A COMPARATIVE ANATOMICAL STUDY OF THE STEMS OF CLIMBING PLANTS FROM THE FOREST REMNANTS OF MARINGA, BRAZIL

ESTUDIO ANATOMICO COMPARATIVO DE LOS TALLOS DE PLANTAS TREPADORAS DE LOS REMANENTES FORESTALES DE MARINGA, BRASIL

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ABSTRACT

Climbing plants differ from self-supporting plants, such as shrubs and trees, in a range of characteristics, most notable are the mechanical properties of the stem. Liana stems as *Pereskia aculeata* Mill. (Cactaceae), *Pisonia aculeata* L. (Nyctaginaceae), *Arrabidaea mutabilis* Bureau et K.Schum. (Bignoniaceae), *Dalechampia stipulacea* Müll.Arg. (Euphorbiaceae) and *Dicella nucifera* Chodat (Malpighiaceae) were collected in Brazilian forest remnants. The objective of this paper is: a) to analyze the comparative stem structure of these species; b) to answer the inquiry whether these species present cambial variants or not and to verify the modes of cambial activities, and c) to contribute with the anatomical identification of the liana species, characterizing mainly the cambial variant types. The stems were sectioned by freehand and in rotation microtome, and stained with safranin, astra blue and toluidine blue. Stem epidermis is uniseriate with tector trichomes. Cortex consists of collenchyma, parenchyma and starch sheath. *Pereskia aculeata* and *Pisonia aculeata* have medullary collateral bundles. Phellogen is subepidermic. The species present stems with cambial variants, except *Dalechampia stipulacea*. *Pereskia aculeata*, *Pisonia aculeata* and *Dicella nucifera* have included phloem in the secondary xylem. *Arrabidaea mutabilis* presents cambial variants in four regions of the secondary vascular cylinder.

KEYWORDS: Anomalous growth, climbing plants, primary growth, secondary growth, cambial variant.

RESUMEN

Las plantas trepadoras difieren de las que se autosoportan, como los arbustos y árboles, en un rango de características, las más notables son las propiedades mecánicas del tallo. Los tallos de lianas como *Pereskia aculeata* Mill. (Cactaceae), *Pisonia aculeata* L. (Nyctaginaceae), *Arrabidaea mutabilis* Bureau et K.Schum. (Bignoniaceae), *Dalechampia stipulacea* Müll.Arg. (Euphorbiaceae) y *Dicella nucifera* Chodat (Malpighiaceae) fueron recolectados en los remanentes forestales brasileños. El objetivo de este trabajo es: a) analizar la estructura comparativa del tallo de estas especies; b) responder la pregunta si estas especies presentan variantes del cambium o no y verificar los modos de actividades del cambium, y c) contribuir con la identificación anatómica de las especies del lianas, caracterizando principalmente los tipos de variantes del cambium. Los tallos fueron cortados a mano y en el micrótomo de rotación y coloreado con safranina, azul de astra y azul de toluidina. La epidermis del tallo es uniseriada con tricomas tectores. La corteza contiene parénquima, colénquima y vaina amilífera. *Pereskia aculeata* y *Pisonia aculeata* tienen haces medulares colaterales. El felógeno es subepidérmico. Los tallos de las especies presentan variantes del cambium, excepto *Dalechampia stipulacea*. *Pereskia aculeata*, *Pisonia aculeata* e *Dicella nucifera* han incluido el floema en el xilema secundario. *Arrabidaea mutabilis* presenta variantes del cambium en cuatro regiones del cilindro vascular secundario.

PALABRAS CLAVES: Crecimiento anómalo, plantas trepadoras, crecimiento primario, crecimiento secundario, variante del cambium.

INTRODUCTION

Climbing plants differ from self-supporting plants, such as shrubs and trees, in a range of characteristics, most notable is the mechanical properties of the stem (Isnard *et al.* 2003b). In lianas, the shift from self-supporting to non-self-supporting growth is accompanied by remarkable changes in anatomical development (Caballé 1993, Rowe & Speck 1996). In the climber, *Clematis vitalba* L., the aerial stem includes young stems with parenchymatous cortex and a cylinder vascular, as well as older stages with significant secondary growth of the vascular cambium and periderm (Isnard *et al.* 2003a, 2003b, Rowe *et al.* 2004).

Considering the tremendous number of possibilities for the functions and structures of stems, it is truly remarkable that there is only one single basic type in all of the vascular plants. In cross section, there is an outermost epidermis that overlies the cortex; the cortex in turn surrounds the vascular tissues (Mauseth 1988). Liana stems in primary growth with this basic type seems to be common. However, many variations of stem structure are usually called anomalies, although the term may be questioned, since the so-called anomalous structure is normal for the species in question. For example, in certain dicotyledons families cortical or medullary bundles are present in addition to the normal cylinder of bundles (Eames & McDaniels 1953, Cutter 1969), such as the Cactaceae and Nyctaginaceae.

