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Morphoanatomy of *Serjania communis* Cambess. seedling (Sapindaceae)

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ABSTRACT. *Serjania communis* Cambess. (Sapindaceae) is a plant with climbing habit and occurs relatively often in Paraná State, Brazil. The fruits were collected at the 'Parque dos Pioneiros' a remnant of subtropical forest in Maringá, Paraná State, Brazil. The seedlings obtained in the greenhouse were described according to traditional techniques in plant morphology. Seedlings were embedded in historesin and sectioned in rotation microtome. The fruit is the samaroid type, the seeds have about 3 mm in length and brown color. Seedlings are epigeal phanerocotylar. The seedlings have a hairy hypocotyl, foliaceous cotyledons, reduced epicotyl, and two opposite eophylls. The root is diarch, the hypocotyl shows root-stem transition structure, stem epicotyl, and dorsiventral and hypostomatous cotyledons and eophylls. 'Tirodendros' with 45 days of age do not develop cambial variant.

Keywords: root, hypocotyl, cotyledon, epicotyl, eophyll.

Morfoanatomia da plântula de *Serjania communis* Cambess. (Sapindaceae)

RESUMO. *Serjania communis* Cambess. (Sapindaceae) é uma planta de hábito lianescente e ocorre com relativa frequência no Estado do Paraná, Brasil. A coleta dos frutos para a obtenção de sementes foi realizada no parque florestal dos Pioneiros (bosque II), um remanescente de floresta subtropical de Maringá, Estado do Paraná, Brasil. As plântulas, obtidas em casa de vegetação foram descritas conforme técnicas usuais em morfologia vegetal. Após a germinação, as plântulas foram descritas morfoanatomicamente e também foi verificada se ocorria a instalação de variante cambial nesta fase inicial de desenvolvimento. O fruto é do tipo samaroide. As sementes têm cerca de 3 mm de comprimento, de cor marrom e com germinação epigeia e as plântulas são fanerocotiledonares. As plântulas foram emblocadas em historresina e secionadas em micrótomo de rotação. As plântulas apresentam o hipocótilo piloso, cotilédones foliáceos, o epicótilo é reduzido, os dois primeiros eofilos são opostos e os demais eofilos apresentam filotaxia alterna helicoidal. A raiz é diarca. O hipocótilo é uma região de transição raiz-caule. O epicótilo tem estrutura caulinar eustélica. Os cotilédones e os eofilos são hipostomáticos e dorsiventrais. Tirodendros com 45 dias de idade não desenvolvem variante cambial.

Palavras-chave: raiz, hipocótilo, cotilédone, epicótilo, eofilo.

Introduction

Serjania Mill. is a genus composed of species with climbing habits, belonging to Sapindaceae and containing approximately 226 species spread from the Southwest United States to the North of Argentina. In Brazil, there are 92 species recognized, of which 47 are endemic. The species are more often found in wet environments than in dry or open environments (ACEVEDO-RODRÍGUEZ, 1990).

Lianas are significant representatives and of great importance for the structure of Tropical Forests (PUTZ, 1984). Most of the climbing species are heliophytes, that is, species that develop where there is abundant light. In order to reach the light over the tree canopies, these species develop a different activity in the vascular cambium, which results in an unusual development of secondary vascular tissues that are not found in most of eudicots.

The structural study of seedlings has great relevance in the population dynamics, and in other areas of botany and ecology, but researches involving anatomical studies are scarce. If there were more studies concerning this issue, it would be easier to understand the processes of establishing seedlings in a determined environment. Souza et al. (2009) define seedling as a plant phase that begins with the seed germination until the formation of the first leaf or eophyll; the subsequent phase called 'tirodentro' by the authors extends to the moment of the appearance of the first metaphyll.

