

# The flower anatomy of five species of Myrteae and its contribution to the taxonomy of Myrtaceae

Luana Martos<sup>1\*</sup>, Adrielle Tairine Oliveira Froemming Galan<sup>1</sup>, Luiz Antonio de Souza<sup>1</sup> and Káthia Socorro Mathias Mourão<sup>1</sup>

Received: November 14, 2016  
Accepted: January 17, 2017

## ABSTRACT

Considerable effort has been spent towards understanding the phylogeny of Myrteae, and based on the phylogenetic data presently available the traditional subdivision of Myrteae into three subtribes is not supported. The present paper aims to assess the usefulness of floral characters in distinguishing five species that represent five of the six South American clades of Myrteae (Myrtaceae). Floral buds and flowers of *Campomanesia adamantium*, *Eugenia pitanga*, *Myrceugenia alpigena*, *Myrcia multiflora* and *Myrciaria cuspidata* were collected from individual plants growing in the Cerrado (Brazilian/Central South American savanna). Among these species, the perianth of *E. pitanga* is the most distinct due to its vascularization and pilosity. The hypanthium is thickest in *C. adamantium* and *M. alpigena*, while *M. delicatula* possesses tangentially elongated cells. Anthers do not exhibit much variation among the studied species, while *M. alpigena* is the only species with trichomes and secretory cavities distributed throughout the mesophyll of the outer wall of the ovary. The ovaries of all of the studied species exhibit vascularization in the form of a single ring of larger-sized bundles. Comparative analysis of these floral structures demonstrates that they are useful in separating these species, and thus the subtribes, of Myrteae.

**Keywords:** anatomical characteristics, Myrtaceae, floral vasculature, flower, phylogeny

## Introduction

In spite of advances in describing morphological characters within Myrtaceae, the taxonomy of the family remains complex and incomplete (Barroso & Peron 1994), especially with regard to distinguishing genera (Landrum & Kawasaki 1997). Numerous studies have found that the distinction of infrafamilial groups is not necessarily clear (Candolle 1828; Schauer 1841; Berg 1855-1856; 1857-1859; Niedenzu 1893; Kausel 1966; McVaugh 1968; Schmid 1980; Johnson & Briggs 1984; Wilson *et al.* 2001; 2005; Lucas *et al.* 2005; 2007; Biffin *et al.* 2007; 2010; Murillo-A. *et al.* 2013).

Johnson & Briggs (1984) were the first to study the phylogeny of Myrtaceae using inflorescence structure. Subsequently, Wilson *et al.* (2001; 2005) investigated the morphology and taxonomy of Myrtaceae based on molecular data, and proposed two subfamilies, the Myrtoideae with 15 tribes, and Psiloxylodeae with two tribes. Myrteae is the largest tribe of Myrtoideae and includes all the American representatives of Myrtaceae except for *Metrosideros stipularis*, which is included in Metrosidereae (Lucas *et al.* 2007).

The traditional subdivision of Myrteae into three subtribes was based on embryo morphology: Myrtinae, with a bent/coiled embryo and small cotyledons;

<sup>1</sup> Programa de Pós-graduação em Biologia Comparada, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, PR, Brazil

\* Corresponding author: lua.martos@hotmail.com

Myrciinae, with an embryo consisting of leaf-like folded cotyledons; and Eugeniinae, with a food-storing embryo and plane-convex cotyledons (Berg 1855-1856; 1857-1859). Lucas *et al.* (2007) revealed the polyphyly of Eugeniinae and Myrtinae, while Myrciinae (excluding *Myrceugenia*) continues to be considered monophyletic. These authors also argued that the subtribal classification reported by Berg (1855-1856; 1857-1859) does not reflect monophyletic groups. Seven informal clades generated by their molecular analysis were proposed: “Australasian group”, “*Eugenia* group”, “*Pimenta* group”, “*Myrteola* group”, “*Myrcia* group”, “*Myrceugenia* group”, and “*Plinia* group”. Additionally, a subsequent phylogenetic analysis by Murillo-A *et al.* (2013) indicated that the subtribes *sensu* Berg are not monophyletic and proposed slightly different subgroups for Myrtinae.

