PROCAMBIAL AND CAMBIAL VARIANTS IN *SERJANIA* AND *URVILLEA* SPECIES (SAPINDACEAE: PAULLINIEAE)

Willian Adriano Lira Lopes,
Luiz Antonio de Souza

Programa de Pós-graduação em Biologia Comparada
Universidade Estadual de Maringá
Av. Colombo, no. 5790
(87020-900) Maringá, PR, BRAZIL
willianliralopes@yahoo.com.br;lasouza@uem.br

Odair José Garcia de Almeida

Programa de Pós-Graduação em Biodiversidade Aquática
São Paulo State University (UNESP)
Institute of Bioscience, São Vicente
Praça Infante Don Henrique, P.O. Box 73601
(11380-972) São Vicente, SP, BRAZIL
odair.almeida@clp.unesp.br

ABSTRACT

For the purpose of comparing the structure of vascular cylinders, and procambial and cambial variants of *Serjania* Miller and *Urvillea* Kunth species, we studied the stems of five species, in order to add to the structural knowledge of these genera belonging to the widely distributed Sapindaceae family. Stems of sampled species were collected at "Estação Ecológica do Caiuá," Diamante do Norte (PR), Brazil; and were analyzed using traditional anatomical techniques. We analyzed five species of two genera from the Paullinieae tribe: three species of *Serjania* (*S. communis*, *S. fuscifolia,* and *S. meridionalis*) and two of *Urvillea* (*U. leavis* and *U. ulmacea*); in which we describe the primary and secondary growths, the number of peripheral cylinders, the type of cylinders concerning its origin, the type of stem, and the origin of the cambial or tissue variant. *Serjania* stems exhibit the compound and corded types, while the cleft and mixed (cleft/corded) types have been recorded in *Urvillea* species. All species have peripheral vascular cylinders in which the stems of *S. communis* and *S. fuscifolia* form the procambial and cambial variants during primary growth, whereas the stems of *S. meridionalis*, *U. leavis,* and *U. ulmacea* develop the cambial variants during secondary growth.

RESUMO

Com o objetivo de comparar os cilindros vasculares e variantes procambiais e cambiais de *Serjania* Miller e *Urvillea* Kunth, nós realizamos estudos na estrutura caulinar de cinco espécies, a fim de contribuir com o conhecimento estrutural desses dois gêneros da família Sapindaceae. O material botânico foi coletado na Estação Ecológica de Caiuá, Diamante do Norte (PR), Brasil, que foram estudadas através de técnicas tradicionais de anatomia vegetal. Nós analisamos cinco espécies de dois gêneros da tribo Paullinieae: três espécies de *Serjania* (*S. communis*, *S. fuscifolia* e *S. meridionalis*) e duas de *Urvillea* (*U. leavis* e *U. ulmacea*); nas quais apresentamos resultados referentes aos crescimentos primário e secundário, o número de cilindros periféricos, o tipo de cilindro conforme a sua origem, o tipo de caule e a origem do câmbio ou do tecido variante. *Serjania* têm caules dos tipos composto e cordado, enquanto as espécies de *Urvillea* apresentam caules tipo fissurado ou misto (fissurado/cordado). Todas as espécies analisadas têm cilindros vasculares periféricos, em que os caules de *S. communize* e *S. fuscifolia* formam variantes procambiais e cambiais durante seu crescimento primário, enquanto os caules de *S. meridionalis*, *U. leavis* e *U. ulmacea* desenvolvem variantes cambiais durante o seu crescimento secundário.

Key Words: *Serjania*, *Urvillea*, lianas, stems, primary growth, secondary growth

INTRODUCTION

Compared with other plant growth forms in tropical forests, lianas have a unique way of developing growth forms of woody non-self-supporting stems which include a diversity of organization systems; these atypical secondary growth systems have always been considered as a factor that is beneficial to the lianescent life form (Caballé 1993). Metcalfe and Chalk (1983) offered a listing of various cambial variants (anomalous stem) patterns in dicot families (sensu lato, i.e., non-monocot species), in which four types of vascular cylinders have been reported in Sapindaceae, which are: compound, divided, corded, and cleft.

The genera *Serjania* Miller and *Urvillea* Kunth are included in the Sapindoideae subfamily, Paullinieae tribe, which have imparipinnate leaves, zygomorphic flowers, petals with a prominent scale, and a unilateral disk as distinctive characters (Buerki et al. 2009). According to them, the liana habit and the development of tendrils and stipules constitute synapomorphies for the Paullinieae tribe, composed mainly by lianas. Cambial variants in *Serjania* stems were relatively well-studied by Fisher and Ewers (1989, 1992) that distinguished the

Serjania stem with a central vascular cylinder and five smaller peripheral cylinders (multiple steles); Acevedo-Rodriguez (1993) reported a detailed revision of *Serjania* wood anatomy showing a unique type of anomaly into the stem's structure in the genus; Caballé (1993) described multiple cylinders for *Serjania brachycarpa* A. Gray ex Radlk. stem; Araújo and Costa (2006) and Tamaio and Angyalossy (2009) reported the development of the compound stem of *Serjania caracasana* (Jacq.) Willd.; León (2009) studied the wood structure of *Serjania atrolineata* C. Wright; Tamaio (2011) analyzed the wood anatomy of six species of *Serjania*; Tamaio et al. (2011) studied the central vascular cylinder and three peripheral vascular cylinders of six *Serjania* species; Borniego and Cabanillas (2014) described the cambial variant in *Serjania meridionalis* Cambess.; and Angyalossy et al. (2015) examined the sieve tubes in *Serjania lethalis* A. St.-Hil., in which those cells are as wide as the small vessels found in the xylem of the same plant. However, there is still missing information about the development of these species regarding cambial and procambial variants. Specifically, in the genus *Urvillea*, stem description is mostly overlooked except for a brief note by Metcalfe and Chalk (1957), Tamaio et al. (2011), and Bastos et al. (2016) looked at the fissured vascular cylinder, but there is still a gap in the formation of the cambial variant that deserves some attention here.

The aim of this investigation was to analyze the structure of the vascular cylinders and the origin of the cambial variants in *Serjania* and *Urvillea* species in order to contribute to the structural knowledge about these Sapindaceae genera. In addition, we also summarize the available and published information on stems of both genera.