Comparison of the differentiated anatomical structures recorded in 448 species of the forest lianas in 35 African and American families and sub-families confirms that lianas show a greater diversity of organization than other plant life forms (Caballé 1993). This anatomical radiation could probably not exist without the achievement of a wide range of secondary growth processes (Caballé 1993). Many dicotyledons, notably those with a climbing habit, show interesting secondary structure which differs from the more usual type described, therefore, sometimes termed anomalous (Cutter 1969). The stems with such secondary growth, which occur commonly in lianas, are referred in the literature as uncommon, atypical, anomalous ones or as presenting cambial variants (Eames & MacDaniels 1953, Sajo & Castro 2006). The anomalous or unusual structure may be a consequence of (1) a cambium of normal type which gives rise to unusual arrangements of secondary xylem and phloem, or (2) a cambium which itself is abnormally situated and so gives rise to abnormal arrangements of tissues, or (3) the formation of accessory or additional cambial zone (Eames & MacDaniels 1953, Cutter 1969).

The variant secondary growth is particularly widespread in tropical lianas. It is speculated that variant growth can increase stem flexibility, protect the phloem, increase storage parenchyma, aid in clinging to supports, limit physical disruption of vascular tissues during twisting and bending, and promote wound healing after girdling (Dobbins & Fisher 1986, Fisher & Ewers 1992). Fisher & Ewers (1992) consider that the major benefits of variant xylem arrangements to lianas are not in their influence upon transport pathways, but rather in their mechanical and regeneration effects.

However, most of the information about cambial variants is based on the mature structure, and only a few developmental studies have been made (Nair 1993, Araújo & Costa 2006). Besides, as suggested by Caballé (1993), the study on the anatomical structure of liana stems should provide a highly efficient descriptive tool for the identification of taxa (families, genera or species).

In Maringá (Brazil) there are lianas as *Pereskia aculeata* Mill. (Cactaceae), *Pisonia aculeata* L. (Nyctaginaceae), *Arrabidaea mutabilis* Bureau et K. Schum. (Bignoniaceae), *Dalechampia stipulacea* Müll. Arg. (Euphorbiaceae), and *Dicella nucifera* Chodat (Malpighiaceae) with stems that were not studied structurally. Thus, the objective of this paper is: a) to analyze the comparative stem structure of these species; b) to answer the inquiry whether these species present cambial variants or not and to verify the modes of cambial activities, and c) to contribute with the anatomical identification of the liana species, characterizing mainly the cambial variant types.

MATERIALS AND METHODS

The stems of *Pereskia aculeata*, *Pisonia aculeata*, *Arrabidaea mutabilis*, *Dalechampia stipulacea* and *Dicella nucifera* were collected from plants in the three of the forest remnants in Maringá city (Brazil). Vouchers of the botanical material were deposited in State University of Maringá Herbarium (HUEM), under respective registers:
Pereskia aculeata Mill.- BRAZIL. Paraná. Maringá, Horto Florestal, S. M. Rosa 5991 HUEM.
Pisonia aculeata L. - BRAZIL. Paraná. Maringá, Horto Florestal, L. A. Souza 8198 HUEM.

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Arrabidaea mutabilis Bureau et K.Schum. - BRAZIL. Paraná. Maringá, Horto Florestal, L. A. Souza 11878 HUEM.
Dalechampia stipulacea Müll.Arg. - BRAZIL. Paraná. Maringá, Bosque dos Pioneiros, L. A. Souza 11720 HUEM.
*Dicella nucifera* Chodat - BRAZIL. Paraná. Maringá, Bosque dos Pioneiros, L. A. Souza 11870 HUEM.

Analyses of the stems were made in fresh and fixed (FAA 50) material. The stems were sectioned by freehand and in rotation microtome between the first (close to the apical bud) and the seventeenth internode. The cross-sections were stained using safranin and astra blue (manual sections) (Souza *et al.* 2005) and toluidine blue (microtome sections) (O' Brien *et al.* 1965), in agreement with usual techniques in plant anatomy (Gerrits 1991).

Microchemical tests were carried out for lipids (Sudan IV), starch (iodine-potassium iodine test) and lignin (phloroglucina test) (Johansen 1940, Berlyn & Miksche 1976).

The illustrations were made by drawings (diagrams), obtained in Willd M20 microscope equipped with camera lucide, and photomicrographs. Photomicrographs were obtained by processing the image captured in Olympus microscope with Cannon digital camera. The respective micrometric scales were prepared in the same optical conditions as the drawings and photomicrographs.

