

Os caracteres anatômicos dos frutos de *Miconia* Ruiz & Pavón podem ter utilidade taxonômica no gênero?

Caracteres anatômicos dos frutos de *Miconia*

Can *Miconia* Ruiz & Pavón fruit anatomical characters have taxonomic usefulness in the genus?

Fruit anatomical characters of *Miconia*

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Abstract

Miconia Ruiz & Pavón is currently recognized as a single genus belonging to the Miconieae tribe, whose taxonomic position is in urgent need of new multidisciplinary studies, including the anatomical investigation of fruits and seeds. Our study investigated the structural ontogeny of fruits and seeds of six species of *Miconia*, from the floral phase. Flowers and developing fruits were obtained from specimens collected in a state park in Paraná, Brazil, and from herbarium specimens. The samples were sectioned in a rotation microtome and analyzed in a light microscope. All species bear berry-subtype pomaceous fruits that originate from inferior or semi-inferior ovaries. The pericarp is fleshy parenchymatous with sclereid idioblasts. The seeds come from anatropous ovules, are testal and exhibit variation in the position of the mechanical cell layer within the testa. Taxonomically consistent features were found, such as position of the ovary and carpel number (inferior or semi-inferior/3 or 4 carpels), exocarp indumentum, mesocarp structure (tissue region), pomaceous fruit with hypanthium totally or partially adnate to the ovary wall, type of seed (exotestal, exomesotestal and mesoendotestal) and sclereid composition of the mechanical layer.

Keywords: Anatropous ovule, Hypanthium, Ontogeny, Ovary position, Pomaceous fruit, Sclereids, Testal seed.

Resumo

Miconia Ruiz & Pavón é reconhecido atualmente como gênero único pertencente a tribo Miconieae, cuja posição taxonômica tem necessidade urgente de novos estudos multidisciplinares, entre os quais a investigação anatômica de frutos e sementes. Nosso estudo investigou a ontogenia estrutural de frutos e sementes de seis espécies de *Miconia*, desde a fase floral. As flores e frutos em desenvolvimento foram obtidos de espécimes coletados em parque estadual do Paraná, Brasil, e de exsicatas de herbários. As amostras foram seccionadas em micrótomo de rotação e analisadas em microscópio de luz. Todas as espécies exibem frutos pomáceos subtipo baga que se originam de ovários íferos ou semi-íferos. O pericarpo é carnoso, parenquimático com idioblastos esclereídes. As sementes provêm de óvulos anátropos, são testais e mostram variação na posição da camada celular mecânica dentro da testa. Foram encontrados caracteres taxonomicamente consistentes, como posição do ovário e número de carpelos (inferior ou semi-inferior/3 ou 4 carpelos), indumento do exocarpo, estrutura do mesocarpo (número de regiões de tecidos), fruto pomáceo com hipanto total ou parcialmente adnato à parede do ovário, tipo de semente (exotestal, exomesotestal e mesoendotestal) e composição de esclereídes da camada mecânica.

Palavras-chave: Esclereídes, Fruto pomáceo, Hipanto, Ontogenia, Óvulo anátropo, Posição do ovário, Semente testal.

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Introduction

Recent publication treats *Miconia* Ruiz & Pavón, Melastomataceae, as the only genus of Miconieae tribe with ca. 1900 species, and it can be considered as seventh largest genus of flowering plants that is restricted to Neotropics (Michelangeli *et al.*, 2022). *Miconia* can be recognized by the same charac-

ters as the tribe, viz. terminal or axillary inflorescences, very rarely cauliflorous (then the inflorescences cymose), each flower subtended by only one pair of bracteoles, or more rarely none (but never by two pairs), anthers without pedoconnectives, leaves without acicular raphides, and fruit a berry (Michelangeli *et al.*, 2022).

According to Michelangeli *et al.* (2022) the *Miconia* recognition as a unique genus within Miconieae requires new multidisciplinary study. Fruit and seed characters have been promising and useful in the taxonomy of eudicots (for example, the classic works of Corner, 1976 and Roth, 1977). Fruits and seeds have been investigated in Melastomataceae with a descriptive, taxonomic and evolutionary purposes (see Baumgratz, 1983-1985; Clausing *et al.*, 2000; Cortez & Carmello-Guerreiro, 2008; Ramirez, 2011; Ocampo & Almeda, 2013; Ocampo *et al.*, 2014; Ribeiro, 2016).

