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MORPHOLOGY AND ANATOMY OF MYRTACEAE SEEDLINGS OCCURRING IN A SEMIDECIDUOUS SEASONAL FOREST

MORFOLOGIA E ANATOMIA DE PLÂNTULAS DE MYRTACEAE OCORRENTES EM FLORESTA ESTACIONAL SEMIDECÍDUAL

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ABSTRACT
Seedlings of six species of Myrtaceae, which occur in Seasonal Semideciduous Forest, were morphologically and anatomically investigated, as a contribution to studies on forest recovery, preservation of native species and for silviculture. Seedlings were obtained from seeds collected in a forest reserve at “Estação Ecológica de Caiuá”, Diamante do Norte, Brazil, and kept in a greenhouse. The seedlings were morphologically described, embedded in histories and sectioned in a rotation microtome. The seedlings are cryptocotylar, hypogeal, with axial root, reserve cotyledons, long epicotyl and two eophylls in general opposite, with limbo that exhibits morphological variation between species. The primary roots are polyarch, with a parenchymatic cortex with exodermis and endodermis not always identifiable, and a central cylinder with reduced vascular secondary growth. Cotyledons exhibit homogeneous starch-rich parenchyma. The epicotyl is stem in nature. Eophylls are dorsiventral, hypostomatic, with hypodermis in only two species. All investigated species have morphologically similar seedlings, showing differences only in eophyll morphology between species. The seedling anatomy is also similar, but with hypodermis only in the eophylls of *Plinia rivularis* and *Myrcianthes pungens*.

KEYWORDS: Cryptocotilar seedlings. Eophylls. Leaf hypodermis. Reserve cotyledons

RESUMO
Plântulas de seis espécies de Myrtaceae, que ocorrem em Floresta Estacional Semidecidual, foram investigadas morfológica e anatomicamente, como contribuição em estudos de recuperação de florestas, preservação de espécies nativas e para a silvicultura. As plântulas foram obtidas de sementes coletadas em reserva florestal da Estação Ecológica de Caiuá, Diamante do Norte, Brasil, e mantidas em casa de vegetação. As plântulas foram descritas morfologicamente, emblocadas em historresina e secionadas em micrótomo de rotação. As plântulas são criptocotiledonares, hipogeias, com raiz axial, cotilédones de reserva, epicótilo longo e dois eofilos em geral opostos e de limbo com variação morfológica entre as espécies. As raízes primárias são poliarcas, com córtex parenquimático provido de exoderme e endoderme nem sempre identificáveis, e cilindro central com crescimento secundário vascular reduzido. Os cotilédones exibem parênquima homogêneo rico em amido. O epicótilo tem natureza caulinar. Os eofilos são dorsiventrais, hipostomáticos, com hipoderme em apenas duas espécies. Todas as espécies investigadas têm plântulas semelhantes morfologicamente, exibindo diferenças particularmente na morfologia dos eofilos entre as espécies. A anatomia das plântulas também é semelhante, mas com hipoderme apenas nos eofilos de *Plinia rivularis* e *Myrcianthes pungens*.

PALAVRAS-CHAVE: Cotilédones de reserva. Eofilos. Hipoderme foliar. Plântula criptocotiledonar

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INTRODUCTION

Works on seedlings “have a considerable scientific value, because they make available data about a stage in the life-cycle of the plant of which little is known, which offers many characters useful for taxonomic classification and for morphological and evolutionary considerations” (VOGEL, 1980). Books and articles have been produced especially in the last century, many of them devoted to tropical tree seedlings (VOGEL, 1980; SOUZA et al., 2009).

The seedling is considered here as a stage of plant development that begins with the germination of the seed and extends to the expansion of the eophyll (SOUZA, 2009a). There is consensus in the literature as to when the seedling stage begins, but there is disagreement among authors when it ends (*sensu* SOUZA et al., 2009). The phase after the seedling is even more controversial, being called young plant, juvenile plant or sapling. We adopt the term “tirodendro” (“tiro”, principiant; “dendro”, plant) which was proposed by Hertel (1968) but modified; the “tirodendro” phase can be considered a stage that extends from the seedling with the eophyll to the formation of the metaphyll (nomophyll-like leaf) (SOUZA, 2009a).