RESULTS

Stem epidermis of the species is uniseriate and presents different tector trichomes. The *Pereskia aculeata* and *Pisonia aculeata* trichomes are simple, long and multicellular (Fig. 1). *Dicella nucifera* and *Dalechampia stipulacea* present unicellular and multicellular tector trichomes; unicellular trichomes (Fig. 2) are long, unbranched and multicellular trichomes (Fig. 3) are formed by two elongated basal cells and one or two apical thick-walled cells. *Arrabidaea mutabilis* presents scales (Fig. 4). The cortex (Figs. 1, 5-7) has similarities among the species, showing collenchyma, parenchyma and starch sheath.
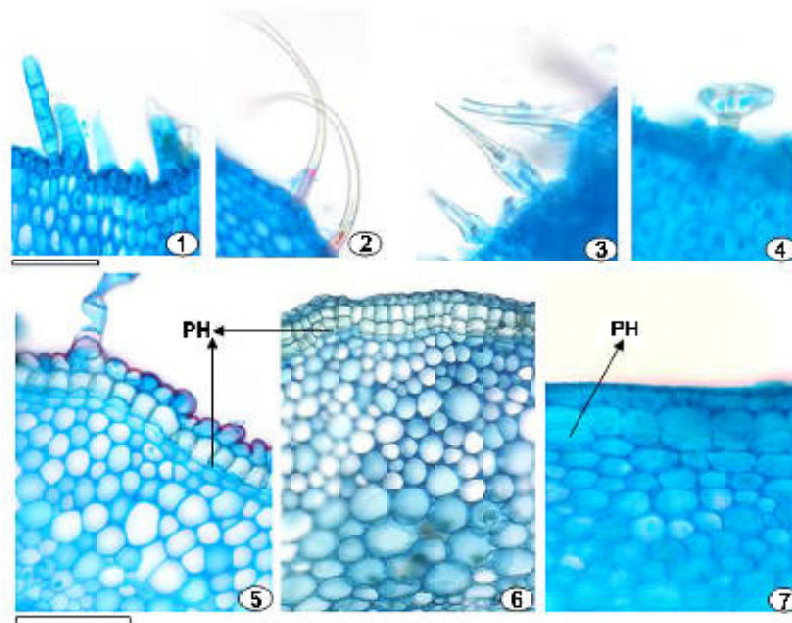
FIGURES 1-7. Details of stem epidermis, peridermis and cortex. Figs. 1-4 show epidermis with trichomes of *Pisonia aculeata* (1), *Dalechampia stipulacea* (2, 3) and *Arrabidaea mutabilis* (4). Figs. 5-7 show cortex and peridermis in *Pereskia aculeata* (third internode), *Pisonia aculeata* (third internode) and *Arrabidaea mutabilis* (seventeenth internode), respectively. (PH = phellogen). Bars = 100 µm.

FIGURAS 1-7. Detalles de epidermis, peridermis y corteza del tallo. Figs. 1-4 muestran epidermis con tricomas de *Pisonia aculeata* (1), *Dalechampia stipulacea* (2, 3) y *Arrabidaea mutabilis* (4). Figs. 5-7 muestran corteza y peridermis en *Pereskia aculeata* (tercer internodio), *Pisonia aculeata* (tercer internodio) y *Arrabidaea mutabilis* (decimoséptimo internodio), respectivamente. (PH = felógeno). Barras = 100 µm.

The *Dicella nucifera* and *Arrabidaea mutabilis* cortical collenchyma differentiates starting from the second internode. *Dalechampia stipulacea* (Fig. 8) and *Arrabidaea mutabilis* (Fig. 20) collenchyma occurs as individual bundles and in *Pereskia aculeata*, (Figs. 10, 11) *Pisonia aculeata* (Figs. 13, 14) and *Dicella nucifera* (Figs. 17, 18) it is a continuous cylinder with 3-4 subepidermic layers.

The primary vascular cylinder possesses pericycle and collateral bundles which enclose the parenchymatous pith (Figs. 8, 13, 16, 19). *Pereskia aculeata* and *Pisonia aculeata* also have medullary collateral bundles (Figs. 10-12, 13-15). *Dalechampia stipulacea* (Fig. 8), *Dicella nucifera* (Fig. 16) and *Arrabidaea mutabilis* (Fig. 19) present fibers towards the outside of the primary phloem forming dense bundles.

All investigated species present secondary growth, with the cambium already installed starting from the first internode (Figs. 8, 10, 13, 16, 19). The phellogen of subepidermic origin (Figs. 5-7) is observed in the third internode in *Pereskia aculeata* (Figs. 5, 11) and *Pisonia aculeata* (Figs. 6, 14) while *Arrabidaea mutabilis* shows a phellogen in the seventh internode (Figs. 7, 21). In the case of *Dalechampia stipulacea* and *Dicella nucifera* the phellogen was not verified to the seventeenth internode.

Dalechampia stipulacea has common pattern of vascular secondary growth, without cambial variant (Figs. 8, 9). The other studied species show cambial variants. *Pereskia aculeata* (Fig. 12), *Pisonia aculeata* (Fig. 15) and *Dicella nucifera* (Fig. 18) have included phloem in the secondary xylem; however, these three species show origin different from included phloem.
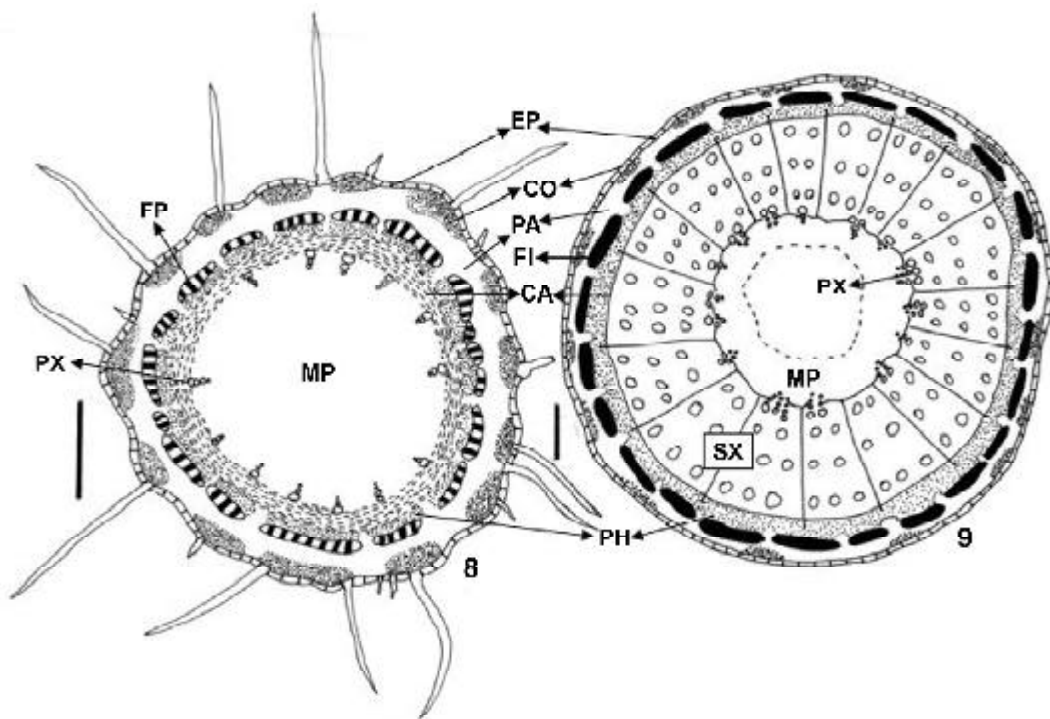