Miconieae fruits are typically berries (indehiscent fruits) usually with fleshy placentas and fused tissues which are characterized by the absence of lignified tissues, few sclereids in the pericarp, and often by an endocarp that collapses early (Clausing *et al.*, 2000). A good example of *Miconia* with fruit of similar structure is shown by Cortez & Carmello-Guerreiro (2008) in *M. albicans* (Sw.) Triana. The taxonomic significance of seed structure has been explored most frequently in Miconieae (Groenendijk *et al.*, 1996; Martin & Michelangeli, 2009; Ocampo & Almeda, 2013; Ocampo *et al.*, 2014; Ocampo *et al.*, 2022); the characters of the tribe's seeds are variable and diverse, especially in *Miconia* (Ocampo & Almeda, 2013).

The main purpose of this investigation was to ascertain the usefulness of fruit and seed characters as a possible criterion for separating species of *Miconia*. Thus, six species of *Miconia* belonging to four different sections were selected.

Material and methods

Plant samples

Flowers and developing fruits of six species of *Miconia* (Table 1) were collected from herbarium specimens and fresh specimens.

The selected species of *Miconia* belong to the vegetation of southern Brazil, especially to the rupestrian fields, which suffer anthropic destructive action. Fresh specimens were collected in Guartelá State Park, located in the municipality of Tibagi, Brazil. Herbarium specimens were obtained from the Herbarium of the Museu Botânico Municipal de Curitiba (MBM), Brazil.

Sample preparation

The herbarium samples were rehydrated with boiling water, immersed in 5% potassium hydroxide (KOH) and submitted to the 10%, 30%, 50% and 70% ethyl series, according to Smith & Smith (1942), with modifications (change in percentage from 2% to 5% KOH and time of exposure to the reagent). Herbarium and fresh samples were fixed in FAA 50 (formaldehyde, acetic acid and ethyl alcohol), and later dehydrated using the series of ethyl alcohol with concentrations of 80%, 90% and 100%.

Analysis of samples under a light microscope

In sequence, the developing flowers and fruits were embedded in Leica historresin, according to the manufacturer's specification. The samples were sectioned on a rotation microtome, whose sections obtained 6-8 μm in thickness were stained in Toluidine Blue in phosphate buffer (pH = 6.8) (O'Brien *et al.*, 1964). Photomicrographs were taken with a Leica ICC50 light microscope with an attached digital camera, using the Leica Application Suite software, version 1.8.

Results and discussion

The fruits of *M. hyemalis* originate from the inferior ovary, while the fruits of the other five species derive from the semi-inferior ovary (Figure 1A,B). The six species have a hypanthium with a free portion

(perigynous hypanthium), whose length varies in different flowers, and another part that is adnate to the ovary (gynoecial hypanthium) (Figure 1A,B). The ovaries generally have 3 carpels and 3 locules, but *M. hyemalis* exhibits 3 or 4 carpels and locules, and *M. ligustroides* (Figure 1C) consists of 4 carpels and locules (Table 2). The placentation is axile and the ovules attach to the septum at different heights.

The ovary wall of all species has a parenchymatous mesophyll (Figure 1A-C), which is generally compact on the outside and spongy or lacunose on the inside. Parenchymatous mesophyll seems to be common in angiosperm ovaries (Fahn, 1990; Souza, 2022), but Gonçalves (2020) reported the presence of collenchyma in the ovary of some species of *Miconia*. The ovary septa (Figure 1A,B) of the six *Miconia* species consist of epidermis and spongy parenchyma, and they can exhibit splits (Table 2), which are considered by Carr & Carr (1961) as compitum in multilocular ovaries of flowering plants. In the anatomical study of *Miconia* flowers, Gonçalves (2020) treated the investigated species as eu-syncarpous (term proposed by Carr & Carr, 1961) with compitum characterized as splits in the septa or a single strand of transmitting tissue in the style.

The fruit wall in the region that has adnate hypanthium (gynoecial hypanthium) (Figure 1D-F) consists of epidermal exocarp and endocarp, and parenchymatous mesocarp. The mesocarp may exhibit different regions of parenchyma (Table 2). In the mesocarp with two parenchyma regions the outermost part is composed of more or less rounded cells with sclereids, and the innermost portion exhibits tangentially elongated cells. In fruits from semi-inferior ovaries (Figure 1D), the upper portion of the fruit wall is essentially carpellary and is sur-

rounded by the perigynous hypanthium. Here the wall is constituted by the papillose exocarp, and the mesocarp consists of more homogeneous parenchyma with few sclereids (Figure 1D).

All *Miconia* fruits investigated here have sclereids in the mesocarp (Figure 1D-F; Table 2), which are sclerenchymatous cells that can occur singly or in clusters in the fleshy pericarp of berries (Roth, 1977; Souza, 2022). Cortez & Carmello-Guerreiro (2008) also found sclereids in the mesocarp of *Miconia albicans* and suggested that they must have originated from sclerification of parenchyma cells and not from sclereid primordia.