The initial stages of seedling/”tirodendro” growth have high mortality rates, which can be caused by several factors, among which predation, disease and competition can be highlighted (FENNER, 1985). The knowledge of the processes of seed germination and seedling/”tirodendro” development is essential for the recovery of forests, for the preservation of plant species and for silviculture. The forests of the northwest region of Paraná, Brazil, have been systematically decimated by humans, leaving less than 1% of vegetation cover, represented by reduced forest remnants. One of these remnants belongs to the Semideciduous Seasonal Forest that harbors species of several plant families, such as Myrtaceae, which was considered one of the families with the highest number of species (SAKURAQUI; JANUNZZI, 2009).

Given this scenario, the present investigation aims at the morphological and anatomical analysis of the seedlings/”tirodendros” of six species of Myrtaceae that occur in Semideciduous Seasonal Forest.

MATERIAL AND METHODS

Seedlings/”tirodendros” of six Myrtaceae species were analyzed. The seeds of *Calycorectes psidiiflorus* (O. Berg) Sobral, *Eugenia blastantha* (O. Berg) D. Legrand, *Eugenia gracillima* Kiaersk., *Hexachlamys edulis* (O. Berg) Kausel & D. Legrand, *Myrcianthes pungens* (O. Berg) D. Legrand e *Plinia rivularis* (Cambess.) Rotman were collected in the forest reserve “Estação Ecológica de Caiuá”, with an area of 1,427.30 ha, which is located in the northwest region of the State of Paraná, in the municipality of Diamante do Norte, Brazil, with approximate coordinates between 52º 49' at 52º 53'W and 22º 34' to 22º 37' S and altitude ranging from 240 to 380 m. (IAP, 1997).

To obtain the seedlings, the seeds were pulped by manual maceration with the aid of a sieve, disinfected with sodium hypochlorite, washed twice in running water and placed in Petri dishes with

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moistened filter paper and left at a temperature of 25ºC in a germination chamber. with a period of 12h until the protrusion of the primary root. The germinated seeds were transferred to a polystyrene tray with vermiculite, kept in a greenhouse and irrigated four times a week. The morphological analysis of the seedlings was based on the terminology of Rizzini (1977), Vogel (1980), Garwood (1996) and Souza et al., (2009).

Anatomical analysis of seedlings was performed on samples fixed in glutaraldehyde (1%) + formaldehyde (4%) and FAA (formaldehyde, acetic acid and ethanol) 50, embedded in historesin (Leica®), sectioned in a rotation microtome and stained with toluidine blue (O'BRIEN et al., 1964). Samples were also sectioned freehand and stained with safranin and astra blue. The anatomical illustrations were made with photomicrographs obtained by image capture in a digital camera coupled to a Leica EZ4D microscope, whose images were processed using Leica Application Suite Version 1.8 software.

RESULTS

Seedling morphology - The seedlings (Figure 1, Table 1) of the six species are cryptocotylar and hypogeal, with axial root, very reduced hypocotyl, and petiolate reserve cotyledons, being globose in most species and reniform only in *M. pungens* and *P. rivularis*. The collet can be distinguished as a thinner or thicker region located between the root and the hypocotyl. The epicotyl is relatively long, with cataphylls, and two opposite eophylls, with a short petiole and a blade that varies in shape between species (Figure 1, Table 1). Table 1 presents morphological features among the species.

Table 1 - Morphological features of seedlings of the six species of Myrtaceae.

