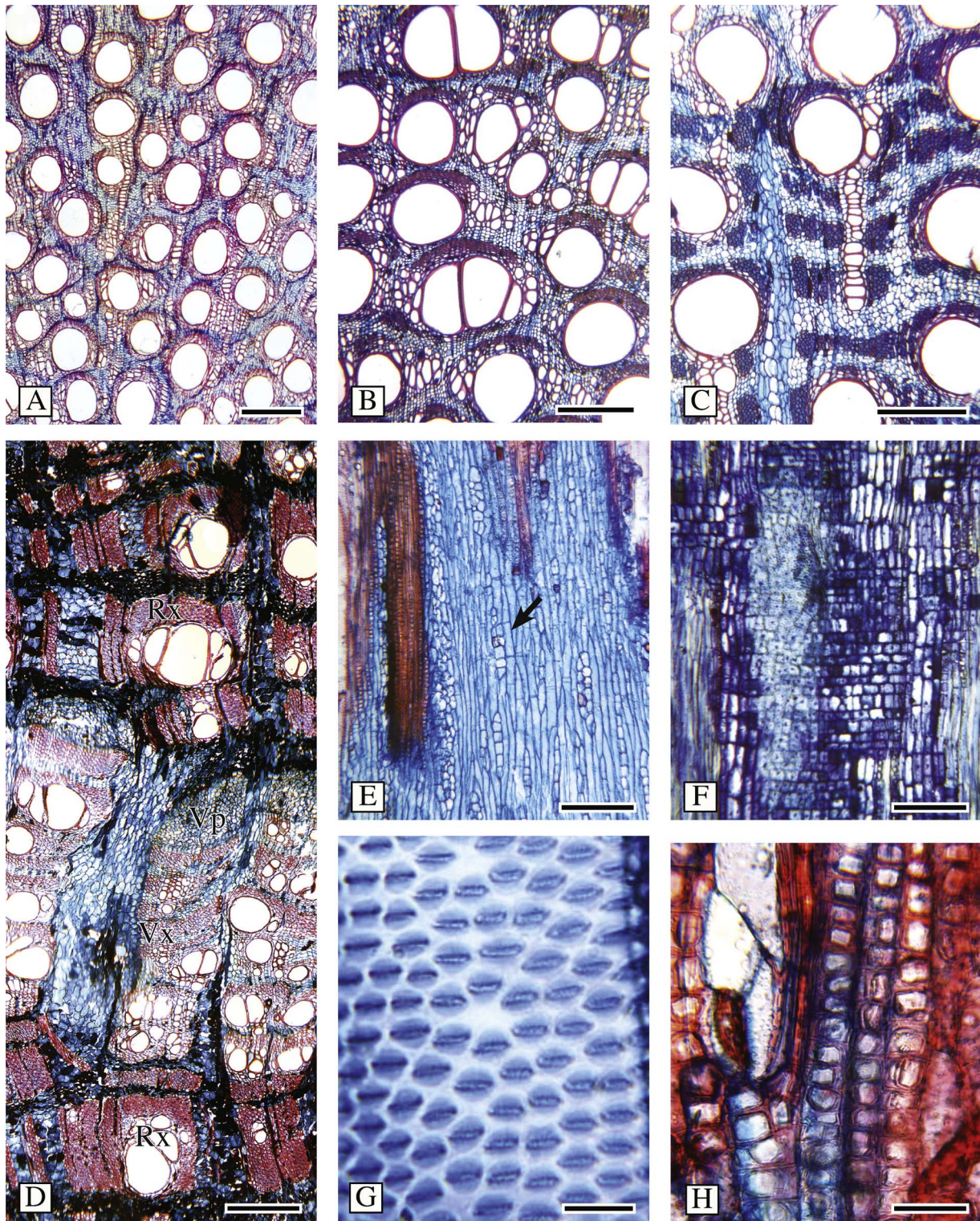
Fig. 6. Wood anatomy. A. *Stigmaphyllon sinuatum* transverse section (TS). Growth rings indistinct. A-C. Vessel dimorphism evident. Wide vessels solitary or in multiples of 2–3, combined with groups of narrow vessels. Non-lignified axial parenchyma in bands evident. B. *Stigmaphyllon blanchetii* TS. C. *Stigmaphyllon saxicola* TS. D. *Banisteriopsis gardneriana* TS. A band of variant xylem (Vx) and variant phloem (Vp) sandwiched by the previously formed, regular xylem (Rx). Note that the regular xylem (Rx) has thicker fibers and more tannins than the variant xylem (Vx), derived from the interxylary cambium. E. *Stigmaphyllon lalandianum* longitudinal tangential section (LT) in an area of non-lignified axial parenchyma. Axial parenchyma in series of 4 cells (arrow). Rays 2–4 seriate. F. *Stigmaphyllon cavernulosum* longitudinal radial section (LR), rays heterocellular mixed. G. *Stigmaphyllon cavernulosum* LR, vestured pits. H. *Stigmaphyllon catingicola* LT, prismatic crystals in chambered axial parenchyma cells. Scale bars: A, D = 400 μm, B–C, E = 200 μm, F = 350 μm, H = 40 μm.

in transverse section. When two companion cells are present, each one lies on opposite sides of the sieve tube (Fig. 7C). Companion cells are fusiform.

Dilatation due to cell expansion and division is evident in the rays, axial parenchyma, and the cortex.

Axial phloem parenchyma constitutes the ground tissue (Fig. 7C), 4 cells per strand (2 cells in *S. abutilifolium*). Undergoing sclerification in the nonconducting phloem.