Figures 8-9. Stem structure in cross-sections of *Dalechampia stipulacea*. Figs. 8-9 show overall diagrams of the first and seventeenth internodes. (CA = cambium; CO = collenchyma; EP = epidermis; FI = fibers; FP = fiber primordia; MP = medullary parenchyma; PA = parenchyma; PH = primary and secondary phloem; PX = primary xylem; SX = secondary xylem). Bars = 200 and 500 µm, respectively.

Figuras 8-9. Estructura del tallo en cortes transversales de *Dalechampia stipulacea*. Figs. 8-9 muestran diagramas de los primero y decimoséptimo internodios. (CA = cambium; CO = colénquima; EP = epidermis; FI = fibras; FP = primordia de fibra; MP = parénquima medular; PA = parénquima; PH = floema primario y secundario; PX = xilema primario; SX = xilema secundario). Barras = 200 y 500 µm, respectivamente.
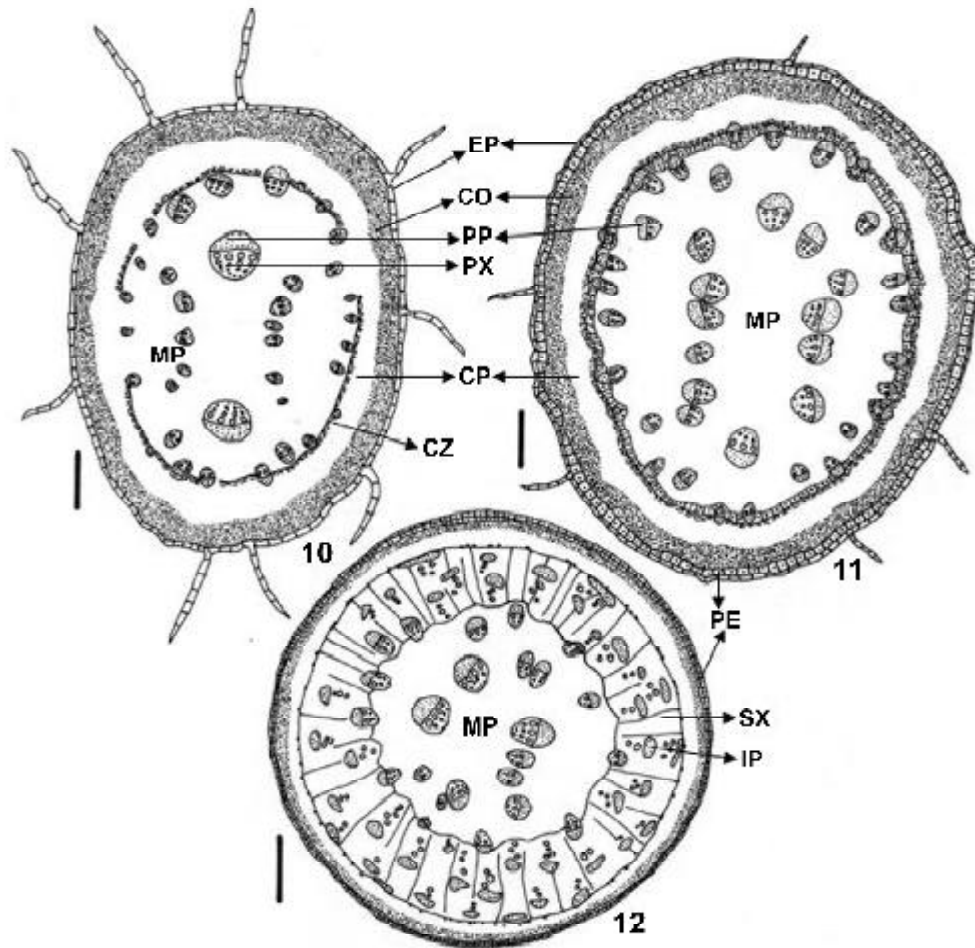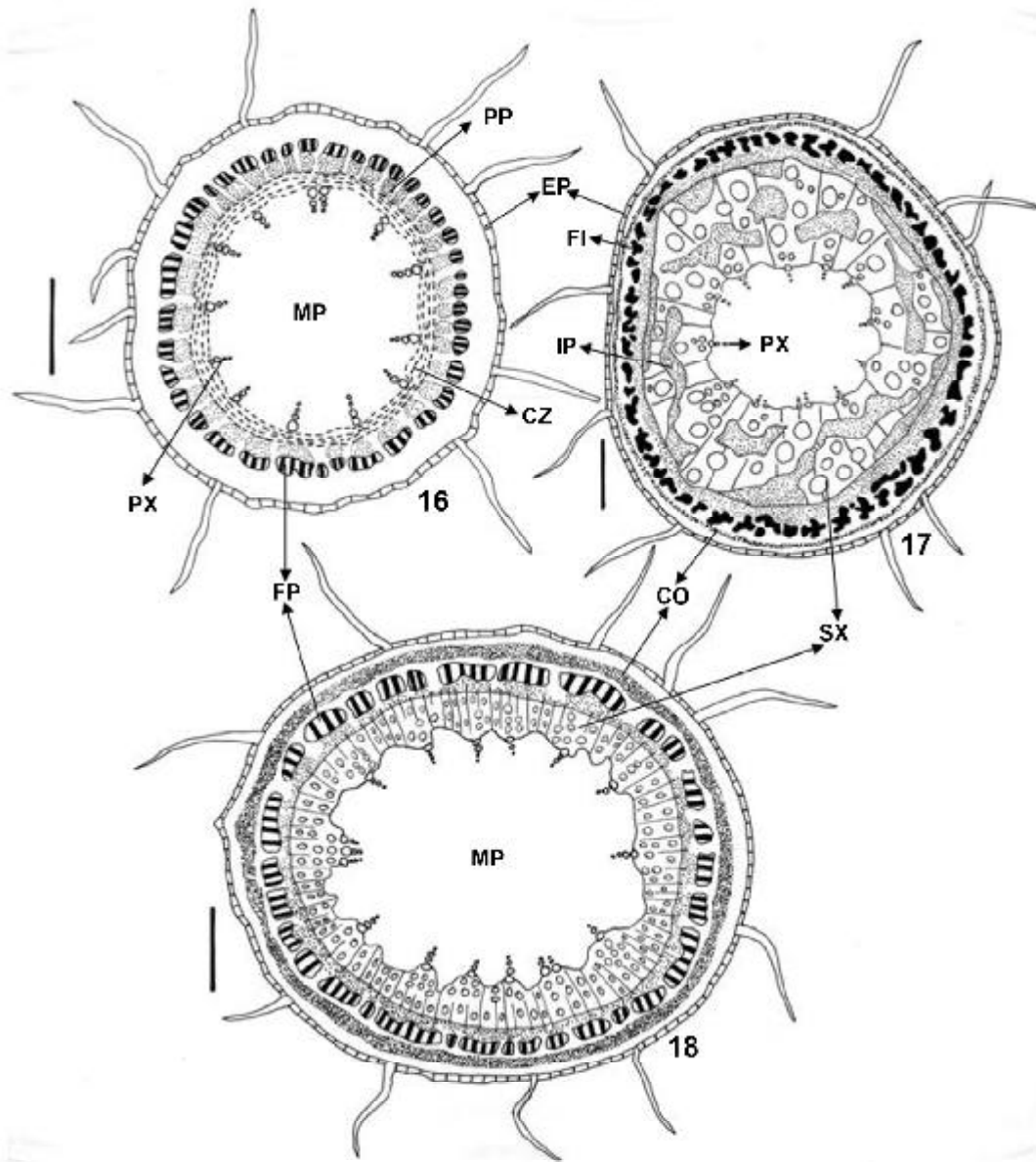
Figures 10-12. Stem diagrams in cross-sections of *Pereskia aculeata* of the first, third and thirteenth internodes, respectively (CO = collenchyma; CP = cortical parenchyma; CZ = cambial zone; EP = epidermis; IP = included phloem; MP = medullary parenchyma; PE = peridermis; PP = primary phloem; PX = primary xylem; SX = secondary xylem). Bars = 250, 250 and 700 µm, respectively.