Species/ Features	*Calycorectes psidiiflorus*	*Eugenia blastantha*	*Eugenia gracillima*	*Hexachlamis edulis*	*Myrcianthes pungens*	*Plinia rivularis*
Seedling type	Cryptocotylar Hypogeal	Cryptocotylar Hypogeal	Cryptocotylar Hypogeal	Cryptocotylar Hypogeal	Cryptocotylar Hypogeal	Cryptocotylar Hypogeal
Primary root	Axial	Axial	Axial	Axial	Axial	Axial
Hypocotyl and collet	Reduced Distinct (thickened region)	Reduced Not identified	Reduced Indistinct	Reduced Not identified	Reduced Distinct (thinnest region between root and hypocotyl)	Reduced Distinct (thinnest region between root and hypocotyl)
Epicotyl and cataphylls	Long, cylindrical Alternate cataphylls	Long, cylindrical Alternate cataphylls	Long, cylindrical Alternate cataphylls	Relatively short, cylindrical Alternate cataphylls	Long, cylindrical Without cataphylls	Long, cylindrical Alternating to subopposite cataphylls
Eophylls	Two opposing eophylls with lanceolate limbo	Opposite or subopposite eophylls, sometimes alternating; ovate limbo	Two opposing eophylls with lanceolate-ovate limbo	Two widely lanceolate opposing eophylls	Two widely lanceolate opposing eophylls	Two opposing eophylls with ovate limbo

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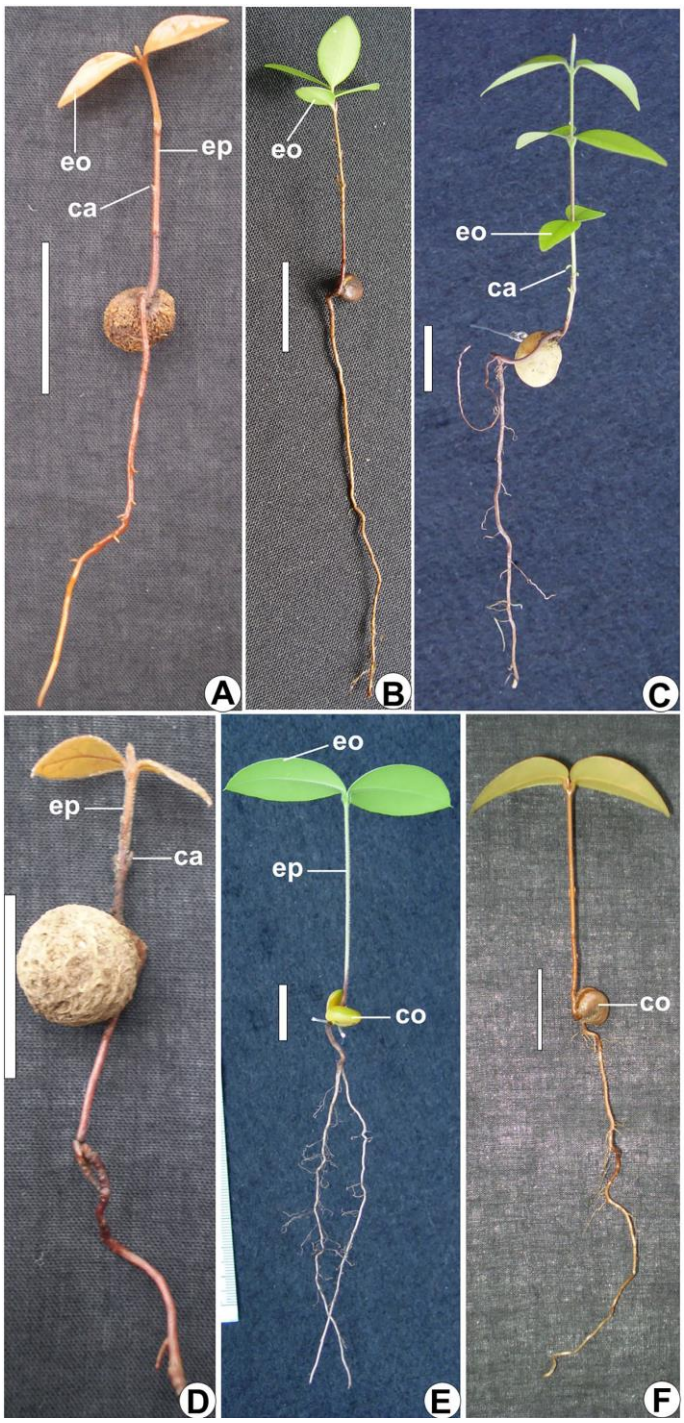

Figure 1 – Seedlings of *Calycorectes psidiiflorus* (A), *Eugenia blastantha* (B), *Eugenia gracillima* (C), *Hexachlamys edulis* (D), *Myrcianthes pungens* (E) and *Plinia rivularis* (F). Seedlings B and C are in the "tirodendro" stage. (ca=cataphyll; co=exposed cotyledons/soil interior; eo=eophyll; ep=epicotyl). Scale bars: 2cm.