Table 1
Quantitative data of the secondary xylem and phloem of *Banisteriopsis nummifera* group and *Stigmaphyllon*.

| Species	Maximum stem diameter sampled (cm)	Secondary xylem						Secondary phloem						
								Regular phloem				Variant phloem		
		Vessel diameter (mean)		Vessel length (μm)	Vessel frequency (wide vessels) mm²	Vessel frequency (with narrow vessels) mm²	Intervessel pit size (μm)	Sieve tube area (μm²)	Sieve tube diameter (μm)	Sieve tube element length (μm)	Sieve pore size at sieve plate (μm)	Sieve tube area (μm²)	Sieve tube diameter (μm)	Sieve pore size at sieve plate (μm)
		Wide vessels (μm)	Narrow vessels (μm)											
Banisteriopsis gardneriana	8.0	147 ± 44	22 ± 9	?	16 ± 4	94 ± 25	?	310 ± 98	20 ± 3	?	?	469 ± 108	24 ± 3	2.08 ± 0.15
Banisteriopsis nummifera	2.3	137 ± 43	24 ± 7	205 ± 33	16 ± 6	70 ± 30	5.9 ± 0.7	380 ± 90	22 ± 3	220 ± 35	2.8 ± 0.4	366 ± 80	21 ± 2	3.7 ± 0.7
Stigmaphyllon abutilifolium	0.9	221 ± 62	21 ± 8	209 ± 27	16 ± 4	70 ± 12	4.5 ± 1.1	?	?	?	?	?	?	?
Stigmaphyllon acuminatum	5.0	146 ± 49	20 ± 8	?	?	?	?	488 ± 104	25 ± 3	?	3.2 ± 0.7	667 ± 84	29 ± 2	3.3 ± 0.5
Stigmaphyllon auriculatum	1.0	110 ± 32	19 ± 8	?	32 ± 6	118 ± 12	?	301 ± 105	19 ± 3	?	?	na	na	na
Stigmaphyllon blanchetii	1.6	150 ± 62	24 ± 7	245 ± 23	16 ± 4	144 ± 30	5.5 ± 0.6	494 ± 134	25 ± 3	241 ± 50	3.0 ± 0.7	na	na	na
Stigmaphyllon bonariense	2.8	151 ± 43	20 ± 7	207 ± 42	15 ± 2	118 ± 33	5.5 ± 0.7	578 ± 126	27 ± 3	276 ± 42	2.1 ± 0.3	397 ± 113	22 ± 3	2.6 ± 0.4
Stigmaphyllon caatingicola	2.1	179 ± 60	24 ± 6	177 ± 70	10 ± 2	65 ± 36	5.8 ± 0.8	585 ± 143	27 ± 3	249 ± 47	?	328 ± 113	20 ± 3	?
Stigmaphyllon cavernulosum	3.8	203 ± 99	27 ± 11	?	4 ± 2	67 ± 14	7.5 ± 0.5	1294 ± 196	40 ± 3	289 ± 51	4.5 ± 0.9	1146 ± 176	38 ± 3	5.7 ± 0.7
Stigmaphyllon ciliatum	0.6	96 ± 31	18 ± 6	?	30 ± 7	137 ± 22	5.7 ± 0.9	283 ± 43	19 ± 1	184 ± 33	3.0 ± 0.3	na	na	na
Stigmaphyllon cordatum	0.6	94 ± 25	21 ± 10	138 ± 24	46 ± 3	136 ± 17	4.5 ± 0.4	224 ± 51	17 ± 2	164 ± 30	2.1 ± 0.1	na	na	na
Stigmaphyllon ellipticum	2.0	126 ± 35	21 ± 7	199 ± 20	28 ± 4	160 ± 46	4.0 ± 0.4	444 ± 82	24 ± 2	225 ± 36	2.2 ± 0.2	230 ± 79	17 ± 3	?
Stigmaphyllon lalandianum	1.1	165 ± 68	23 ± 9	237 ± 9	16 ± 6	46 ± 27	6.8 ± 0.9	650 ± 131	29 ± 3	200 ± 38	3.2 ± 0.5	1007 ± 209	36 ± 4	4.5 ± 0.6
Stigmaphyllon palmatum	1.8	209 ± 80	25 ± 9	269 ± 18	11 ± 3	105 ± 44	6.8 ± 0.4	988 ± 190	35 ± 4	253 ± 37	?	814 ± 218	32 ± 4	3.9 ± 0.4
Stigmaphyllon saxicola	3.1	197 ± 65	28 ± 8	233 ± 35	10 ± 4	79 ± 38	5.8 ± 0.5	863 ± 235	33 ± 5	248 ± 40	3.4 ± 0.7	368 ± 278	29 ± 6	?
Stigmaphyllon sinuatum	2.0	236 ± 70	28 ± 7	356 ± 32	6 ± 2	154 ± 45	6.1 ± 0.8	1899 ± 288	49 ± 4	315 ± 62	4.0 ± 0.3	na	na	na