The structure of the vegetative organs of Sapindaceae, which includes the species *Serjania*, is well known in the literature (METCALFE; CHALK, 1957; ARAÚJO; COSTA, 2007; TAMAIO; SOMNER, 2010; TAMAIO, 2011). On the other hand, the study of seedlings seems to be restricted to the morphological analysis of species belonging to *Dodonaea* Mill., *Erioglossum* Blume, *Ganophyllum* Blume, *Pometia* J.R. Forst. & G. Forst., *Schleichera* Willd. (BURGER-HZN, 1972), *Cubilia* Blume, *Dimocarpus* Lour., *Harpulia* G. Don, *Lepisanthes* Blume, *Nephelium* L. (VOGEL, 1980), *Allophylus* L. (BURGER-HZN, 1972; LEONHARDT et al., 2008), *Cupania* L. (LEONHARDT et al., 2008), *Pseudima* Radlk. (PAOLI; BIANCONI, 2008).

The structural research of Sapindaceae seedlings appears to have escaped the attention of the researchers, but the interesting structure of the seedling may reveal characters that are important in studies on species preservation, seedling establishment and ecological aspects for the regeneration of the vegetation. Thus, the main purpose of this study was to analyze the seedling structure of *Serjania communis* Cambess. (Sapindaceae) (the liana species that grows in a forest fragment in the Northwest of Paraná State, Brazil), with particular reference to the installation of cambium variants in the phase of seedling/tirodendro.

Material and methods

Fruits were collected from three different individuals at the 'Parque Florestal dos Pioneiros (Bosque II)', a forest fragment in the city of Maringá, Paraná, Brazil, with an area of 59 ha, located in the urban area. Voucher materials were stored at the Herbarium (HUEM) of the State University of Maringá with collection number 11733 HUEM.

Seeds (256) were placed to germinate in substrate for plants in two seeders of expanded polystyrene (Styrofoam), with 128 cells each seeder. The age of the seedlings was considered with their sprouting from the soil. After 15 days, the seedlings were planted in a mixture of soil and organic substrate, with equal proportions, in plastic bags in a greenhouse.

The morphological and anatomical analyses were carried out for seedlings/tirodendros within 5-45 days after the emergence. The roots, hypocotyls, epicotyls, cotyledons and the eophylls were fixed in FAA 50 (formaldehyde, glacial acetic acid and ethyl alcohol) (JOHANSEN, 1940) and Glutaraldehyde (KARNOVSKY, 1965), embedded in historesin (GERRITS, 1991), cross-sectioned on a rotation microtome and stained with toluidine blue (O'BRIEN et al., 1964). The material was also sectioned manually (cross-sections) and stained using safranin and astra blue (SOUZA et al., 2005). A specific microchemical test for starch (iodine-potassium iodide test) was carried out (JOHANSEN, 1940).

The seedlings were illustrated by drawings. The anatomical drawings were made using a light microscope (Willd M20) equipped with a camera lucida. Photographs of light microscope were taken using a digital camera Leica EZ4D and subsequently processed using a Leica Application Suite software version 1.8.

After washing in 0.1 M sodium cacodylate buffer, the samples (seedlings) were dehydrated in a graded acetone series, critical point dried with CO_2, mounted on aluminum stubs, and then coated with gold, being subsequently examined using scanning electron microscopy (Shimadzu SS-550 Superscan), obtaining digital images.

Results

Diaspore and seedling/'tirodendro' morphology

A schizocarpic fruit, of samaroid subtype, being a dry winged tricarpellate fruit, represents the diaspore (Figure 1A-C). There are three small and bright seeds (Figure 1D) (approximately 3 mm in length) per fruit.

The seedling (Figure 1E-G) is phanerocotylar and epigeal, but the envelopes remain temporarily around the cotyledons. The root is axial (Figure 1E-G) and it shows secondary branches in the seedling with five days of age. The collet is almost indistinguishable showing coloration different between root and hypocotyl. The cotyledons (Figure 1E-G) are foliaceous, petiolate, with blade from obovate to oblong, with truncated apex, obtuse base and smooth margin.

In the seedling, the epicotyl is greatly reduced (Figure 1F,G), green and hairy. There are two simple eophylls with opposite position (Figure 1E-G). They have petiole and blade; the blade is lanceolate with serrated margin. The simple eophylls of the tirodendro phase are all spirally arranged (Figure 1F,G).