Although phylogenetic analyses have made considerable progress in understanding the relationships of Myrteae, morphological, anatomical and ontogenetic studies still need to be brought to bear on the phylogeny of the family. Different floral characters have been investigated with regard to the phylogeny Myrtaceae, such as studies of the gynoecium (Pimentel *et al.* 2014) and floral anatomy (Schmid 1972b; c; Volgin & Stepanova 2001; 2002a; b; 2004; Lopes 2008; Costa *et al.* 2010; Pimentel 2010; Moreira-Coneglian 2011; Pires & Souza 2011).

The inflorescences of Myrteae were found to be of the panicle type, or reductions of this, and developed to form a racemic, dichasial or single flower (Briggs & Johnson 1979; Wilson 2011). The flowers can be uni- or bisexual (Wilson *et al.* 2005; Wilson 2011), and usually possess numerous stamens with tetra- or bilocular anthers (Wilson *et al.* 2005). The ovary varies from being inferior to being semi-inferior (Wilson *et al.* 2005; Wilson 2011).

The main purpose of this investigation was to determine the usefulness of floral characters of five species of Myrteae (Myrtaceae), which represent five of the six South American clades of the family, for: a) understanding species morphology and taxonomy; and b) identifying character states of phylogenetic relevance to the tribe.

## Materials and methods

Floral buds and flowers of five species representing five clades of Myrteae (*sensu* to Lucas *et al.* 2007) were collected in Cerrado (South American savanna) of “Parque Estadual de Vila Velha - PEVV” (25°14’09”W, 50°00’17”S) and “Parque Estadual do Guartelá - PEG” (50°10’W, 24°37”S), in the municipalities of Ponta Grossa and Tibagi. Voucher specimens were deposited in the Herbarium (HUPG) of Universidade Estadual de Ponta Grossa (Tab. 1).

Analyses of floral buds and flowers were done using material fixed in FAA 50 (formaldehyde, acetic acid and ethylic alcohol), following the protocol of Johansen (1940). Fixed material was embedded in historesin (Guerrits 1991), sectioned (cross- and longitudinal sections) in a rotation microtome and stained in Toluidine Blue (0.1 M acetate buffer, pH 4.7) (O’ Brien *et al.* 1964). Photographs were taken using a Leica EZ4D digital camera coupled to a light microscope, and subsequently processed using the software Leica Application Suite version 1.8.