? = unkown, na = not applicable
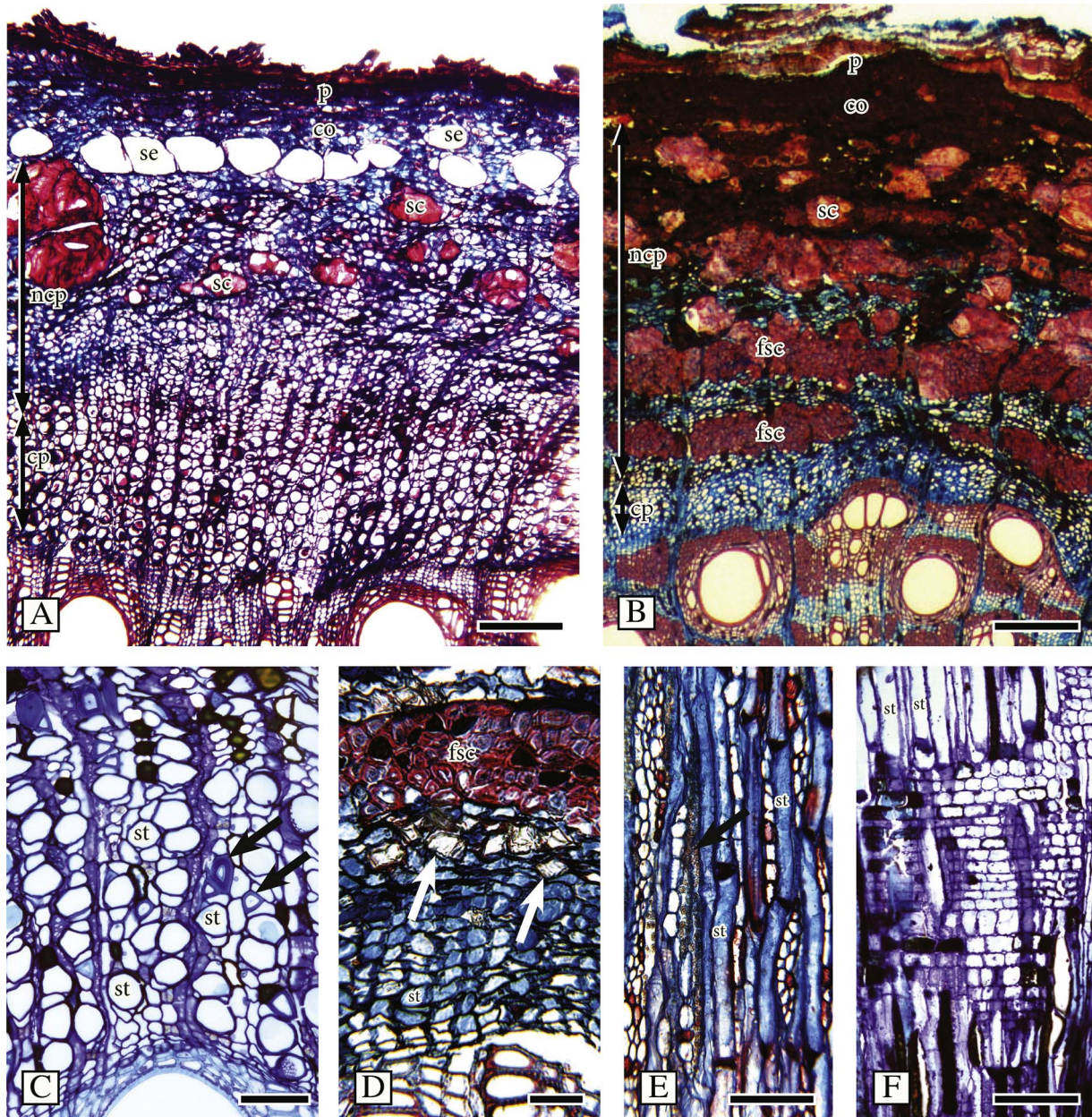
Fig. 7. Seconday phloem anatomy. A. *Stigmaphyllon caatingicola* transverse section (TS), secondary phloem has a conducting area (cp) with turgid sieve tube elements, and a non-conducting area (ncp), where large sclereid clusters develop (sc). Right beyond the limits of the vascular cylinder, large secretory cells are present, forming a continuous circle across the stem girth. B. *Banisteriopsis nummifera* TS. Secondary phloem has a short conducting area with turgid sieve tubes, and a large nonconducting area, where fiber-sclereids bands (fsc) develop, constituting a stratified phloem. Sclereids develop in the outermost parts of the nonconducting phloem. Large amounts of phenolic substances stain as dark red in all parenchyma cells of the cortex and phelloderm. C. *Stigmaphyllon cavernulosum* TS, conducting secondary phloem. Sieve tubes diffuse or in groups of 2, axial parenchyma constitutes the ground tissue, fiber-sclereids with gelatinous layer (arrows). D. *Banisteriopsis gardneriana* TS. Prismatic crystals associated to the fiber-sclereid bands. E. *Stigmaphyllon caatingicola* longitudinal tangential section. Phloem nonstoried, sieve tubes with simple sieve plates, transverse to slightly inclined. Slime plug evident (stained in red), druses in chambered axial parenchyma cells (arrow). F. *Stigmaphyllon cavernulosum* longitudinal radial section. Ray heterocellular mixed, with procumbent, erect and square cells in alternating layers. p = periderm, co = cortex, se = secretory cells, ncp = nonconducting phloem, cp = conducting phloem, sc = sclereids, fsc = fiber-sclereids, st = sieve tubes. Scale bars: A = 200 μm, B = 350 μm, C–E = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Rays with a straight course (Fig. 7A–B), slightly dilated (funnel shaped), ray width, height, and composition equal to those of secondary xylem (Fig. 7D–E), undergoing massive sclerification in the nonconducting phloem, and giving rise to large sclereids and sclereid clusters.

Sclerenchyma is represented by both fiber-sclereids (Fig. 7B–D) and sclereids (Fig. 7A) in most species. Both cell types appear more common in the regular than in the variant phloem.These fibers are round to polygonal in transverse section, and are gelatinous in *Stigmaphyllon cavernulosum*, *S. palmatum*, *S. saxicola* (Fig. 7C). In members of the *B.*

nummifera group, fiber-sclereids form alternating bands in the nonconducting phloem, constituting a stratified phloem (Fig. 7B). In *Stigmaphyllon* fiber-sclereids and sclereids form large clusters, generally in the nonconducting phloem (Fig. 7A). In some cases, sclereids differentiate within the conducting phloem, directly from cambial derivatives (e.g., *S. cavernulosum*, *S. lalandianum*).