Seedling anatomy

A more pronounced enlargement of cells is evident in the root protodermis, which is covered by the root cap cells (Figure 2B). In the cortical fundamental meristem, a pro-endodermis is distinguishable, and pericycle and procambium cylinder are also evident in the primary root apex (Figure 2B).

The primary root is diarch (Figure 2C,D), and consists of uniseriate epidermis with rectangular, oval and obovate cells. A parenchymatous cortex underlies the epidermis, with endodermis (Figure 2D) devoid of Casparian strips and starch. The central cylinder is composed of parenchymatous uniseriate pericycle and alternate bundles of primary xylem and phloem (Figure 2D).

The hypocotyl is made up of uniseriate epidermis with unicellular non-glandular trichomes, and collenchymatous and parenchymatous cortex. The hypocotyl vasculature is complex, consisting chiefly of the root-stem transition region. In the root and collet region, each primary xylem bundle breaks into a median protoxylem and a pair of metaxylems, and in this portion a pith begins to appear (Figure 2E, F). The hypocotyl is characterized by primary xylem that is spread into a continuous tangential ring surrounding the pith (Figure 2G, H). The primary phloem bundles are also divided into four bundles (Figure 2F). In the cotyledonary node, the switch for the exarch typical condition of the root to the endarch (stem) condition occurs. In this node, the epicotyledonary traces, which is composed of two phloem bundles, and also each cotyledonary double trace, median protoxylem, and a pair of metaxylem bundles were verified.

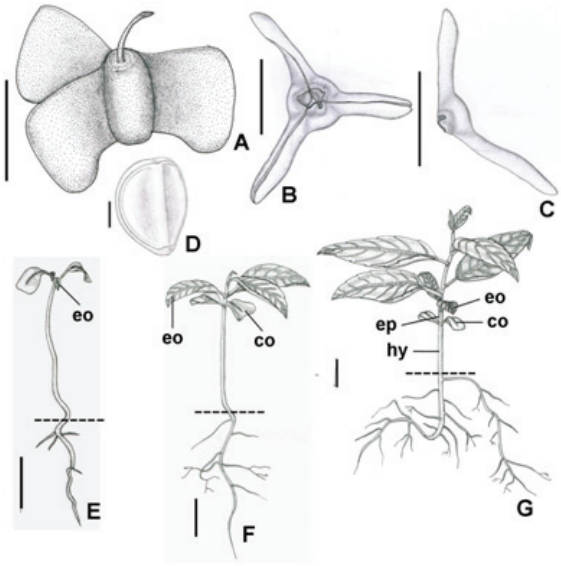

Figure 1. Diaspore and seedling/tirodendro of *Serjania communis*. A-C. Schizocarpic fruit, front view of the fruit, and fruit from the side view. D. Seed. E. Seedling. F,G. Tirodendros with various eophylls. (co=cotyledon; eo=eophyll; ep=epicotyl; hy=hypocotyl). Bars = 0.1cm (D), 0.5cm (A-C), 1cm (e-g).

A cross-section of the cotyledon petiole shows a hairy uniseriate epidermis, collenchyma, parenchyma and the double trace. The cotyledon blade has heterogeneous mesophyll which is composed of a compact palisade parenchyma with narrow cells (Figure 3A, C) and multilayered spongy parenchyma. The adaxial face of the epidermis of the cotyledon blade is glabrous and devoid of stomata (Figure 3D); and non-glandular trichomes (Figure 3E) and stomata are only found on the abaxial surface of the leaf.