Seedling anatomy – All species have polyarch primary roots (Figure 2A, B) that consist of uniseriate epidermis, usually hairy (unicellular hairs), parenchymatous cortex, central cylinder with primary and

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secondary vascular tissues, and parenchymatous central region; this region is made up of sclerified cells in *P. rivularis*.

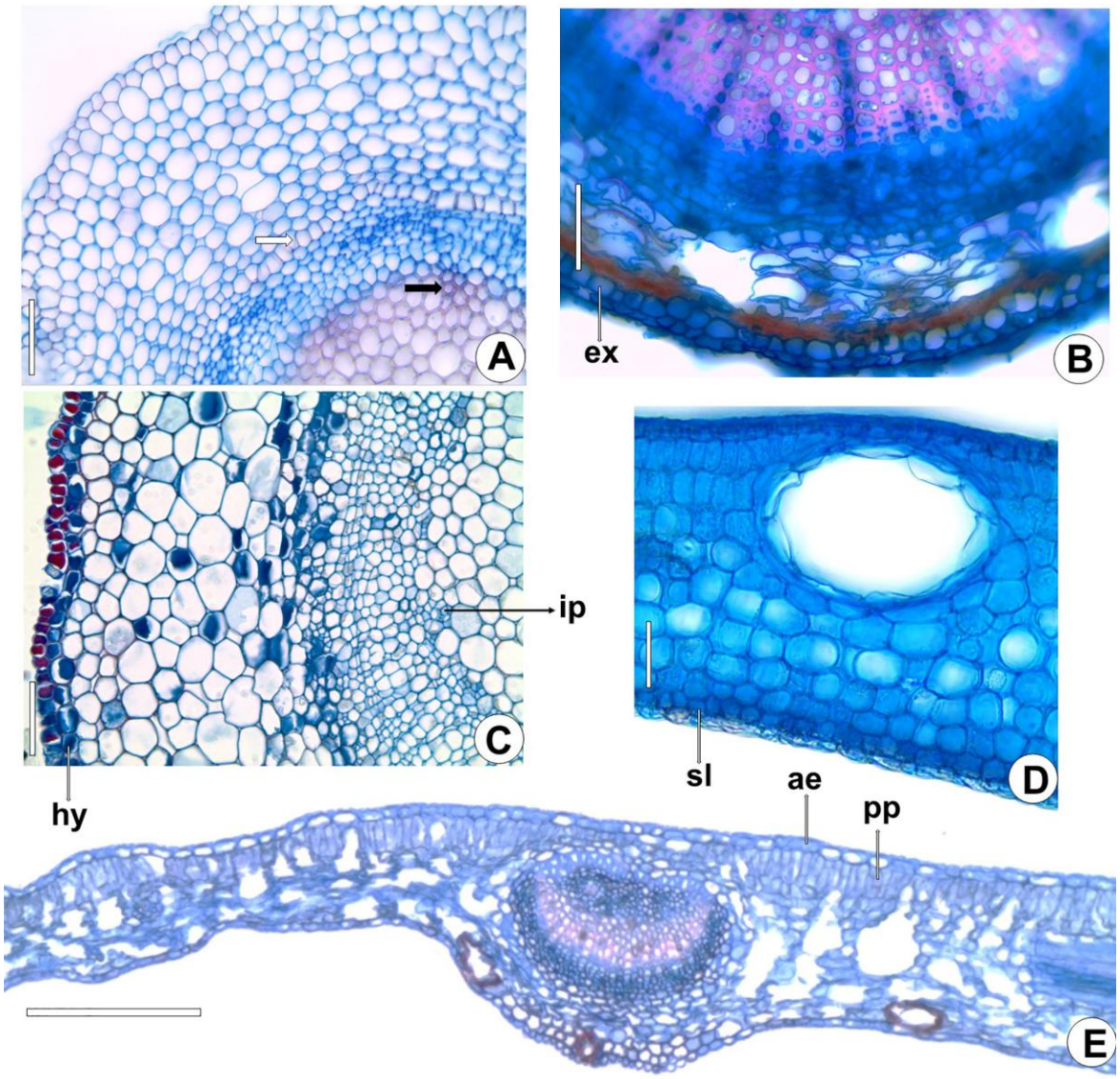

Figure 2 – Seedling structure of *Plinia rivularis* (A,D), *Calycorectes psidiiflorus* (B,E), *Hexachlamys edulis* (C), in cross-sections. A - Axial root in primary growth (white arrow indicates endodermis, black arrow metaxylem). B - Axial root in secondary growth; note also the cortex with exodermis. C – Epicotyl in early secondary growth, with internal phloem. E,F - Limbos of eophylls, with mesophyll showing atypical palisade parenchyma and dorsiventral mesophyll, respectively. (ae=adaxial epidermis; ex=exodermis; hy=hypodermis; ip=internal phloem; pp=palisade parenchyma; sl=subepidermal cell-layer). Scale bars: 50µm (A-D), 200µm (E).

The root cortex (Figure 2A, B) consists of uniseriate or biseriate exodermis, not evident in *H. edulis* and *P. rivularis*; exodermis cells are compressed in *E. blastantha*. Cortex exhibits parenchyma that may present more or less compressed cells at a more advanced stage of root development; the endodermis (Figure 1A) usually exhibits Casparian strip and thick-walled cells, which are suberified

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only in *E. gracilima*. The central cylinder of the root of 35-to-50-day old seedlings shows reduced secondary growth (Figure 2B).

The reserve cotyledons (Figure 1E, F) can be partially fused and are formed by the epidermis of cells with relatively thick walls, homogeneous parenchyma rich in starch and vascular bundles immersed in the mesophyll. In this parenchyma, idioblasts with druses and secretory cavities can be observed.