MATERIAL AND METHODS

Stems of sampled species were collected at "Estação Ecológica do Caiuá" (EEC), Diamante do Norte, Brazil (Atlantic Forest at the Northwest of Paraná), with an area of 1449.4834 hectares. Vouchers of *Serjania communis* Cambess. (*HUEM 26186*), *Serjania fuscifolia* Radlk. (*HUEM 26185*), *Serjania meridionalis* Cambess. (*HUEM 183047*), *Urvillea leavis* Radlk. (*HUEM 26215*), and *Urvillea ulmacea* Kunth (*HUEM 26187*) were deposited at Universidade Estadual de Maringá Herbarium (HUEM).

Longitudinal sampling of stem fragments was done at several distances from the tip (up to 25th internode), with the intention of detecting the ontogenetic origin of multiple cylinders.

Specimens for microscopic examination were prepared either for historesin sectioning (permanent slides) or by hand-sectioning (semi-permanent slides). Stem fragments from the tip until the 25thinternode were fixed in glutaraldehyde (1% in 0.1 M phosphate buffer, pH 7.2) (Karnovsky 1965) and sectioned (rotation microtome and hand cut). All staining was accomplished with toluidine blue O (O'Brien et al. 1964) (permanent slides) and safranin along with astra blue (semipermanent slides) (Souza et al. 2005).

Older branches (diameter 0.5–3.5 cm) were softened with 10% ethylenediamine (Carlquist 1982), submerged in a solution of water, detergent, and glycerin (Alcorn & Ark 1953), cut freehand, and stained with safranin along with astra blue (Souza et al. 2005).

Light microscope photographs were taken on LeicaEZ4D digital camera and subsequently processed using the software Leica Application Suite version 1.8.

RESULTS

We analyzed five species from two genera from the Paullinieae tribe: three species of *Serjania* (*S. communis*, *S. fuscifolia,* and *S. meridionalis*) and two of *Urvillea* (*U. leavis* and *U. ulmacea*). Results concerning the primary and secondary growths, the number of peripheral cylinders, the type of cylinders concerning its origin, the type of stem, and the origin of the cambial or tissue variant are given in Table 1.

Serjania stems

The vascular structure of *S. communis,* near the apical meristem, consists of a wide procambial central cylinder and three small peripheral procambial cylinders (Figs. 1, 2), with differentiated protoxylem and protophloem cells. All procambial cylinders are surrounded by faint pluriseriate ground tissue. This tissue is composed of thick-walled cells in the third and fourth internodes (Fig. 3), where the fascicular cambium is distinguished

Table 1. Stem structure and cambial/tissue variants of Paullinieae species (Sapindaceae). The classification of the type of stem was based on Metcalfe and Chalk (1957) and Tamaioet al. (2011).

Species	No. of peripheral cylinders	Primary or secondary growth	Origin of the cylinders	Origin of the procambial or cambial variant	Type of stem
Serjania communis	5–6 cylinders	Peripheral cylinders in the primary growth	Procambial (primary growth)	–	Compound vascular cylinder
Serjania fuscifolia	9 cylinders	Peripheral cylinders in the primary growth	Procambial (primary growth)	–	Compound vascular cylinder
Serjania meridionalis	9 or more cylinders	Peripheral cylinders in the secondary growth	Cambial variant (secondary growth)	Ground tissue	Corded vascular cylinder
Urvillea leavis	Variable, in older internodes	Neo-formed peripheral cylinders in the secondary growth	Origin of new cambia	Cambium	Cleft vascular cylinder
Urvillea ulmacea	Variable	Neo-formed peripheral cylinders in the secondary growth	Origin of new cambia	Cambium and pericycle	Corded and cleft vascular cylinder

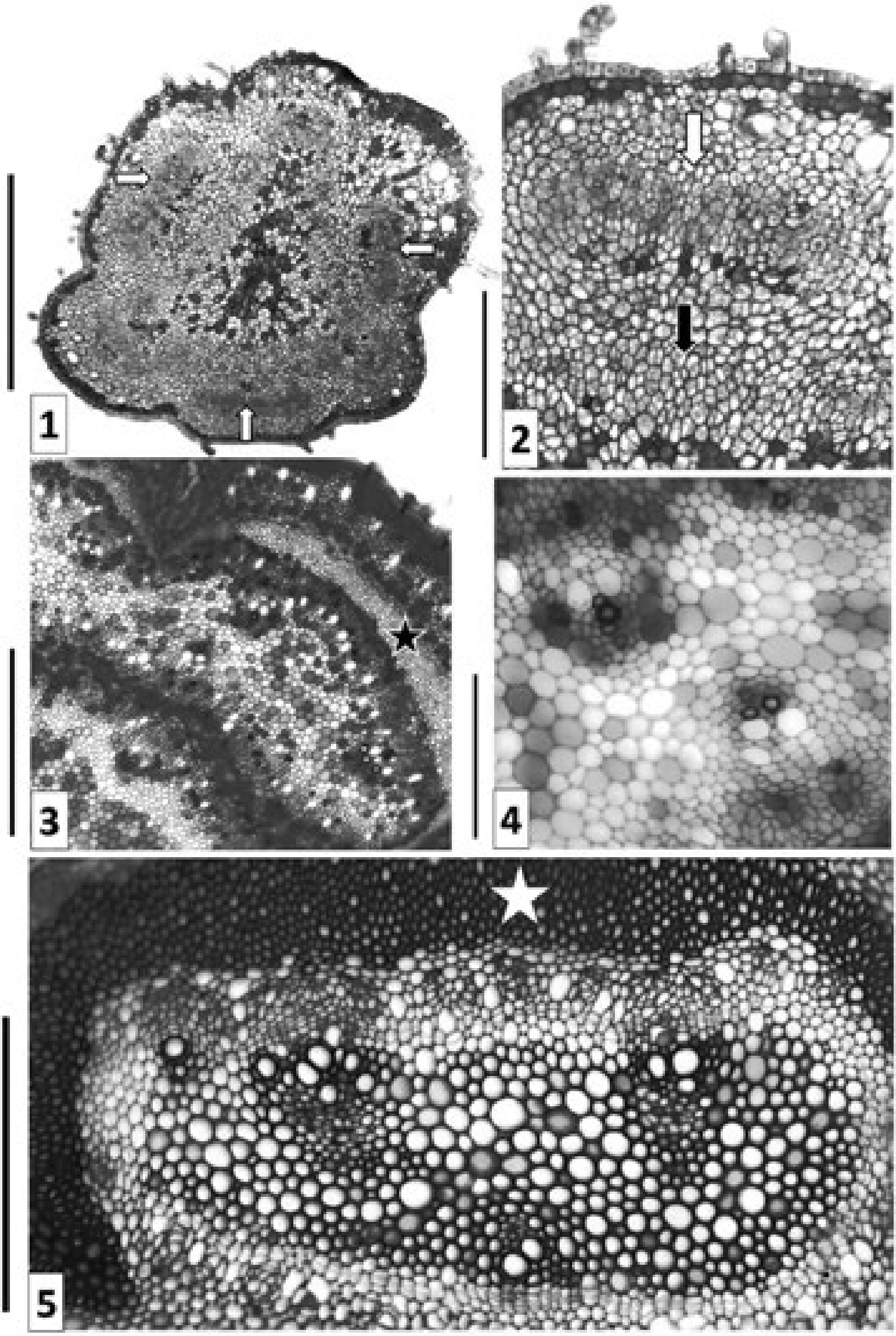
(Fig. 4). In the tenth internode (Fig. 5) all cylinders show mature primary vascular tissues; however, they still have meristematic activity in the fascicular cambium. Internodes with advanced secondary growth have shown up to six or seven separate cylinders, one wider central cylinder and five or six smaller peripheral cylinders (Table 1), all surrounded by the sclerenchymatous ground tissue (Figs. 14, 15).