Phloem elements are non-storied (Fig. 7E).

Druses are generally abundant (Fig. 7E; scarce in *Banisteriopsis nummifera*), and are present in chambered axial parenchyma cells and in individual axial and ray parenchyma cells of the phloem and cortex.

Table 2
Unpaired *t*-test comparing sieve tube areas of regular and variant phloem. Significant P values (at alpha < 0.05) are in boldface.

Species	Mean difference (regular x variant sieve tubes)	df	t	P
Banisteriopsis gardneriana	−159.13	47	5.32	**< 0.0001**
Banisteriopsis nummifera	13,44	53	0.58	0.5608
Stigmaphyllon acuminatum	−185.37	49	6.89	**< 0.0001**
Stigmaphyllon bonariense	181.25	39	4.73	**< 0.0001**
Stigmaphyllon caatingicola	257.29	43	7.34	**< 0.0001**
Stigmaphyllon cavernulosum	147.83	53	2.94	**0.0048**
Stigmaphyllon ellipticum	214.68	30	6.91	**< 0.0001**
Stigmaphyllon lalandianum	−357.11	54	7.52	**< 0.0001**
Stigmaphyllon palmatum	173.77	43	2.84	**0.0067**
Stigmaphyllon saxicola	165.52	40	2.08	**0.0434**

Druse crystals are generally larger in nonconducting phloem and cortex than in the conducting phloem (Fig. 8A). Prismatic crystals are abundant in *B. gardneriana* (Fig. 7D), located in individual parenchyma cells of the nonconducting phloem, especially in the parenchyma cells surrounding the fiber-sclereid bands (Fig. 7D).

Tannin cells are abundant, especially in early stages of stem development or in the outer regions of nonconducting secondary phloem (Fig. 7B).

Large secretory cells are present in the confines of secondary phloem and the cortex of *Stigmaphyllon* (Fig. 7A); absent in members of the *B. nummifera* group. These large secretory cells form a concentric ring around the vascular cylinder in *Stigmaphyllon* (Fig. 7A).

3.5. Pericyclic fibers

Pericyclic fibers form a more or less continuous ring of cells, interrupted by a single layer of parenchyma cells every 3–5 cell files (Fig. 4A). Clearly visible only in young stages of development; primary fibers get scattered as the stem thickens due to parenchyma proliferation between fibers as stem goes through secondary growth.

3.6. Periderm

First periderm originates in the cortex sub-epidermal layers (Fig. 8A). Sequent periderms give rise to a concentric rythidome (Fig. 8B) in thick, mature stems of all species. The rythidome was usually not very thick, except in species collected near bodies of water, such as *Stigmaphyllon bonariense*, and *S. ciliatum*, where the rythidome resembled that of an *Aristolochia*. Phellem cells evenly thick-walled in members of the *B. nummifera* group and thin to thick-walled in *Stigmaphyllon*, generally with periclinal walls thicker-walled and lignified (Fig. 8A). Phellem non-stratified in both genera (Fig. 8A–B), lenticels with non-stratified filling tissue (homogeneous) and suberized (Fig. 8C). Phelloderm is evenly thin walled (Fig. 8A).

4. Discussion

Here we described in detail the ontogeny of a type of cambial variant described by 19th century botanists, but later overlooked in general studies on cambial variants and never named. This type of cambial variant is found in two Malpighiaceae lineages, *Stigmaphyllon* and the *Banisteriopsis nummifera* group and is here named ***interxylary cambia***. We also describe for the first time the wood and bark anatomy of both lineages.
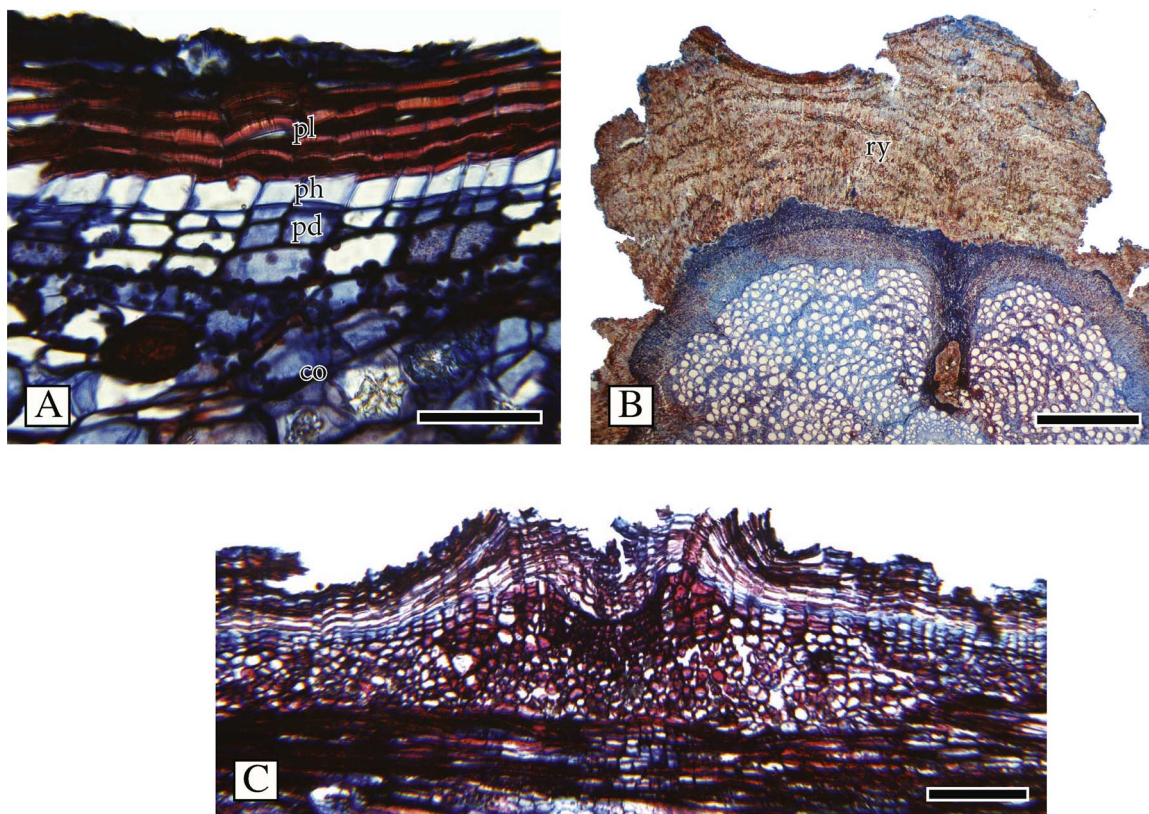