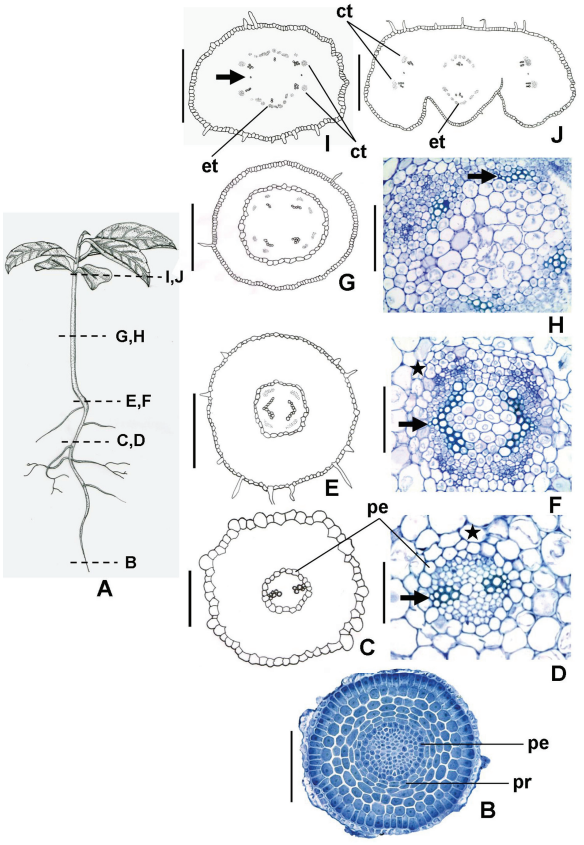

Figure 2. Root and root/stem transition region of *Serjania communis* seedling in cross sections. A. Seedling. B. Root apex. C,D. Primary root. E,F. Collet region. G,H. Hypocotyl. I,J. Cotyledonary node region. (asterisk indicates endodermis; black arrow indicates protoxylem; ct=cotyledonary trace; et=epicotyledonary trace; pe=pericycle; pr=pro-endodermis). Bars = 50 μm (D), 100 μm B,F,H), 150 μm (C), 300 μm (E,G,I,J).

A cross-section of the cotyledon petiole shows a hairy uniseriate epidermis, collenchyma, parenchyma and the double trace. The cotyledon blade has heterogeneous mesophyll which is composed of a compact palisade parenchyma with narrow cells (Figure 3A, C) and multilayered spongy parenchyma. The adaxial face of the epidermis of the cotyledon blade is glabrous and devoid of stomata (Figure 3D); and non-glandular trichomes (Figure 3E) and stomata are only found on the abaxial surface of the leaf.

The epicotyl (Figure 4A) consists of uniseriate epidermis with unicellular non-glandular trichomes,

collenchymatous and parenchymatous cortex, and central cylinder composed of two collateral vascular bundles and phloematic strands that surround the parenchymatous pith. The first eophyll node is unilacunar with a single trace (Figure 4B).

The eophyll petiole (Figure 4C) is made up of hairy uniseriate epidermis, wtih collenchymatous and parenchymatous cells that underlie the epidermis. A prominent single vascular bundle runs the petiole (Figure 4C). The eophyll blade is hypostomatous and has dorsiventral mesophyll, with a funnel-shaped adaxial layer (Figure E). The mid-vein shows a single vascular bundle; collenchyma and parenchyma (Figure 4D).