The *S. fuscifolia* stem has practically the same structure as *S. communis*, except for having more peripheral vascular cylinders (nine) and prominent edges in the third internode (Figs. 6, 16). The sclerification of the ground tissue begins at the third internode (Fig. 7). The parenchyma of the fifth internode (Fig. 8), located between primary xylem strands, also undergoes sclerification, and a more pronounced cell sclerification occurs in the twentieth internode (Fig. 9), in which the isolated sclereids or group of sclereids are formed between vascular cylinders. The stem, having completed secondary growth, has a round shape with nine peripheral cylinders and one central cylinder (Fig. 17; Table 1).

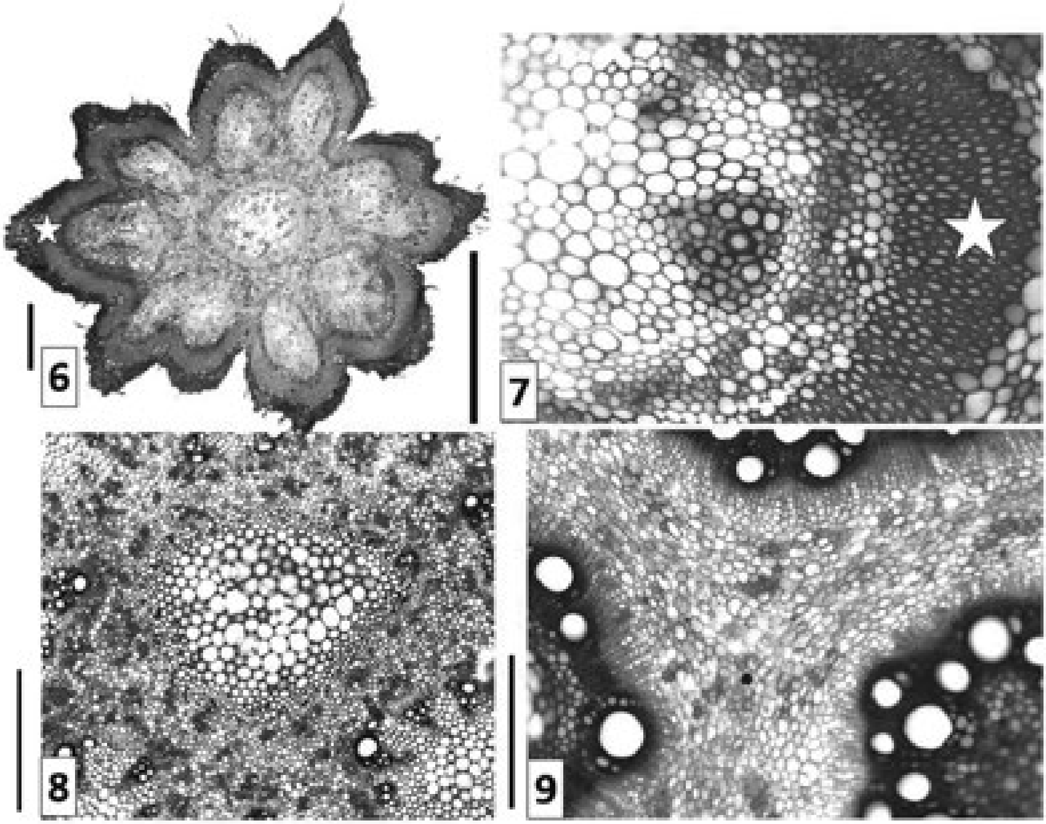
Compared with both species previously described, the *S. meridionalis* stem is strikingly different. The pattern of arrangement of vascular tissues seen in this species is made of collateral bundles, which enclose the pith, and devoid of additional cylinders surrounding the central one. The differentiation of fascicular and interfascicular cambia occurs from the third until the tenth internode. In the twentieth internode (Fig. 10), the secondary vascular growth is still reduced, and it is surrounded by ground tissue that has inner layers with thin-walled cells and outer layers with thickened cell walls. Subsequent internodes with 2.0 cm diameter show secondary phloem with secretory cells and wide parenchymatous rays. External to these rays the differentiation of cambial variants begins, which is noted by divisions of the ground tissue cells, originating at the peripheral cylinders (Figs. 11, 18) with bidirectional cambial activity, and they are devoid of parenchymatous pith (Figs. 11, 12). The stem with advanced secondary vascular growth (Fig. 13) has the same structure as the other two *Serjania* species, that is, the various small peripheral cylinders and the wide central cylinder (Fig. 19; Table 1).