Fig. 8. Periderm. A. *Stigmaphyllon blanchetii* transverse section (TS), periderm originated in the cortex (co), thick-walled phellem cells. Phelloderm thin, composed of 2–3 cell layers, evenly thin-walled cells. B. *Stigmaphyllon bonariense* TS, thick rhytidome. C. *Stigmaphyllon blanchetii* longitudinal radial section, lenticel with filling tissue nonstratified (homogeneous) and suberized. pl = phellem, ph = phellogen, pd = phelloderm, co = cortex, ry = rhytidome. Scale bars: A = 60 μm. B = 4 mm, C = 200 μm.

4.1. Interxylary cambia

The presence of a cambial variant in the stems of *Stigmaphyllon* has been long documented, since the first comprehensive treatments of Malpighiaceae (Gaudichaud, 1841; Jussieu, 1843; Niedenzu, 1928), where the general anatomy of *Stigmaphyllon* was described as a dispersed xylem mass structure; however, without mentioning any developmental details. The first authors to describe in detail the stem anatomy of *Stigmaphyllon* and other Malpighiaceae were Van Tieghem (1884) in France and Schenck (1893) in Germany, in their anatomical studies of Neotropical lianas. These authors independently studied *Stigmaphyllon* stems, describing for the first time the phenomenon of new cambia derived from xylem axial parenchyma. Solereder (1908) and Metcalfe and Chalk (1950), in their general surveys of plant anatomy, mentioned this peculiar cambial configuration based on the work of Schenck (1893). While Schenck (1893) described the phenomenon without naming it, Van Tieghem (1884) called this phenomenon *tertiary growth,* a term coined by him. The name tertiary growth aimed to express that these new cambia derived from secondary tissues, i.e., the xylem axial parenchyma. The phloem and xylem produced by these new cambia were hence called *"tertiary phloem"* and *"tertiary xylem".* The use of tertiary growth by Van Tieghem (1884) encompassed not only cambia derived from secondary xylem, but also those derived from axial parenchyma of the nonconducting phloem. Van Tieghem (1884) classified as *"tertiary tissues",* for instance, those derived from phloem parenchyma within Bignoniaceae and Sapindaceae, a phenomenon that has been called "new vascular cylinders" or "neoformation of vascular cylinders" by more recent authors (Tamaio and Angyalossy, 2009; Angyalossy et al., 2012, 2015; Bastos et al., 2016; Cunha Neto et al., 2018). Formation of new vascular cylinders from nonconducting phloem parenchyma is apparently not uncommon, being found in lianas of other plant families as well, such as Cucurbitaceae, Euphorbiaceae, and Rubiaceae (see photos in Acevedo-Rodríguez, 2017 onwards). Multiple cambia derived from xylem axial parenchyma, however, is a more rare phenomenon, reported here for Malpighiaceae, but likely present also in other lianescent lineages. For instance in the lianas *Ipomoea pes-caprae* (L.) R. Br. (Convolvulaceae) and *Coccinia indica* L. (Cucurbitaceae) new cambia derived from wood parenchyma was found producing fair amounts of secondary phloem (Patil et al., 2011; Rajput et al., 2013). These new cambia derived from xylem parenchyma are interxylary cambia. The term "tertiary growth" is here rejected since primary and secondary growth involve two different meristems, procambium and cambium, respectively, which differ both in structure and products. The variant cambia described here, derived from non-lignified xylem axial parenchyma, are similar in structure and products to any regular cambium, with the sole exception of appearing with a crenate pattern, and not forming a concentric ring.

Interxylary cambia involve segments of *cambia* in a single stem and should not be confused with interxylary phloem, which denotes the presence of phloem embedded within the secondary xylem and derived from a single cambium (Carlquist, 2013; Angyalossy et al., 2016), encountered in Malpighiaceae for instance in *Dicella* (Chodat 1892). This distinction is fundamental: interxylary phloem involves just one cambium. The phloem that appears in the stems of *Stigmaphyllon* and members of the *Banisteriopsis nummifera* group is not interxylary phloem, since it derives from multiple new cambia differentiated from the xylem parenchyma within an already established secondary xylem.