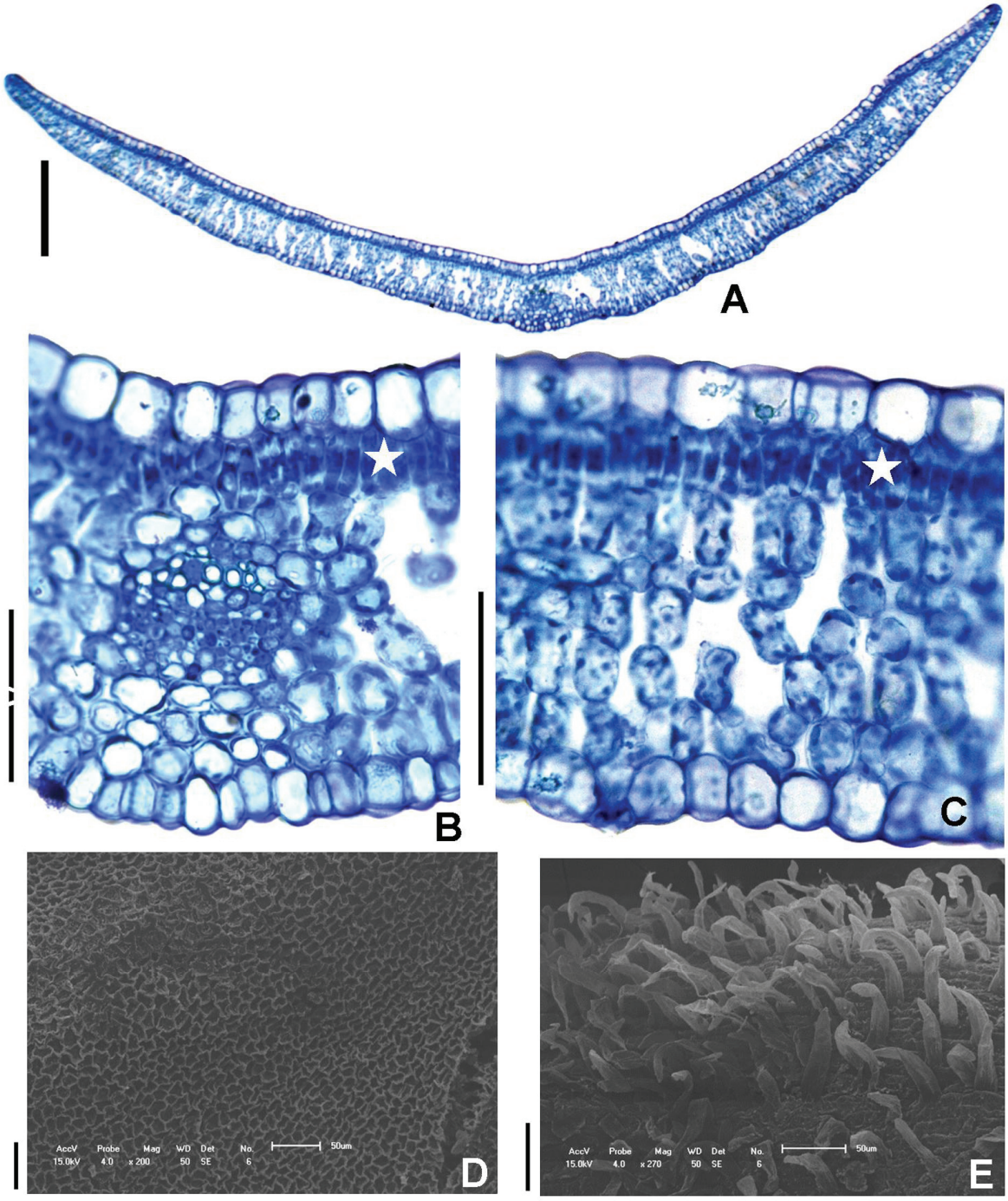

Figure 3. Cotyledon structure of *Serjania communis*. A. General view of the lamina in cross section. B. Portion of cross section of the midrib. C. Portion of cross section of the lamina. D,E. Scanning electron micrographs of the adaxial and abaxial cotyledon surfaces. (asterisks indicate palisade parenchyma). Bars=50 μm (D,E), 100 μm (B,C), 300 μm (a).