Urvillea stem

Urvillea leavis stems show that up to approximately the twentieth internode, the primary and secondary vascular growth is similar to most of the eudicots, with ground tissue partially sclerified around the vascular cylinder (Figs. 20, 27). Beyond the twentieth internode, certain cambial cells undergo more intense divisions than other cambial regions (Fig. 21), producing extensive amounts of phloem. This way, as the formation of cambial variants continues, subsequent grooves are formed in the vascular cylinder (Figs. 22, 28). Peripheral vascular cylinders may be formed in older internodes through the activity of these cambial variants (Table 1).



Figs. 1–5. Transverse sections of *Serjania communis* stem. **1.** Near the apical meristem, showing the procambial central cylinder and three peripheral procambial cylinders (white arrows). **2.** Detail of the procambial central (black arrow) and peripheral cylinders (white arrow). **3.** Third internode (black star indicates pericycle). **4.** Fourth internode showing differentiated vascular cells, and fascicular cambium. **5.** Tenth internode evidencing sclerenchymatous ground tissue (white star), fascicular and interfascicular cambia. Scale bars: 1, 3, and 5 = 300 µm; 2 and 4 = 100 µm.

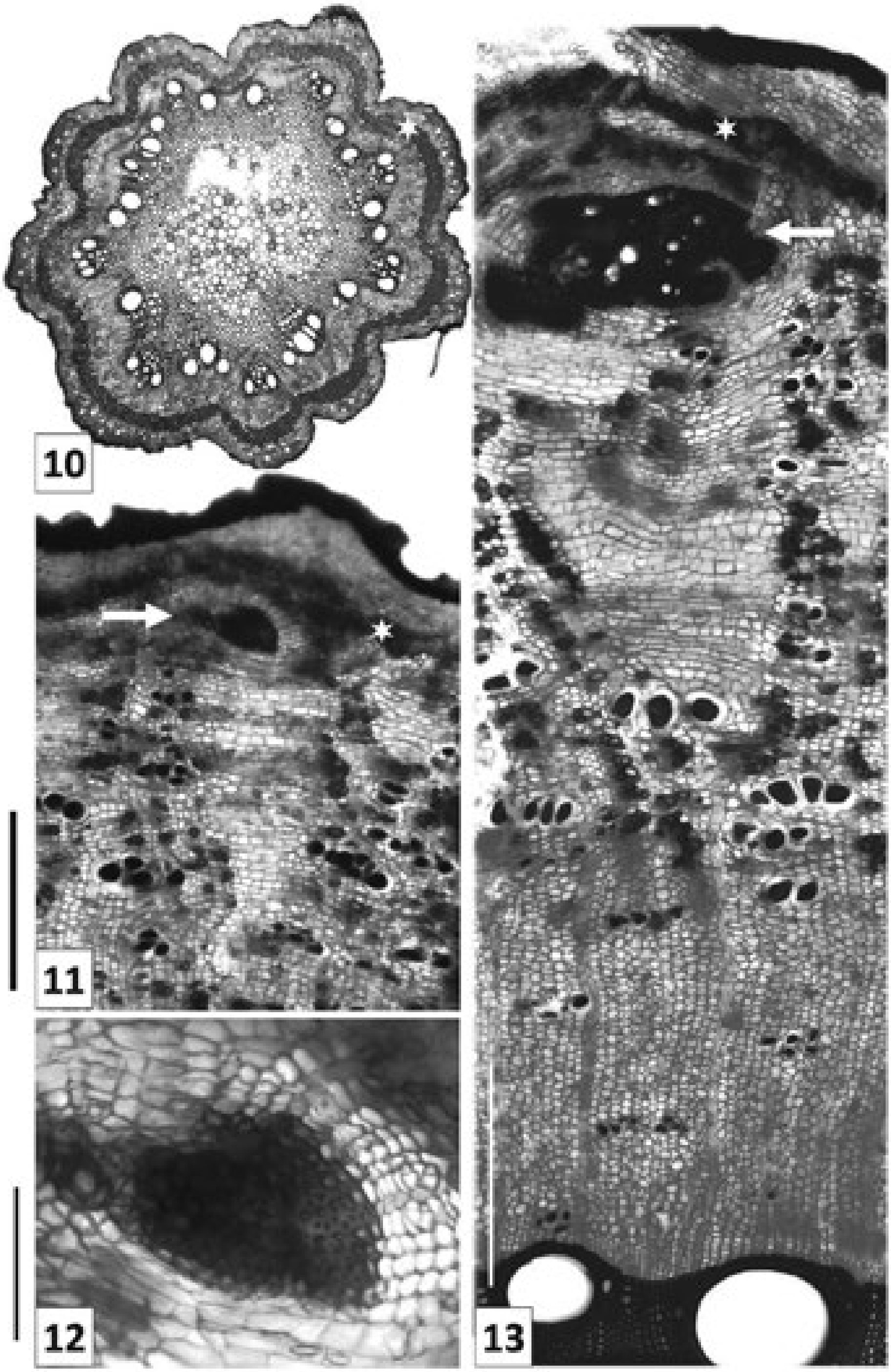


Figs. 6–9. Transverse sections of *Serjania fuscifolia* stem. 6. Third internode with evident edges (white star shows the pericycle). 7. Detail of a peripheral cylinder and sclerenchymatous ground tissue (white star) of the third internode. 8. Fifth internode; parenchymatous cells in sclerification among the xylem strands. 9. Twentieth internode with sclereids in the parenchyma. Scale bars: 6, 8, and 9 = 300 µm; 7 = 100 µm.