Other types of cambial variants that also involve multiple cambia are successive cambia, stems with neoformed vascular cylinders derived from phloem parenchyma, compound stems, and corded stems, the two latter being exclusive of Sapindaceae (Angyalossy et al., 2015). Compound stems develop from a different procambial organization peculiar to the primary growth of several *Serjania* and *Paullinia* species (Schenck, 1893; Van der Walt et al., 1973; Carlquist, 1991; Acevedo-Rodríguez, 1993; Tamaio and Angyalossy, 2009), while all other cambial variants derive from multiple cambia developed after a phase of regular secondary growth. Neither *Stigmaphyllon,* nor *Banisteriopsis* fit any of the previously described cambial variants. Members of the *Banisteriopsis nummifera* group were suggested as having successive cambia (Angyalossy et al., 2015) as they resemble the final aspect of successive cambia. However, because the way these cambia are formed within existing xylem, they cannot be classified as such. Successive cambia, typical of many Caryophyllalean families, Convolvulaceae, Menispermaceae, and Fabaceae among others, involves the formation of new cambia from the outside of the original vascular system. Successive cambia may derive from 1) the pericycle (Eames and MacDaniels, 1925; Tamaio et al., 2009; Terrazas et al., 2011; Rajput and Marcati, 2013; Cunha Neto et al., 2018); 2) the cortex (Carlquist, 2007; Rajput et al., 2012); 3) a meristem named "master cambium" by Carlquist (2007) in cortical regions, and 4) more rarely from the secondary phloem (Nair and Mohan Ram, 1990; Dias-Leme, 1999) or the primary phloem (Pace et al., in press); but not from within the secondary xylem.

The tissues derived from interxylary cambia also differ from other cambia variants such as fissured stems (– dispersed xylem masses of the earlier authors), a common cambial variant found in Malpighiaceae. Fissured stems are described as derived from the activity of a single cambium with irregular activity, forming deep phloem wedges that coalesce, which in later stages breaks into discrete portions. Each of the vascular portions remains with part of the original cambium, which starts to act independently (Obaton, 1960; Cabanillas et al., 2017). However, the cambial variant of *Stigmaphyllon* and the *Banisteriopsis nummifera* group cannot be included within fissured stems, since fissured stems involve phloem wedges combined with massive proliferation of axial parenchyma (Obaton, 1960; Carlquist, 2001; Angyalossy et al., 2012, 2015; Cabanillas et al., 2017). Such parenchyma proliferation typically breaks up the lignified cells of the secondary xylem and forms a continuous matrix of non-lignified parenchyma, named disruptive parenchyma (Cabanillas et al., 2017), connecting the cortex, phloem, non-lignified xylem parenchyma, and the pith. Fissured stems also differ from interxylary cambia in general macroanatomical structure with large, discrete lignified vascular portions largely separated by disruptive parenchyma. Interxylary cambia, on the other hand, maintain the structure of a single vascular cylinder, but with a crenate, fish scale-like pattern.

Interxylary cambia are found in all members of the *Banisteriopsis nummifera* group, but unknown from any other species of *Banisteriopsis.* The *nummifera* group was established by Gates in 1982 based on morphology, and emerged as monophyletic in the most recent phylogenetic analysis (Davis and Anderson, 2010). The shared presence of this cambial variant gives further support to the recognition of this group. Within *Stigmaphyllon* so far, 14 out of the 20 species showed presence of this cambial variant, which was lacking only in species under 1 cm diameter (except for *S. sinuatum,* which had 2 cm). Among the species lacking the cambial variant is *Stigmaphyllon abutifolium,* formerly *Ryssopterys abutifolium* (C. Anderson, 2011). In phylogenetic analyses, *Ryssopterys,* which is exclusively found in the Old World, emerged nested within *Stigmaphyllon,* making *Stigmaphyllon* paraphyletic (Davis and Anderson, 2010). This led to the synonimization of *Ryssopterys* in *Stigmaphyllon,* and the creation of two subgenera, *Stigmaphyllon* subgenus *Ryssopterys* for the Old World and *Stigmaphyllon* subgenus *Stigmaphyllon* for the New World (C. Anderson, 2011). Analyses of thicker stems and within a more comprehensive phylogeny for the genus are needed to confirm the presence and perhaps distribution of this cambial variant in *Stigmaphyllon* as a whole, in order to determine whether the interxylary cambia represent a synapomorphy supporting the clade with both subgenera or alternatively is something exclusive to the Neotropical lineage of *Stigmaphyllon.*

4.2. Secondary xylem, phloem, and periderm

Secondary xylem and phloem in *Stigmaphyllon* and members of the *Banisteriopsis nummifera* group conform in many features that have been

described for Malpighiaceae (Metcalfe and Chalk, 1950; Domingues, 2008; Guimarães et al., 2016; Amorim et al., 2017; Cabanillas et al., 2017) or with general features described for lianas (Carlquist, 1985, 1991; Angyalossy et al., 2012, 2015).

These general features of lianas have been referred to as the "*lianescent vascular syndrome*", and clearly represent the convergence of anatomical features due to constraints imposed by the climbing habit (Pace and Angyalossy, 2013). These include the association of wide and narrow vessels (vessel dimorphism), abundant non-lignified axial parenchyma, and the common occurrence of dimorphic, heterocellular mixed rays (Carlquist, 1985; Angyalossy et al., 2012, 2015). The evolution of these features seems to be involved with the generation of more hydraulic efficient vasculature (Carlquist, 1985; Angyalossy et al., 2012, 2015), a more flexible structure given by the combination of soft tissues mixed to the stiff cells of the secondary xylem (Rowe et al., 2004, 2006; Ménard et al., 2009), and a structure capable of repairing injuries more rapidly (Dobbins and Fisher, 1986; Fisher and Ewers, 1989, 1991).

Typically, Malpighiaceae have woods with vessels in radial disposition, simple perforation plates, alternate intervessel pits of small dimensions, vestured pits, heterocellular rays less than 1 mm high, commonly paratracheal axial parenchyma (apotracheal also present in several taxa), parenchyma strands of 2–4 cells, septate fibers, perforated ray cells, and abundant prismatic crystals in both axial and ray parenchyma (Metcalfe and Chalk, 1950; Amorim et al., 2017; Cabanillas et al., 2017). *Stigmaphyllon* and members of the *B. nummifera* group differ from previous descriptions by the lack of septate fibers and perforated ray cells. It also differs in that the vessels are in radial disposition only for the narrow vessels, while the large vessels are solitary or in short multiples of 2–3. Also, the rays are dimorphic, a feature not always present in the trees.