Figuras 10-12. Diagramas del tallo en cortes transversales de *Pereskia aculeata* del primero, tercero y decimotercero internodios, respectivamente (CO = colénquima; CP = parénquima cortical; CZ = zona del cambium; EP = epidermis; IP = floema incluido; MP = parénquima medular; PE = peridermis; PP = floema primario; PX = xilema primario; SX = xilema secundario). Barras = 250, 250 y 700 µm, respectivamente.
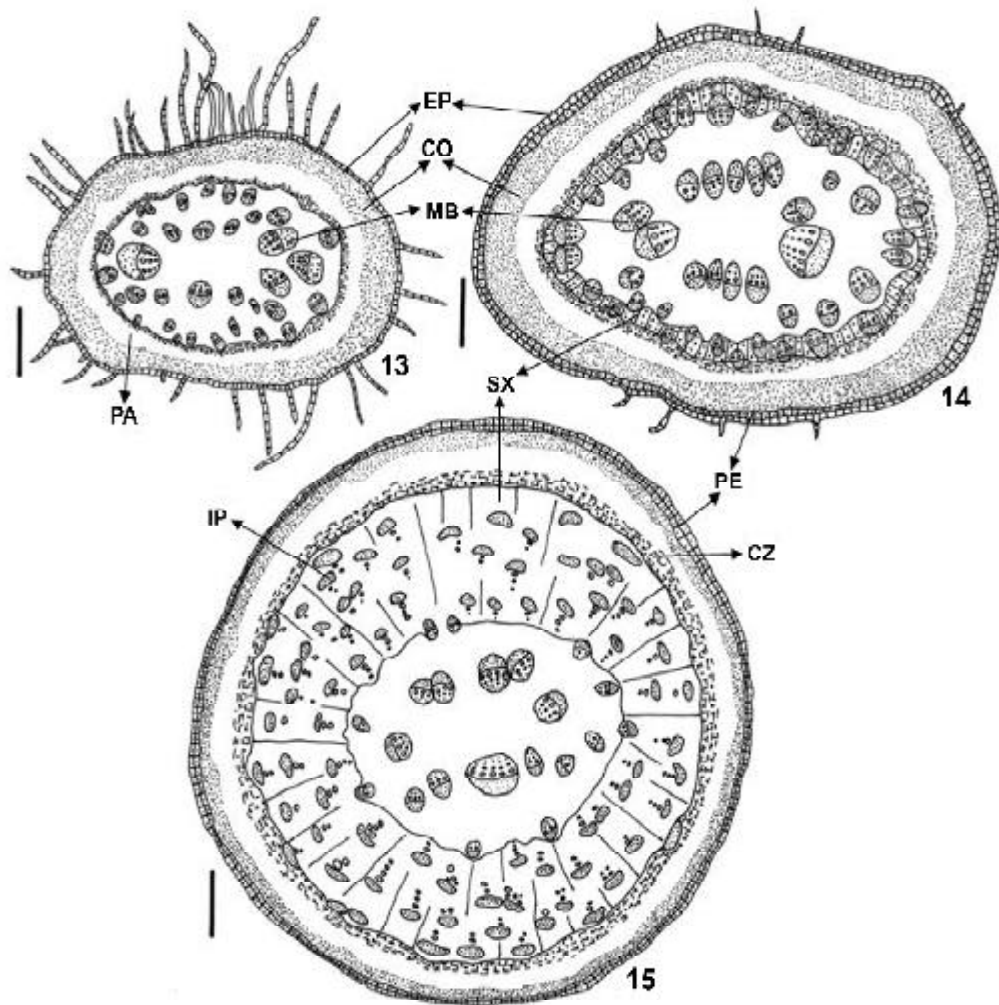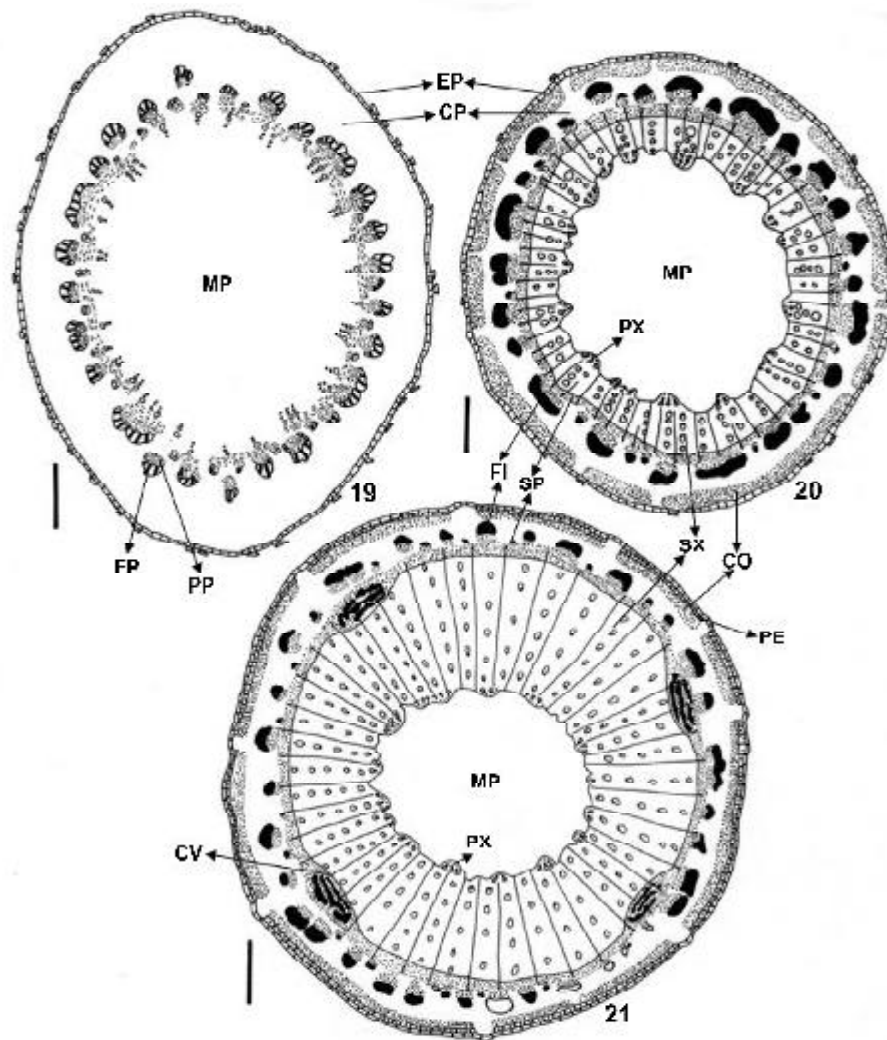
Figures 13-15. Stem diagrams in cross-sections of *Pisonia aculeata* of the first, third and basal internodes, respectively. (CO = collenchyma; CZ = cambial zone; EP = epidermis; IP = included phloem; MB = medullary vascular bundles; PA = parenchyma; PE = peridermis; SX = secondary xylem). Bars = 750, 400 and 400 µm, respectively.