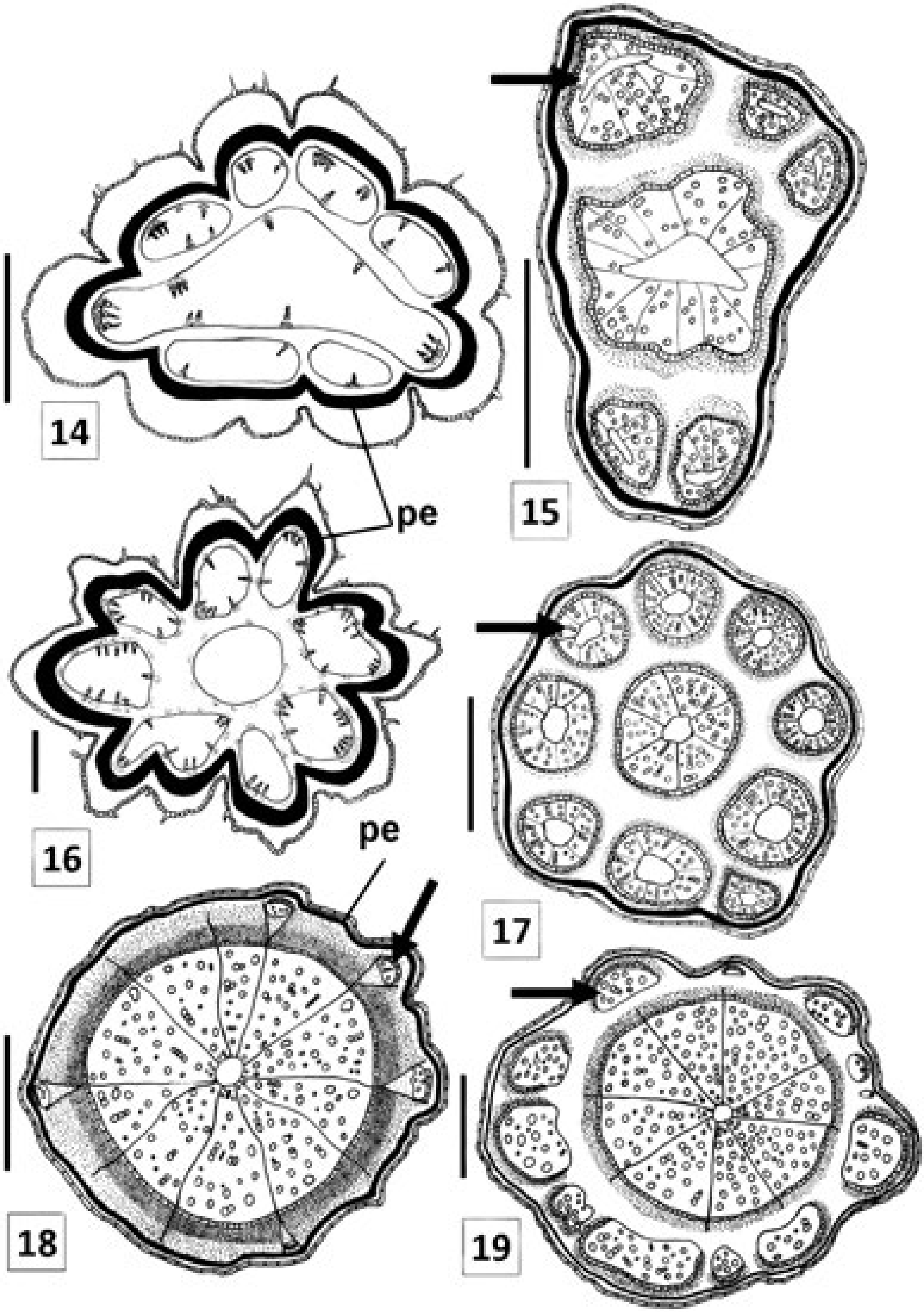
Urvillea ulmacea stems up to 0.5 cm in diameter are similar to the stem of *U. leavis*, which in cross-section has a cylinder of primary and secondary vascular tissues, parenchymatous pith, and partially sclerified ground tissue (Figs. 23, 29). Inferior internodes develop cambial variants from the ground tissue that produce the peripheral cylinders with narrow pith (Figs. 24, 30). As in *U. leavis*, cambial variants are also formed in the central cylinder (Figs. 25, 26), producing more phloem that may result in the production of additional peripheral cylinders (Fig. 30).

DISCUSSION

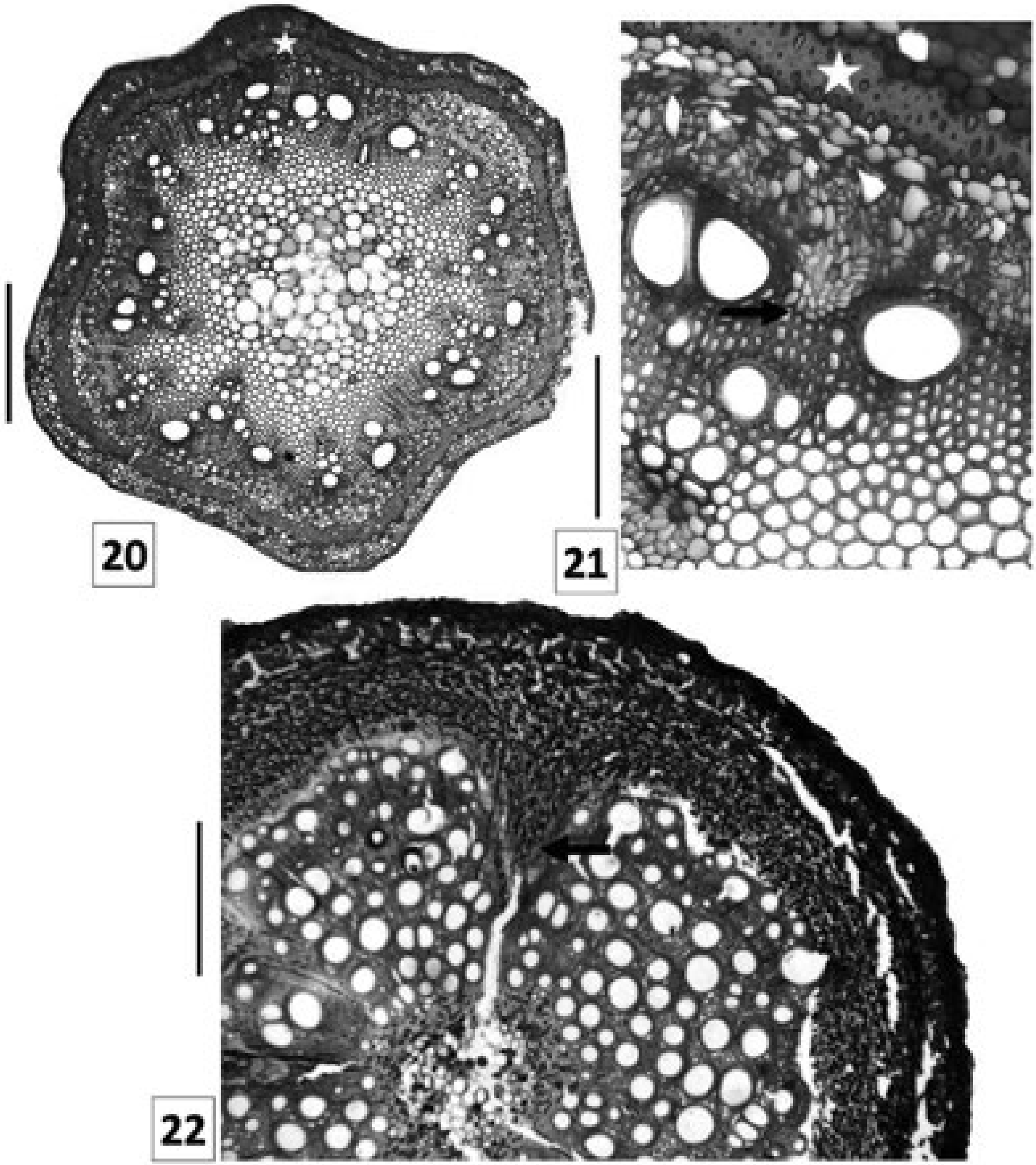
Procambial and cambial variants may occur in either primary or secondary growth of stems of the Paullineae members (Tamaio & Angyalossy 2009; Tamaio & Somner 2010), creating a cable configuration. In the corded vascular cylinder, which has a normal configuration during the primary growth and most of the secondary growth, the cable structure is consolidated with the appearance of peripheral vascular cylinders, creating a final configuration that is similar to the compound vascular cylinder (Tamaio & Somner 2010). Stems of *S. communis* and *S. fuscifolia* form their final anatomical configuration from multiple procambial cylinders during the primary growth (Table 1), as already reported by Tamaio and Angyalossy (2009) and Tamaio et al. (2011). On the other hand, the stems of *S. meridionalis*, *U. leavis,* and *U. ulmacea* develop the cambial variants during secondary growth, but from different origins according to the species.