Secondary phloem was only studied in *Callaeum* (Cabanillas et al., 2017), a genus that belongs to a lineage fairly distant from both *Stigmaphyllon* and *Banisteriopsis* (Davis and Anderson, 2010). The secondary phloem in *Stigmaphyllon* and the *B. nummifera* group share with *Callaeum* the presence of diffuse sieve tubes, which are solitary or in short radial multiples, immersed in axial parenchyma, sieve plates slightly inclined to transverse, simple sieve plates, fusiform companion cells, which lay in one or two corners of the sieve tubes, rays with a straight course, equal in height and composition to the secondary xylem, abundant druse crystals, and tanniniferous cells. The nonconducting phloem is similar in the dilatation phenomena involving both the axial and ray parenchyma, the presence of larger druse crystals in the nonconducting phloem when compared to its conducting portion, and in the formation of large sclereid clusters. In spite of these similarities, there are important anatomical differences with phylogenetic significance for these genera. The secondary phloem in members of the *Banisteriopsis nummifera* group uniquely share the formation of fiber-sclereids disposed in bands, creating a conspicuous stratified phloem. Bands of fiber-sclereids are less common than fiber bands, but have also been reported in other taxa, such as in apples (i.e., *Malus domestica* Borkh (Evert, 1963)). *Banisteriopsis* also contain prismatic crystals in addition to druse crystals, while the phloem of all other studied Malpighiaceae has druse crystals exclusively (Pace, 2015; Cabanillas et al., 2017). In *Stigmaphyllon*, all studied species share sclereids in clusters, similarly to *Callaeum*. However, these sclereids are sometimes also encountered in the conducting phloem. While the most traditional definitions of sclereids would entail their differentiation exclusively in the nonconducting phloem (Holdheide, 1951; Trockenbrodt, 1990; Den Outer, 1993), here we encountered an exception. Other reports have also indicated the possibility of sclereid formation within the conducting phloem; these studies later determined that the form of the sclerenchyma cell can be considered sufficient to differentiate sclereids from fibers (Evert, 2006; Pace et al., 2015; Angyalossy et al., 2016). Another conspicuous feature of *Stigmaphyllon* is the presence of large secretory cells forming a concentric ring, likely of laticifers, in the outer

zone of the vascular system. Further detailed studies are being carried out to determine the nature of these cells. Laticifers in Malpighiaceae have been reported only in the Galphimioids, a basal clade of trees and shrubs (Vega et al., 2002), and were interpreted as a possible indication of a close relationship between Malpighiaceae and Euphorbiaceae within the Malpighiales. This latter suggestion seems not to hold true, since more recent phylogenetic hypotheses suggest Malpighiaceae as sister to the mostly aquatic plant family Elatinaceae (Davis and Chase, 2004), which also has laticifers, while Euphorbiaceae is sister to the Rafflesiaceae, and both are sister to Peraceae (Wurdack and Davis, 2009). Rafflesiaceae lacks laticifers, but they also possess the most reduced body in the plant kingdom, peculiar to endoparasites (Nikolov et al., 2014).

Given the formation of multiple cambia, several phloem regions are present in stems with interxylary cambia. These phloem regions are qualitatively similar, but are shown to be quantitatively different. In plants with interxylary phloem or plants with phloem wedges interrupting the xylem, it is know that the variant phloem typically has wider sieve tubes than the regular phloem (Pace et al., 2009, 2011; Carlquist, 2013), a phenomenon suggested to represent a division of labor between variant and regular portions, with the variant phloem taking over the function of transport and the regular phloem (with more abundant parenchyma) assuming the function of storage. In this study, we did not encounter this pattern. In some species the sieve tubes of the regular phloem were on average wider, while in others the opposite occurred. Similarly, in *Ipomoea pres-capraea* (Convolvulaceae), which we interpret as another example of interxylary cambia, the phloem derived from these cambia was not wider than that derived from the regular cambium (Rajput et al., 2013). Therefore, it is likely that when more than one phloem type, regular and variant phloem, is present in the stem derived from a single cambium, differences exist between regular and variant phloem. However, when these two phloem types, regular and variant, derive from multiple cambia, no such difference is present. Detailed studies with the phloem of other plants with multiple cambia are needed to confirm this suggestion.

Pericyclic fibers in this study form a ring of discrete fiber strands across the stem girth, as reported also for several Malpighiaceae such as *Galphimia*, *Heteropterys* (Metcalfe and Chalk, 1950), and *Callaeum* (Cabanillas et al., 2017). These fibers are described as absent in *Byrsonima* and *Malpighia* (Metcalfe and Chalk, 1950), and their presence therefore is phylogenetically significant. We found that the periderm in these two genera is superficial in origin, similar to what was described by other authors (Solereder, 1908; Metcalfe and Chalk, 1950; Guimarães et al., 2016), and derived from the outer portions of the cortex. A concentric rhytidome is typically present, while thicker in some species than in others, especially in species associated to riverbanks such as *Stigmaphyllon bonariense* (associated to the Paraná river delta) and *S. ciliatum* (associated to mangroves and riverbank areas of eastern Brazil). Phellem and lenticels are nonstratified, homogeneous, and the phelloderm thin walled. Additional studies of the periderm are necessary to understand whether they may possess diagnostic value in differentiating or confirming the identity of the genera in Malpighiaceae.