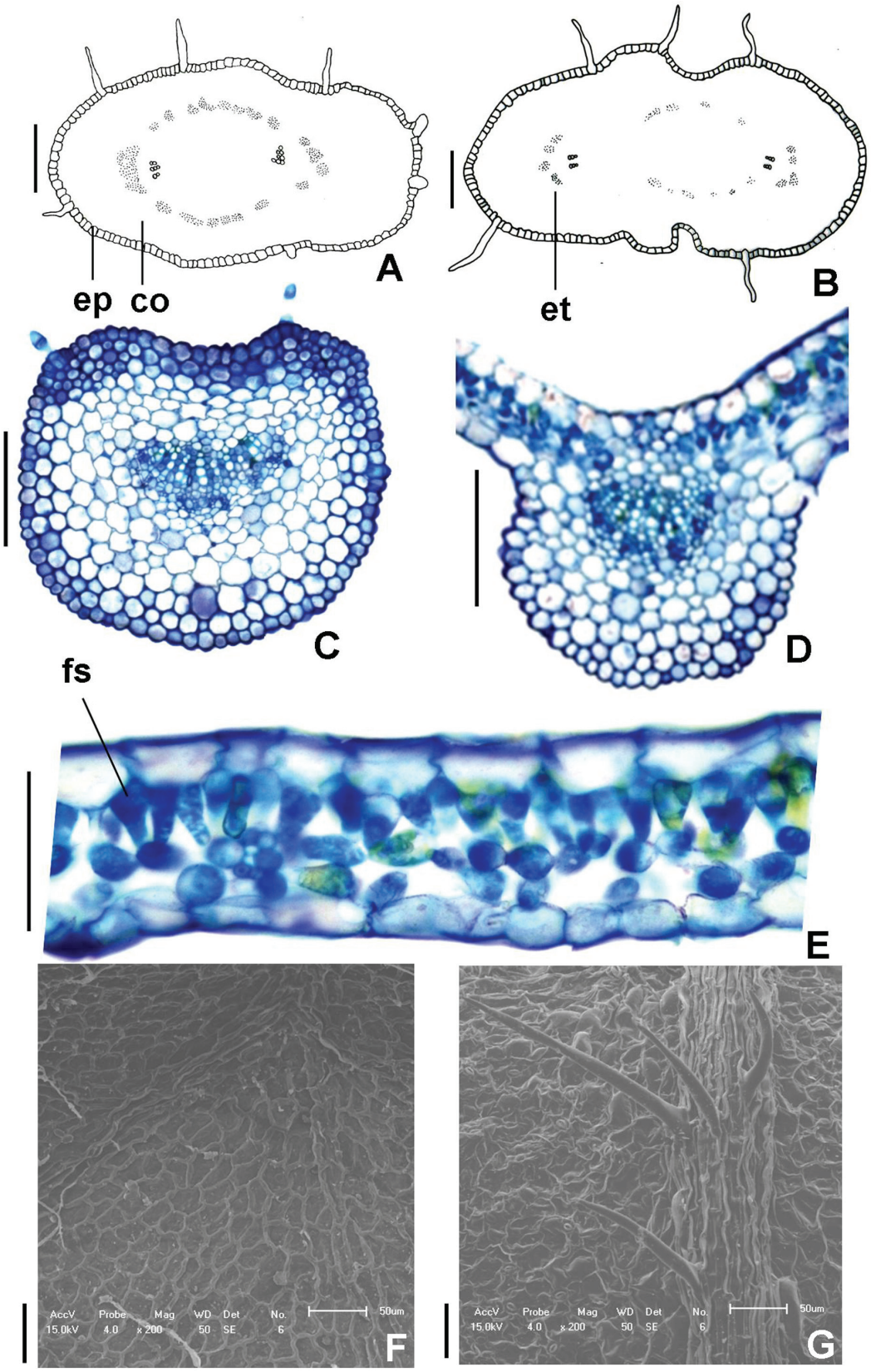

Figure 4. Structure of the epicotyl and eophyll of *Serjania communis*, in cross sections (A-E) and scanning electron micrographs (F-G). A,B. Epicotyl and eophyll node, respectively. C. Petiole. D. Midrib. E. Portion of cross section of the lamina. F,G. Adaxial and abaxial surfaces of the eophyll, respectively. (co=cortex; ep=epidermis; et=eophyll trace; fs=funnel-shaped cells). Bars=50 μm (E-G), 100 μm (A,B), 300 μm (C,D).

Discussion

The phanerocotylar seedlings observed for *Serjania communis*, which are similar to those seedlings of *Allophylus edulis* (Saint-Hilaire) Radlkofer, are not common in Sapindaceae. The existence of another seedling type, the cryptocotylar type, may occur in several species, such as *Matayba elaeagnoides* Radl., *Cupania vernalis* Cambess. (LEONHARDT et al., 2008) and *Pseudima frutescens* (Aubl.) Radlk. (PAOLI; BIANCONI, 2008). The seedling traits in which were described for 1744 species of Australian plants show that the characters phanerocotyly and cryptocotyly are registered within one single family or genus (WRIGHT et al., 2000). According to these authors, the type of germination did not appear to be 'taxonomically conservative', with 16, 25 and 42% of the genera, families and orders, respectively recorded as polymorphic.