Figs. 10–13. Transverse sections of *Serjania meridionalis* stem. **10.** Twentieth internode (white star indicates sclerenchymatous tissue). **11.** Internode with 2.0 cm in diameter showing peripheral cylinder (white arrow) and ground tissue (white star). **12.** Detailed view of a peripheral cylinder devoid of pith. **13.** Internode with advanced secondary vascular growth with peripheral cylinder (white arrow) and ground tissue (white star). Scale bars: 10, 11, and 13 = 500 µm; 12 = 100 µm.



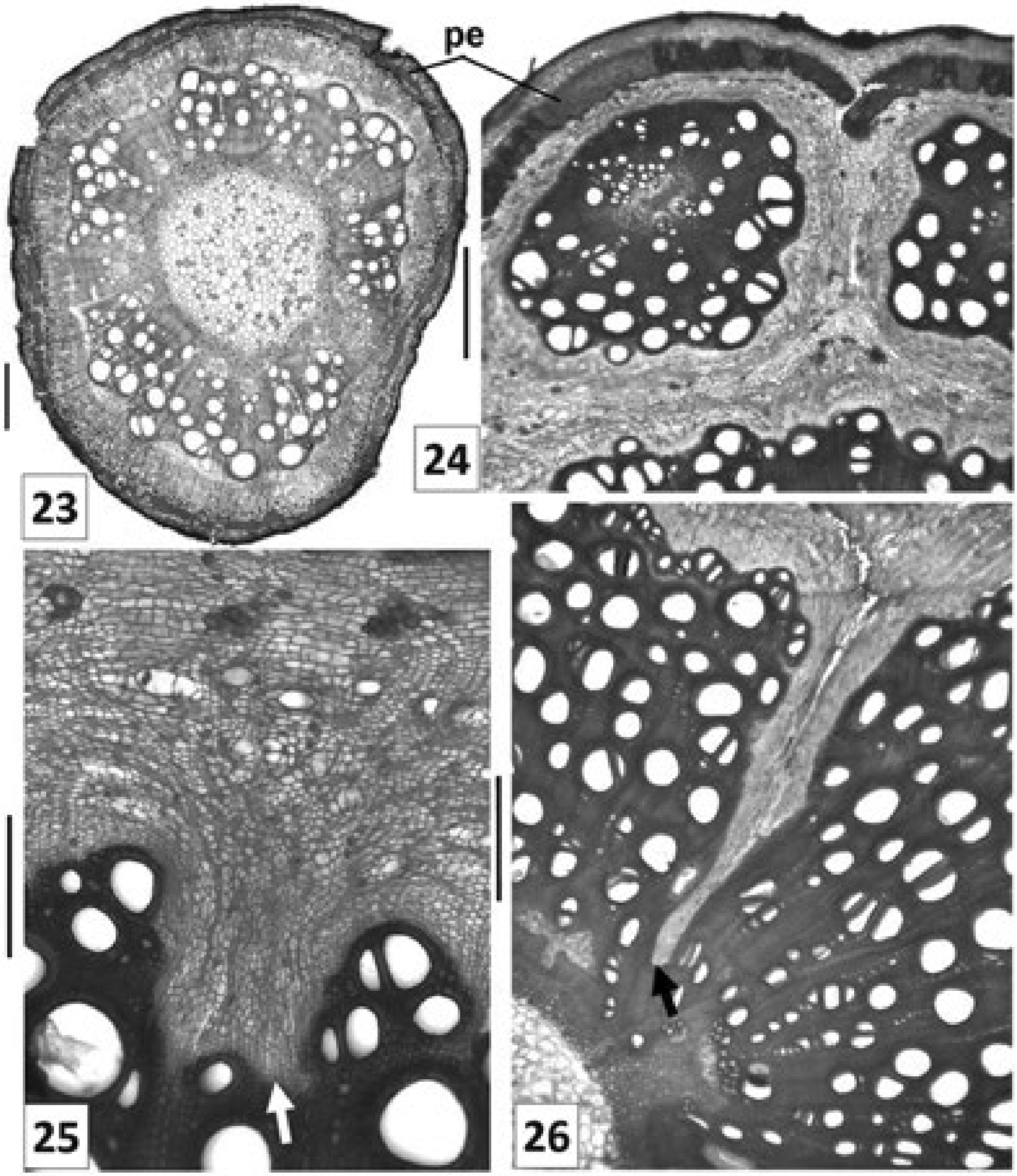
FIGS. 14–19. Diagrams of transverse sections of *Serjania* stem. **14–15.** *Serjania* communis stems. **16–17.** *Serjania fuscifolia* stems. **18–19.** *Serjania meridionalis* stems. Black arrow indicates peripheral cylinders; pe = pericycle. Scale bars: 14 and 16 = 300 µm; 15, 17–19 = 500 µm.