The epicotyl (Figure 2C) is stem in nature, and it consists of uniseriate epidermis with stomata, and non-glandular trichomes, uni to pluricellular and tapered end. In the epidermis of the epicotyl of *C. psidiiflorus* the cells have very thick walls. Hypodermis with cells rich in phenolic derivative was evidenced in the epicotyl of *H. edulis* (Figure 2C). The cortex (Figure 2C) is parenchymatic and may present typical collenchyma and secretory cavities. There was no evidence of endodermis with Casparian strip or starchy content. The central cylinder (Figure 2C) already has cambium that forms little secondary vascular tissue between the primary xylem and phloem. All species have internal phloem strands (Figure 2C) and also cambium in early development, located between the protoxylem and these phloem strands. The pith (Figure 2C) is parenchymatic in all species, but with sclerified cells in *C. psidiiflorus*.

Two eophylls consist of uniseriate epidermis (Figure 2D, E) covered by a cuticle which is relatively thick in the adaxial surface in *E. blastantha*, *E. gracilima* and *M. pungens*. Stomata are present in the epidermal abaxial face, and the non-glandular hairs can occur in the eophyll epidermis. Eophylls are dorsiventral (Figure 2E) with a layer of palisade parenchyma and spongy pluriseriate parenchyma, which is made of about 12 cell-layers in the eophyll of *M. pungens*. Mesophyll with atypical palisade parenchyma and more compact parenchyma was recorded in *P. rivularis* (Figure 2D). Secretory cavities may occur in the mesophyll (Figure 2D, E), which may occupy 1/3 of this mesophyll in *M. pungens*. Subepidermal cell-layer (Figure 2D) was found in the eophylls of *P. rivularis* and *M. pungens*.