Figuras 13-15. Diagramas del tallo en cortes transversales de *Pisonia aculeata* del primero, tercero y internodios de la base, respectivamente. (CO = colénquima; CZ = zona del cambium; EP = epidermis; IP = floema incluido; MB = haces vasculares medulares; PA = parénquima; PE = peridermis; SX = xilema secundario). Barras = 750, 400 y 400 µm, respectivamente.

Figures 16-18. Stem diagrams in cross-sections of *Dicella nucifera* of the first, fourth and twelfth internodes, respectively. (CO = collenchyma; CZ = cambial zone; EP = epidermis; FI = fibers; FP = fiber primordia; IP = included phloem; MP = medullary parenchyma; PP = primary phloem; PX = primary xylem; SX = secondary xylem). Bars = 200, 500 and 200 µm, respectively.

Figuras 16-18. Diagramas del tallo en cortes transversales de *Dicella nucifera* del primero, cuarto y duodécimos internodios, respectivamente. (CO = colénquima; CZ = zona del cambium; EP = epidermis; FI = fibras; FP = primordia de fibra; IP = floema incluido; MP = parénquima medular; PP = floema primario; PX = xilema primario; SX = xilema secundario). Barras = 200, 500 y 200 µm, respectivamente.

Figures 19-21. Stem diagrams in cross-sections of *Arrabidaea mutabilis* of the first, third and seventh internodes, respectively (CO = collenchyma; CP = cortical parenchyma; CV = cambial variant; EP = epidermis; FI = fibers; FP = fiber primordial; MP = medullary parenchyma; PE = peridermis; PP = primary phloem; PX = primary xylem; SP = secondary phloem; SX = secondary xylem). Bars = 400, 500 and 400 µm, respectively.

Figuras 19-21. Diagramas del tallo cortes transversales de *Arrabidaea mutabilis* del primero, tercero y séptimo internodios, respectivamente (CO = colénquima; CP = parénquima cortical; CV = variante del cambium; EP = epidermis; FI = fibras; FP = primordio de fibra; MP = parénquima medular; PE = peridermis; PP = floema primario; PX = xilema primario; SP = floema secundario; SX = xilema secundario). Barras = 400, 500 y 400 µm, respectivamente.
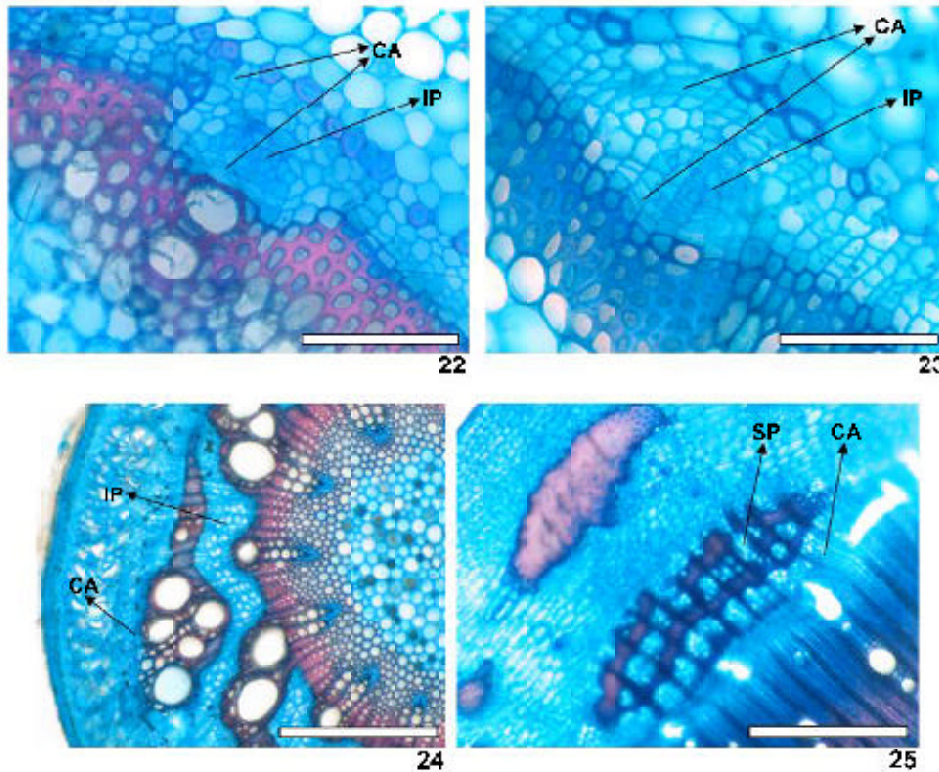
FIGURES 22-25. Details of stem in cross-sections. Figs. 22-24 show cambium (CA) and included phloem (IP), in *Pereskia aculeata*, *Pisonia aculeata* and *Dicella nucifera*, respectively. Fig. 25 shows cambium (CA) and larger amount of secondary phloem (SP) in *Arrabidaea mutabilis*. Bars = 100 µm.

FIGURAS 22-25. Detalles de tallo en cortes transversales. Figs. 22-24 muestran cambium (CA) y floema incluido (IP), en *Pereskia aculeata*, *Pisonia aculeata* y *Dicella nucifera*, respectivamente. Fig. 25 muestra el cambium (CA) y cantidad más grande de floema secundario (SP) en *Arrabidaea mutabilis*. Barras = 100 µm.