Figs. 20–22. Transverse sections of *Urvillea leavis* stem. **20–21.** Twentieth internode and showing the detailed sclerenchymatous ground tissue (white star) and early stage of the cambial variant (black arrow). **22.** Stem about 0.4 cm in diameter with cambial variant (arrow). Scale bars: 20 = 300 µm; 21 = 100 µm; 22 = 500 µm.

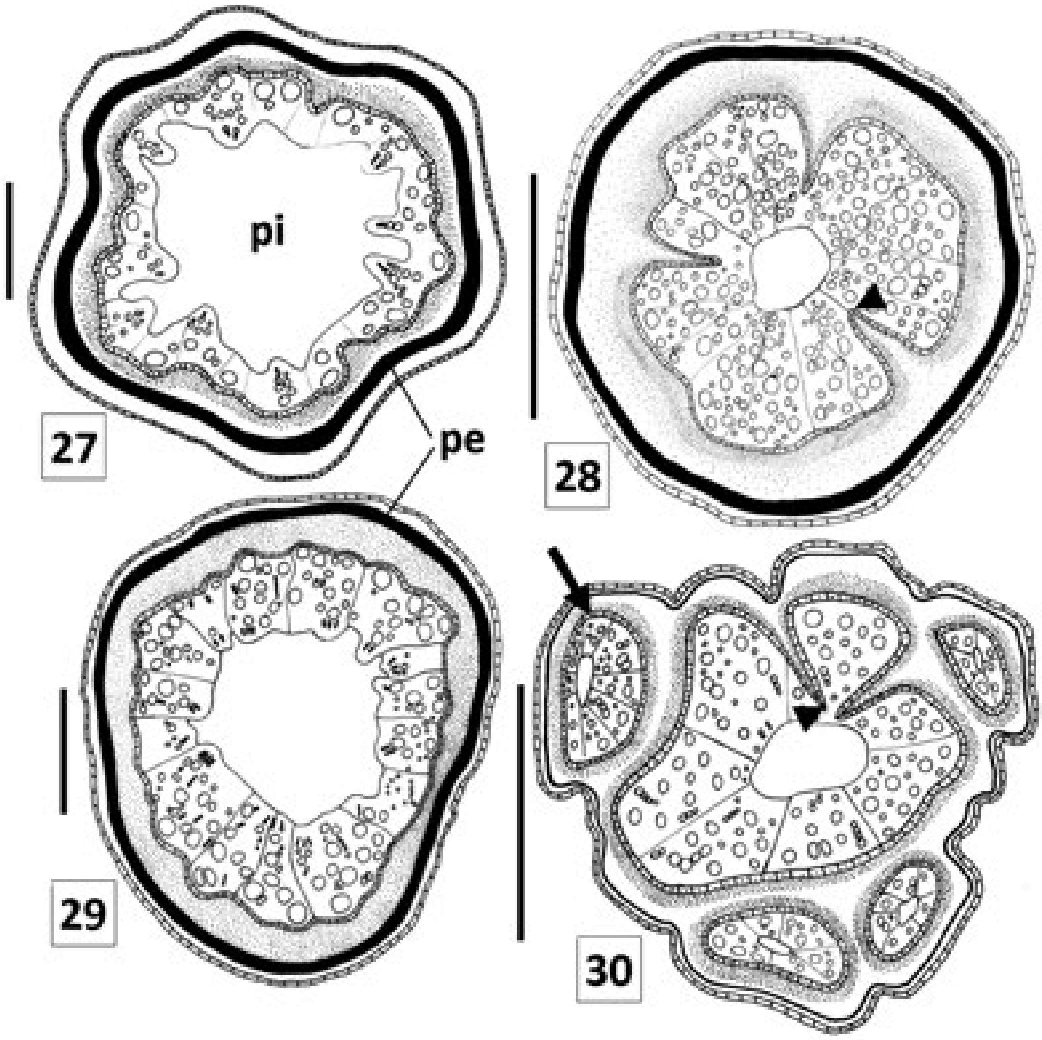
The cambial variant of *S. meridionalis* differentiates from the non-sclerified ground parenchyma (this tissue is considered a pericycle by Angyalossy et al. 2015) that remains active during the growth period, whereas the cambial variant of *U. leavis* originates from the cambium (Table 1). The organization in *U. ulmacea* is intermediate between the *S. meridionalis* and *U. leavis*, in which the cambial variant initially originates from the cambium, and then also from the pericycle cells.

Metcalfe and Chalk (1957) and Tamaio et al. (2011) gave a valuable description of four types of cambial



Figs. 23–26. Transverse sections of *Urvilleau lmaceastem*. **23.** Stem about 0.5 cm in diameter devoid of cambial variant. **24–26.** Stem about 1.5 cm in diameter showing peripheral cylinders (24) and the cambial variant in the central vascular cylinder (25–26; arrow). (pe = sclerenchymatous ground tissue). Scale bars: 23, 24, and 26= 500 µm; 25 = 300 µm.

variants in Sapindaceae, a compound, divided, corded, and cleft vascular cylinder, in which the first type is confined to the primary growth, and the remaining three occur during the secondary growth. The *S. communis* and *S. fuscifolia* stems show the compound vascular cylinder, according Tamaio et al. (2011) and Bastos et al. (2016). On the other hand, *S. meridionalis* is reported as the corded vascular cylinder type (Table 1), which has a central cylinder and 3–10 peripheral vascular cylinders devoid of pith. *Urvillea leavis* stems are cleft type, as



Figs. 27–30. Diagrams of transverse sections of *Urvillea* stem. 27–28. *Urvillea leavis* stems, without and with cambial variant (head arrow), respectively. 29–30. *Urvillea ulmacea* stems, without and with cambial variant (head arrow) and neo-formed peripheral cylinders (arrow), respectively. (pe = sclerenchymatous ground tissue; pi = pith). Scale bars: 27 = 300 µm; 28–29 = 100 µm; 30 = 500 µm.

is reported by Metcalfe and Chalk (1957) (lobed/fissured sensu Bastos et al. 2016), whereas the *U. ulmacea* stem has an intermediate type, that is, corded type with peripheral vascular cylinders and cleft type (Table 1).