The eophyll midrib (Figure 2E) protrudes only on the abaxial surface, except in *P. rivularis* which protrudes on both sides. It consists of a single bicolllateral vascular bundle, with a small amount of cambium and secondary vascular tissue. This bundle has thin-walled fibers on the phloem face in *E. blastantha*, *H. edulis* and *P. rivularis*, and thick-walled fibers on the phloem face in *C. psidiiflorus* or involving the entire bundle, as in *E. gracilima* and *M. pungens*. Collenchyma is entirely wanting in *P. rivularis*.

DISCUSSION

No doubt in the six Myrtaceae species investigated here there is a seedling pattern, and it is distinguished on the basis in the features, as reserve cotyledons involved by the seminal integument (cryptocotyledonary), taproot, reduced hypocotyl, cataphylls in the epicotyl and opposite eophylls. However, in certain species of the Myrtaceae variation of morphological characters can be observed in the seedlings (DUKE, 1969; BURGER-HZN, 1972; GOGOSZ et al., 2010). On the other hand, when

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analyzing some species of the same genus of Myrtaceae, uniformity of morphological characters of the seedlings is registered. The two *Eugenia* species studied here, for example, have the same characters as other investigated species of the genus (DUKE, 1965; 1969), such as cryptocotyledonary plants, reserve cotyledons and opposing eophylls.

The seedlings of the six species belong to the “Horsfieldia” type, “Horsfieldia” subtype, which were formulated by Vogel (1980); according to the author, this type is common in woody angiosperms, and is mainly characterized by a reduced hypocotyl, reserve cotyledons surrounded by the seed coat, cataphylls in the epicotyl and two opposing eophylls. In the classification of seedlings by Garwood (1996) the six species can be considered as type “CHR” (Cryptocotylar, Hypogeal and Reserve storage). This author analyzes the possibility proposed by Duke (1965) that the percentage of cryptocotyledonary species increases according to the evaluated forest moisture gradient. In this analysis Garwood (1996) concludes that, given the available data, it is not possible to know the contribution of the floristic region, forest type or environmental factors that limit the abundance of morphofunctional types of seedlings.

All investigated Myrtaceae species have polyarch primary roots, although the most eudicot seedlings studied do not have this type of root (SOUZA, 2009b); most of the eudicots either have diarch or tetrarch roots (EAMES, 1961). The polyarch condition is usually registered in monocots (DUKE, 1969), but Souza (2009b) admits polyarchy in primary roots of Bignoniaceae seedlings, pentarch-heptarch in *Tabebuia avellanedae* Lor. ex Griseb. and hexarch in *T. chrysotricha* (Mart. ex DC.) Standl. (SOUZA; OLIVEIRA, 2004). Polyarch primary root (pentarch/hexarch) was also reported in the Myrtaceae *Campomanesia xanthocarpa* O. Berg. by Gogosz et al., (2010).

Eophylls of the six Myrtaceae species are dorsiventral, which differ from the basic pattern of Myrtaceae which has isobilateral or centric leaves (METCALFE; CHALK, 1957). It may, however, be pointed out here that dorsiventrality appears to be a uniform structural character in the nomophylls of *Eugenia* L. species (METCALFE; CHALK, 1957; DONATO; MORRETES, 2008; SILVEIRA; HARTHMAN, 2010; ARMSTRONG et al., 2012), although Donato and Morretes (2007) considered *Eugenia brasiliensis* Lam. nomophylls as dorsiventral with a tendency to isobilaterality, comparing leaves collected from restinga and forest individuals.

Subepidermal cell-layer was found in the eophylls of *P. rivularis* and *M. pungens*, particularly considered by Metcalfe and Chalk (1957) as a hypodermis that occurs in the leaf lamina of *Metrosideros* Banks ex Gaertn. and *Psidium* L. As pointed out by Dickison (1975), a hypodermis in leaves may have taxonomic value at the specific level.

CONCLUSION

The six Myrtaceae species investigated have seedlings of similar morphology but differ in the morphology of the eophylls. The anatomical analysis of the seedlings revealed a similar structure of the different organs and tissues, deserving attention the presence of hypodermis in eophylls of *P. rivularis* and *M. pungens*.

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