Miconia fruits were classified as type pomaceous subtype berry based on the fruit classification proposed by Souza (2022) and Souza *et al.* (2022). Pomaceous fruit for these authors originates from an inferior or semi-inferior ovary, and exhibits several subtypes. Clausen *et al.* (2000) considered the fruit of Miconieae, particularly a species of *Clidemia* D. Don, as a soft berry that is characterized by the absence of lignified tissues and a dearth of sclereids in the fruit walls. Cortez & Carmello-Guerreiro (2008) analyzed the ontogeny of the fruit of *Miconia albicans*, and also considered its fruit as a berry, originating from a semi-inferior ovary. One of the characters considered diagnostic for the tribe Miconieae and the genus *Miconia* by Michelangeli *et al.* (2022) is the type of fruit like berry. Berry definition for Souza (2022) and Souza *et al.* (2022) is a simple fruit that must originate from the superior ovary.

Miconia seeds originate from anatropous, bitegmic and crassinucellate ovules. Seeds have non-multiplicative testa and the tegmen usually collapses. Seeds are exotestal (Figure 1D,E) in *M. albicans*,

M. collatata, *M. hyemalis* and *M. sellowiana*, exomesotestal in *M. inconspicua* and mesoendotestal in *M. ligustroides* (Table 2). In exotestal and exomesotestal seeds the outer epidermis (exotesta) consists of macrosclereids. Outer epidermal cells of *M. ligustroides* are prismatic, but with thin walls.

Several studies have shown that seed morphology in Miconieae has taxonomic value, but almost all investigations describe seeds under scanning electron microscopy (Groenendijk *et al.*, 1996; Martin & Michelangeli, 2009; Ocampo & Almeda, 2013; Ocampo *et al.*, 2022). The anatomical analysis of the Miconieae seed is still little explored. In general, the seed coat characteristics as testa not multiplicative with outer epidermis that is made up of cuboid or shortly radially elongated lignified cells, and tegmen as pellicle no lignified are typical of *Miconia* (Corner, 1976). *Miconia albicans* seeds were also described by Cortez & Carmello-Guerreiro (2008) as having the sclerenchymatous palisade exotesta, and tegmen absent. In the case of the *Miconia* species studied here, we verified that the mechanical layer of the testa can occur in the outer epidermis (exotesta) or in the mesophyll (mesotesta) (Table 2).

It is not surprising, therefore, that the fruit and seed ontogeny possesses many features of potential taxonomic significance. Ontogeny of fruits and seeds of six *Miconia* species provide many important features which are of taxonomic value at the specific level (Table 2). Features such as the position of the ovary (inferior or semi-inferior), carpel number (3 or 4 carpels), axile placentation (ovules attaching to the base, middle or apical region of the septum), exocarp indumentum, mesocarp structure (tissue region), type of pomaceous fruit (with hypanthium entirely adnate to the fruit wall or fruit with perigynous hypanthium), type

of seed (exotestal, exomesotestal, mesoendotestal), and structure of the mechanical cell layer when it occurs in the testa (macroclereid or cuboidal sclerenchyma cells) should be highlighted.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Author contributions

RG: Collection, sample preparation, fixation, embedding and histological sections of plant material; preliminary writing of the manuscript. AFS: Preliminary writing of the manuscript. CHM: Sample preparation, fixation, embedding and histological sections of plant material. LAS: Final writing of the manuscript.

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Table 1 - Species investigated with information on sample type and herbarium accession number.

Species	Sample types	Herbarium registrations
<i>Miconia albicans</i> (Sw.) Triana	Fresh sample	HUEM 24929
<i>Miconia collatata</i> Wurdack	Exsiccate	MBM 312353
<i>Miconia hyemalis</i> A. St-Hil. & Naudin	Fresh sample	HUEM 32868
<i>Miconia inconspicua</i> Miq.	Exsiccate	MBM 242419
<i>Miconia ligustroides</i> (DC.) Naudin	Fresh sample	HUEM 24927
<i>Miconia sellowiana</i> Naudin	Fresh sample	HUEM 24957

HUEM - Herbarium at the State University of Maringá; MBM - Herbarium of the Municipal Botanical Museum of Curitiba

Table 2 - Taxonomically important features that may be useful in separating the six species of *Miconia*.