Cambial or procambial variants of the stems of *Serjania* and *Urvillea* species investigated here differ in a few details, such as the origin of the variant and their structural types. *Serjania* has stems that belong to the following types: the compound vascular cylinder (it is formed in the primary growth) and corded vascular cylinder (it is also compound, but it originates in the secondary growth), while *Urvillea* has the stem distinguished by cleft and mixed vascular cylinder (cleft/corded vascular cylinder) types. The stem of *S. communis* can be distinguished from the *S. fuscifolia* by the number of peripheral cylinders, being five or six in the former and nine in the latter. The *S. meridionalis* stem is similar to both *Serjania* species in its final structure (nine peripheral cylinders or more), but differs in the origin of the cambial variants (the cylinders are formed during the secondary growth) (Table 1). In the analysis of the stem of *S. meridionalis*, Borniego and Cabanillas (2014) found similar results, in which the external vascular cylinders originate from the parenchymatous pericycle. The stems of *Urvillea* species have the same final structure, but the most striking distinction between *U. ulmacea* and *U. leavis* involves the development of the cambial variant, which in the former has one additional variant cambial type originating from the neo-formed peripheral vascular cylinders.

ACKNOWLEDGMENTS

We thank CNPq (National Council of Scientific and Technological Development, Brazil) for the support granted to complete this study. We are grateful to Sherwin Carlquist and an anonymous reviewer for their valuable comments and suggestion on the early version of this manuscript, and to Denver Falconer for review of the final version.

REFERENCES

Acevedo-Rodriguez, P. 1993. Systematics of *Serjania* (Sapindaceae) – Part 1: A revision of *Serjania* Sect. *Platycoccus*. The New York Botanical Garden, New York, U.S.A.

Alcorn, S.M. & P.A. Ark. 1953. Softening paraffin-embedded plant tissues. Stain. Tech. 28(2):55–56.

Angyalossy, V., M.R. Pace, & A.C. Lima. 2015. Liana anatomy: A broad perspective on structural evolution of the vascular system. In: S.A. Schnitzer, F. Bongers, R.J. Burnham, & F.E. Putz, eds. Ecology of lianas. John Wiley & Sons, New Jersey, U.S.A. Pp. 253–287.

Araújo, G.U.C. & C.G. Costa. 2006. Cambial variant in the stem of *Serjania corrugata* (Sapindaceae). IAWA J. 27:269–280.

Bastos, C.L., N. Tamaio, & V. Angyalossy. 2016. Unravelling roots of lianas: A case study in Sapindaceae. Ann. Bot. 118:733–746. doi:10.1093/aob/mcw091

Borniego, M.L. & P.A. Cabanillas. 2014. Desarrollo de la variante cambial en *Serjania meridionalis* (Sapindaceae, Paullinieae). Darwiniana 2:144–153.

Buerki, S., F. Forest, P. Acevedo-Rodríguez, M.W. Callmander, J.A.A. Nylander, M. Harrington, I. Sanmartín, P. Küpper, & N. Alvarez. 2009. Plastid and nuclear DNA markers reveal intricate relationships at subfamilial and tribal levels in the soapberry family (Sapindaceae). Molec. Phylogen. Evol. 51:238–258.

Caballé, G. 1993. Liana structure, function and selection: A comparative study of xylem cylinders of tropical rainforest species in Africa and America. Bot. J. Linn. Soc. 113:41–60.

Carlquist, S. 1982. The use of ethylenediamine in softening hard plant structures for paraffin sectioning. Stain. Tech. 57:311–317.

Ferrucci, M.S. 2000. Cytotaxonomy of Sapindaceae with special reference to the tribe Paullinieae. Genet. Molec. Biol. 23:941–946.

Fisher, J.B. & F.W. Ewers. 1989. Wound healing in stems of lianas after twisting and girdling injuries. Bot. Gaz. 150:251–265.

Fisher, J.B. & F.W. Ewers. 1992. Xylem pathways in liana stems with anomalous secondary growth. Bot. J. Linn. Soc. 180:181–202.

Karnovsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell. Biol. 27:137–138.

León, H.W.J. 2009. Anatomía de la madera de 20 especies de lianas del lote boscoso El Dorado-Tumeremo (Estado Bolívar, Venezuela). Ernstia 19:137–170.

Lombello, R.A. & E.R. Forni-Martins. 1998. Chromosomal studies and evolution in Sapindaceae. Caryologia 51(1):81–93.

Metcalfe, C.R. 1979. The stem. In: C.R. Metcalfe & L. Chalk, eds. Anatomy of the dicotyledons. Clarendon Press, Oxford, U.K. Pp. 166–180.

Metcalfe, C.R. & L. Chalk. 1957. Anatomy of the dicotyledons (leaves, stem, and wood in relation to taxonomy with notes on economic uses). Clarendon Press, Oxford, U.K.

Metcalfe, C.R. & L. Chalk. 1983. Anatomy of the dicotyledons(wood structure and conclusion of the general introduction). Clarendon Press, Oxford, U.K.

O'Brien, T.P., N. Feder, & M.E. McCully. 1964. Polycromatic staining of plant cell walls by toluidine blue O. Protoplasma59:368–373.

Souza, L.A., S.M. Rosa, I.S. Moscheta, K.S.M. Mourao, R.A. Rodella, D.C. Rocha, & M.I.G.A. Lolis. 2005. Morfologia e anatomia vegetal – técnicas e práticas. Editora UEPG, Ponta Grossa, Brasil.

Tamaio, N. 2011. Caracterização anatômica das madeiras de lianas de Sapindaceae utilizadas comercialmente em São Paulo. Cerne 17:533–540.

Tamaio, N. & V. Angyalossy. 2009. Variação cambial em *Serjania caracasana* (Sapindaceae); enfoque na adequação terminológica. Rodriguésia 60:651–666.

Tamaio, N., M.F. Neves, A.F.N. Brandes, & R.C. Vieira. 2011. Quantitative analyses establish the central vascular cylinder as the standard for wood-anatomy studies in lianas having compound stems (Paullinieae: Sapindaceae). Flora 206:987–996.