5. Conclusions

Stigmaphyllon and the species in the *B. nummifera* group have a unique type of cambial variant here called interxylary cambia in which new vascular tissue differentiate from the wood parenchyma, generating a crenate, fish scale-like pattern. Further studies are needed to determine whether this feature is a synapomorphy of *Stigmaphyllon s.l.* or solely shared by species in the core Neotropical *Stigmaphyllon* clade. We establish that this cambial variant is important to support *B. nummifera* group as a monophyletic group. Wood anatomy of Malpighiaceae is quite homogeneous in the lianas due to convergent evolution driven by the climbing habit. Nevertheless, the phloem seems to carry

significant phylogenetic information, such as the presence of stratified phloem and prismatic crystals in the *Banisteriopsis nummifera* group and a concentric ring of cortical secretory cells in *Stigmaphyllon*.

Appendix A. Taxa, collectors and localities of the *Banisteriopsis nummifera* group and *Stigmaphyllon* (Malpighiaceae) species. Voucher specimens are deposited in the herbaria as indicated in parenthesis after the locality. Acronyms according to Thiers (2017).

Banisteriopsis gardneriana (A.Juss.) W.R.Anderson & B.Gates, *Pace 328*, Estrada para São João da Chapada, Diamantina, Minas Gerais, Brazil (SPF); *Ceccantini 3724*, Almas, Tocantins, Brazil (SPF). *Banisteriopsis nummifera* (A.Juss.) B. Gates, *Pace 258*, Usina São José, Igarassu, Pernambuco, Brazil (SPF); *Pace 337*, Caminho para Lagoa Encantada, Ilhéus, Bahia, Brazil (SPF); *Ceccantini 3748*, Almas, Tocantins, Brazil (SPF). *Stigmaphyllon* (= *Rhyssopterys*) *abutilifolium* (A.Juss) C.E. Anderson, *Evans 1401*, Track from Nob Hill to main island ridge, Micronesia, Caroline Islands, Moen Island (US). *Stigmaphyllon acuminatum* A.Juss., *Pace 377,* Dario Meira, Bahia, Brazil (SPF, CEPEC). *Stigmaphyllon auriculatum* (Cav.) A.Juss., *Pace 397*, Maracás, Bahia, Brazil (SPF); *Almeida 595*, Itaberaba, Bahia, Brazil (HUEFS) *Stigmaphyllon blanchetii* C.E. Anderson, *Almeida 532*, Reserva Biológica Córrego Grande, Conceição da Barra, Espírito Santo, Brazil (SP); *Pace 239*, Usina São José, Igarassú, Pernambuco, Brazil (SPF). *Stigmaphyllon bonariense* (Hook. & Arn.) C.E. Anderson, *Pace 541, 560*, Isla Martín García, La Plata, Buenos Aires, Argentina (SPF). *Stigmaphyllon caatingicola* R.F.Almeida & Amorim, *Almeida 577* (type specimen), Arapiranga, Rio de Contas, Bahia, Brazil (HUEFS, CEPEC, MICH, P, RB, SP). *Stigmaphyllon cavernulosum* C.E. Anderson, *Pace 357*, Jussari, Bahia, Brazil (SPF). *Stigmaphyllon ciliatum* (Lam.) A.Juss., *Pace 338*, Caminho para Lagoa Encantada, Ilhéus, Bahia, Brazil (SPF, CEPEC). *Stigmaphyllon cordatum* Rose ex Donn.Sm., *Pace 142*, Jardim Botânico Plantarum, Nova Odessa, São Paulo, Brazil (SPF). *Stigmaphyllon ellipticum* (Kunth) A.Juss., *Pace 403*, Cerro del Barro, Jalcomulco, Veracruz, Mexico (SPF); *Acevedo-Rdgz 16395*, Along road 200, Sern entrance of Arriaga, Chiapas, México (US). *Stigmaphyllon hispidum* C.E. Anderson, *Amorim* s.n., location unknown. *Stigmaphyllon lalandianum* A.Juss., *Almeida 816*, Fazenda Campininha, Mogi Guaçu, São Paulo, Brazil (HUEFS). *Stigmaphyllon macedoarum* C.E. Anderson, *Camargo-Rosa 1*, Ladário, Mato Grosso do Sul, Brazil (SPF). *Stigmaphyllon macropodum* A.Juss., *Pace 352*, Olivença, Bahia, Brazil (SPF, CEPEC); *Pace 371*, Fazenda de Manoel Crente, Guararema, Bahia, Brazil (SPF). *Stigmaphyllon palmatum* (Cav.) A.Juss, *Sato 45*, Pará, Brazil (SPF). *Stigmaphyllon retusum* Griseb, *Acevedo-Rodríguez 16427*, along road 199, ca. 5.6 km west of

entrance to Palenque, Chiapas, Mexico. *Stigmaphyllon saxicola* C.E. Anderson, *Almeida 568*, Reserva Biológica Mata Escura, Jequitinhonha, Minas Gerais, Brazil (HUEFS). *Stigmaphyllon sinuatum*(DC.) A.Juss., *Pace 516*, Reserva Allpahuayo Mishana, Loreto, Iquitos, Peru (SPF). *Stigmaphyllon tomentosum* A.Juss., *Pace 180*, *186*, *187*, São Sebastião, São Paulo, Brazil (SPF, US, MO, CEPEC); *Pace 236*, Reserva Biológica de Poço das Antas, Silva Jardim, Rio de Janeiro, Brazil (SPF); *Pace 378*, Dario Meira, Bahia, Brazil (SPF, CEPEC, MICH). *Stigmaphyllon urenifolium* A.Juss., *728*, Correntera, Bahia, Brazil (SPF, HUEFS).

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