In *Pereskia aculeata* and *Pisonia aculeata* vascular bundles of cambial origin are formed, which soon afterwards are included again in the secondary xylem by the action of the cambium that is formed in the periphery of the phloem. This cambium turns to produce secondary xylem internally (Figs.11, 12, 14, 15, 22, 23).

Dicella nucifera has cambium that just originates secondary phloem in certain regions of the vascular cylinder towards the pith, interrupting the production of secondary xylem temporarily. When this cambium retakes the production of secondary xylem, the phloem stay included in this xylem (Figs. 17, 18, 24).

Arrabidaea mutabilis stem shows cambial variants in four regions of the secondary vascular cylinder (Figs. 21, 25). In these regions the cambium reduces the formation of secondary xylem and it originates larger amount of secondary phloem. This secondary phloem presents tangential strips of sclerenchymatous cells in the axial system.

DISCUSSION

A marked feature of the lianoid development in *Clematis* L. is that mechanical support is provided by a primary structure composed of fibers and collenchyma localized at the outside of the stem (Rowe *et al*. 2004). Mechanical strategy among woody lianas is which young stages show a self-supporting phase and are adapted to grow across gaps and reach host supports whereas flexible older stages can absorb and reduce potentially catastrophic mechanical stresses resulting from

movement of the host plant (Isnard *et al.* 2003a). *Dalechampia stipulacea*, *Dicella nucifera* and *Arrabidaea mutabilis* with collenchyma and fibres towards the outside of the primary phloem seem to be more efficient in the self-supporting phase than the *Pereskia aculeata* and *Pisonia aculeata* that just present collenchyma. But only biomechanical studies can confirm this supposition.

Many variations of stem primary structure are usually called anomalies (Cutter 1969). The occurrence of medullary bundles in stems, as verified in *Pereskia aculeata* and *Pisonia aculeata*, can be considered as anomalous structure type (Eames & MacDaniels 1953, Cutter 1969). Medullary bundles can be present in Cactaceae and Nyctaginaceae species and the role or function of these bundles is completely unknown (Mauseth 1988).

In the stem of the studied species, the phellogen has subepidermic or cortical origin which is common in stem dicotyledons (Esau 1959, Mauseth 1988). In the investigated species, the phellogen arises after the cambium installation, what seems also to be frequent in dicotyledons (Mauseth 1988). A delay in forming a phellogen in *Dalechampia stipulacea* and *Dicella nucifera* may be related to the photosynthetic activity of the stem cortex, as Mauseth (1988) verified for other dicotyledons.

Pereskia aculeata, *Pisonia aculeata* and *Dicella nucifera* have included phloem in the secondary xylem, for action of a single cambium. Carlquist (2002) stands out that, in dicotyledons, interxylary or included phloem is not confused with the phloem produced by successive cambia; in the latter, phloem is produced outwardly from each of the successive cambia and therefore lies between conjunctive tissue and the secondary xylem produced by each of the cambia.

Nyctaginaceae (*Pisonia* family) is notable because of occurrence of secondary thickening of the anomalous type in its axis (Esau & Cheadle 1969). Metcalfe & Chalk (1957) describe this thickening as one derived from successive cambia and producing a tissue composed of successive increments of vascular bundles. *Bougainvillea spectabilis* Willd. cv. Lateritia, Nyctaginaceae, presents anomalous cambia that arise successively in centrifugal order, each originating among the derivatives of the preceding cambium (Esau & Cheadle 1969). On the other hand, *Pisonia aculeata* differs of other Nyctaginaceae for not forming successive cambia, but a single cambium.

The interruption or reduction of the cambial activity in forming xylem but maintaining the phloem formation, in at least four regions of the secondary vascular cylinder, as verified in *Arrabidaea mutabilis* stem, seems to be common in Bignoniaceae stems (Chalk & Chattaway 1937, Metcalfe & Chalk 1957, Dobbins 1971, Gabrielli 1993). Fisher & Ewers (1992) affirm that many species of Bignoniaceae have arrangement of secondary tissues, with phloem wedges and discontinuous vascular cambium: four arcs of the vascular cambium become physically separated from the initially cylindrical cambium; in these four sectors much more phloem than xylem is produced. In Bignoniaceae (*Arrabidaea* DC., *Bignonia* L., *Pyrostegia* C.Presl, *Macfadyena* A. DC.) shoots with decussate phyllotaxy there are four cambium segments, each opposite one of the major vascular strands (Dobbins 1981). Still, Dobbins (1981) suggests that the number and positioning of these unidirectional cambium segments supports the hypothesis of morphogenetic correlation between anomalous secondary growth and the vascular pattern of the primary body.

Pfeiffer (1926) recognized eight types of anomaly (cambial variants) in dicotyledons, depending on the organization and activity of the cambium (Chalk & Chattaway 1937). In agreement with the Pfeiffer classification *Pereskia aculeata*, *Pisonia aculeata*, and *Dicella nucifera* stems in which phloem is included within the xylem can be considered as foraminate ("corpus lignosum foraminatum") type; *Arrabidaea mutabilis* stem can be classified as interrupted ("corpus lignosum interruptum") type, in that the cambial ring becomes broken into separate arcs as a result of the formation of unequal amounts of xylem and phloem in different parts of the ring. *Dalechampia stipulacea*, unlike the other species, has usual vascular secondary growth for dicotyledons, with a single cambium which produces secondary phloem to the outside and secondary xylem to the inside.

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