In species with compound foliage (leaves), such as the species *Serjania*, both simple and compound eophylls may be borne on a seedling (SOUZA et al., 2009). *Serjania communis* showed only simple eophyll in the seedling/'tirodendro' phase, but the simple and compound eophylls may be found in seedlings of Sapindaceae, which were examined and described. *Matayba elaeagnoides* and *Cupania vernalis* (LEONHARDT et al., 2008) have simple eophylls, whereas the *Allophylus edulis* (LEONHARDT et al., 2008; NOGUEIRA et al., 2011) and *Pseudima frutescens* (PAOLI; BIANCONI, 2008) show compound eophylls.

In the classification of Vogel (1980), it was adopted the type Macaranga for seedlings of *Serjania communis*, which consist of phanerocotylar and epigeal seedlings, and foliaceous cotyledons. The opposite phyllotaxis for the eophylls of the seedlings of *Serjania communis* may be considered as exceptional in the Macaranga type, since the first few leaves of the Macaranga are, in most cases, all spirally arranged. The seedlings of *Serjania communis* can also belong to the PEF (Phanerocotylar, Epigeal and Foliaceous) type, according to the Garwood (1996) classification. In agreement with Garwood (1996) the PEF type is usually more common in woody tropical floras.

The diarch type of primary root of seedlings of *Serjania communis* is coommonly observed in dicotyledons (eudicots) (EAMES, 1961; DUKE, 1969). However, Souza (2009), who examined seedlings of other eudicots, found that primary roots may present from two (diarch) to five (pentarch) protoxylem poles.

The root-stem transition region of *Serjania communis* begins in the collet region and progresses slowly all the way up to the hypocotyl, characterizing the intermediate type that was proposed by Compton (1912). Studies on the transition structure among the dicots show that the intermediate type is common in the epigeal seedlings with long hypocotyls (SOUZA, 2009).

It was observed unilacunar nodal structure with double trace in cotyledonary node of *Serjania communis*, which is repeatedly found in various others flowering plants. According to Takhtajan (1980), some authors consider this condition as phylogenetic ancestral in all seed plants. This author, interpreting Benzing (1967, p. 805-820), "[…] appropriately points out that the anatomy of the cotyledonary nodes does not necessarily reflect ancestral conditions in the mature stem". The eophyll node of *Serjania communis* is also unilacunar with a simple trace, which is characteristic, mainly for advanced taxa, according to Takhtajan (1980). In addition, he concludes, finally, that the unilacunar type of nodal structure is secondary in flowering plants, originated from the basic type tri-pentalacunar.

The epicotyl of *Serjania communis* has simple stem structure with cortical collenchyma and two collateral vascular bundles that are derived from the epicotyledonary traces of the hypocotyl. This epicotyl structure with age of 10 days is strikingly different from the young stem of Sapindaceae, which is characterized by, among other features, cork arising from the layers below the epidermis, pericycle usually containing strands of sclerenchyma, xylem in the form of a continuous cylinder, and pith generally lignified in tropical species (METCALFE; CHALK, 1957).

Compared to the Sapindaceae foliage (leaves) (METCALFE; CHALK, 1957), the cotyledons and eophylls of *Serjania communis* are also dorsiventral and hypostomatous leaves. On the other hand, characters such as the hypoderm and sclerenchymatous fibers in the mesophyll of the foliage (leaves) (METCALFE; CHALK, 1957) were registered for seedling leaves of *Serjania communis*.

Conclusion

The seedlings of *Serjania communis* belong to the types Macaranga and PEF ('Phanerocotylar, Epigeal and Foliaceous'), with intermediate root-stem transition region, which is typical in seedlings with epigeal and long hypocotyl. The seedling leaves are dorsiventral, which is a character usually registered in Sapindaceae. The cambium variants are not installed in the axes of the seedlings.

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