Species	Ovary position/carpel number	Septum split	Exocarp	Mesocarp	Hypanthium	Seed type/mechanical layer
<i>M. albicans</i>	Semi-inferior/ 3 carpels	Present	Sparse hairs	Parenchymatous (2 tissue regions) Sclereids	Adnate to the ovary wall Perigynous	Exotestal Macrosclereids
<i>M. collatata</i>	Semi-inferior/ 3 carpels	Absent	Sparse hairs	Parenchymatous (several tissue regions) Sclereids	Adnate to the ovary wall Perigynous	Exotestal Wide cuboidal cells
<i>M. hyemalis</i>	Inferior/ 3-4 carpels	Present	Hairy	Parenchymatous Sclereids	Adnate to the ovary wall	Exotestal Macrosclereids
<i>M. inconspicua</i>	Semi-inferior/ 3 carpels	Present	Hairy	Parenchymatous (2 tissue regions) Sclereids	Adnate to the ovary wall Perigynous	Exome-sotestal Macrosclereids (exotesta) Brachysclereids (mesotesta)

<i>M. ligustroides</i>	Semi-inferior/ 4 carpels	Present	Glabrous	Parenchymatous (2 tissue regions) Sclereids	Adnate to the ovary wall Perigynous	Mesoendotestal Sclereids (mesotesta and endotesta)
<i>M. sellowiana</i>	Semi-inferior/ 3 carpels	Absent	Hairy	Parenchymatous Sclereids	Adnate to the ovary wall Perigynous	Exotestal Macrosclereids

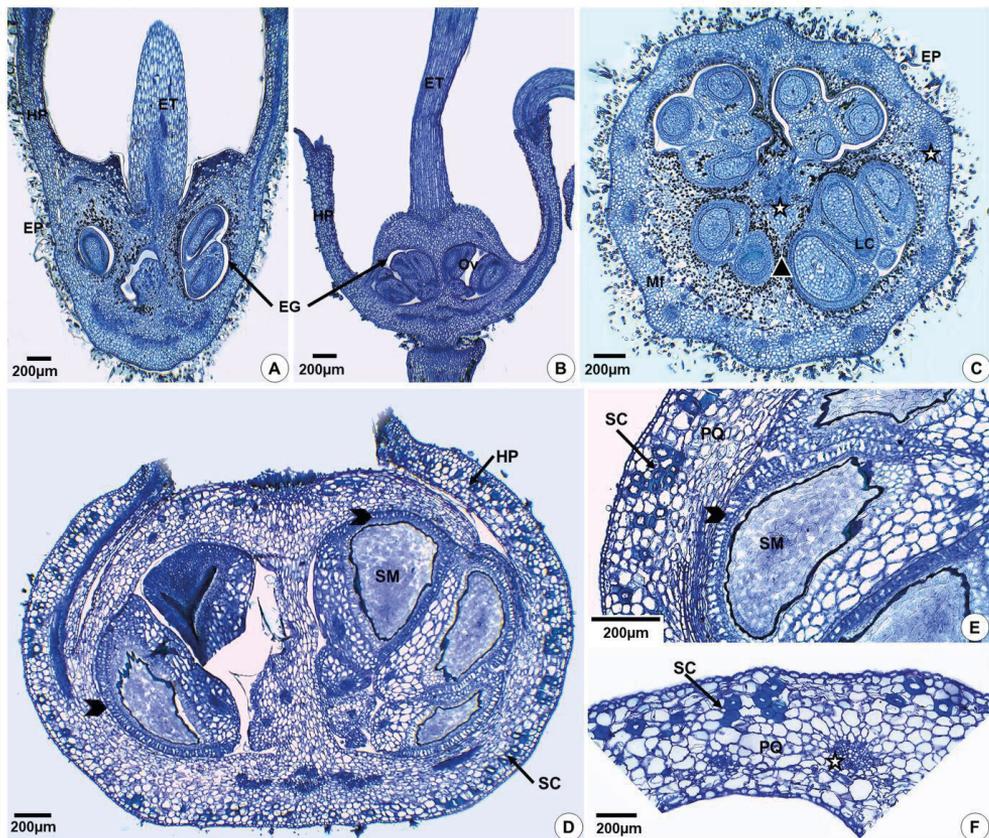


Fig. 1 - Ovary and fruit structure of *Miconia hyemalis* (A,F) and *Miconia sellowiana* (B-E) in longitudinal (A,B,D,E) and cross- (C,F) sections. A,B - Inferior and semi-inferior ovary. C - Ovary with 4 carpels and locules. D - Fruit originating from semi-inferior ovary showing hypanthium adnate to pericarp and free hypanthium (perigynous). E - Detail of the pericarp and seed showing sclereids and exotesta of macrosclereids (arrowhead). F - Detail of the pericarp with sclereids. (EG=glabrous inner epidermis; EP=hairy outer epidermis; ET=style; HP=hypanthium; LC=locule; Mf=mesophyll; Ov=ovule; PQ=parenchyma; SM=seed; SC=sclereids; stars indicate vascular bundles).