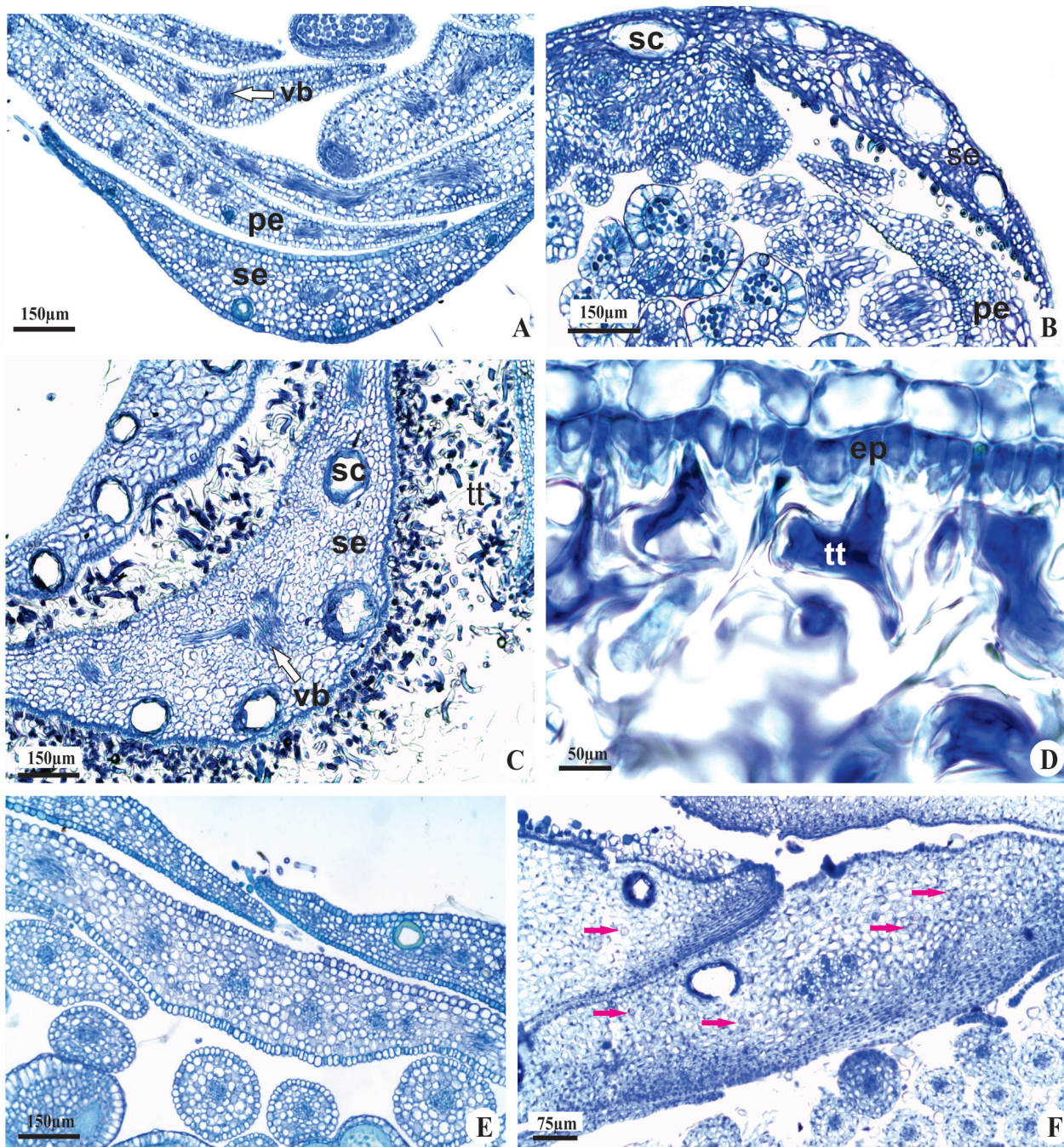
## Results

**Perianth** – Both surfaces of the sepals have a stomatous uniseriate epidermis. Non-glandular trichomes were not found in *Eugenia pitanga* (Fig. 1A), but all the other species are hairy with glandular trichomes (Fig. 1B-C). Trichomes can be distinguished on both epidermal faces of *Myrceugenia alpigena* trichomes (Fig. 1C), while in *Myrcia multiflora* (Fig. 1B), *Campomanesia adamantium* and *Myrciaria cuspidata* they are restricted to the adaxial surface. Trichomes were found to be composed of one (*C. adamantium*) or two cells (*M. multiflora* and *M. cuspidata*), or are T-shaped (Fig. 1D) (*M. alpigena*). Mesophyll is parenchymatous, varying slightly in cell shape and length (Fig. 1A-C), with the presence of secretory cavities (all species) and druse idioblasts (*M. alpigena* and *M. cuspidata*). Sepal vasculature can consist of a single collateral bundle (Fig. 1B) (*M. multiflora* and *M. cuspidata*); three bundles, in which the main bundle

**Table 1.** Studied species, ordered according to the informal groups proposed by Lucas *et al.* (2007), and voucher information. (PEVV: Parque Estadual de Vila Velha; PEG: Parque Estadual do Guartelá)

Clade	Taxon	Locality	Voucher	Collector
Pimenta	<i>Campomanesia adamantium</i>	PEVV/PEG	HUPG 19421, 19751	L. Martos & A. T. O. F. Galan 24 A. T. O. F. Galan & L. Martos 66
Eugenia	<i>Eugenia pitanga</i>	PEVV/PEG	HUPG 19442, 19444	A. T. O. F. Galan & L. Martos 54 L. Martos & A. T. O. F. Galan 14
Myrceugenia	<i>Myrceugenia alpigena</i>	PEVV/PEG	HUPG 19400, 19753	A. T. O. F. Galan; N. C. Machado & L. Martos 41 A. T. O. F. Galan & L. Martos 74
Myrcia	<i>Myrcia multiflora</i>	PEVV/PEG	HUPG 19401, 19754	N. C. Machado; A. T. O. F. Galan & L. Martos 10 MRB Carmo 1809
Plinia	<i>Myrciaria cuspidata</i>	PEVV/PEG	HUPG 19454, 19781	L. Martos & A. T. O. F. Galan 21 L. Martos <i>et al.</i> 78





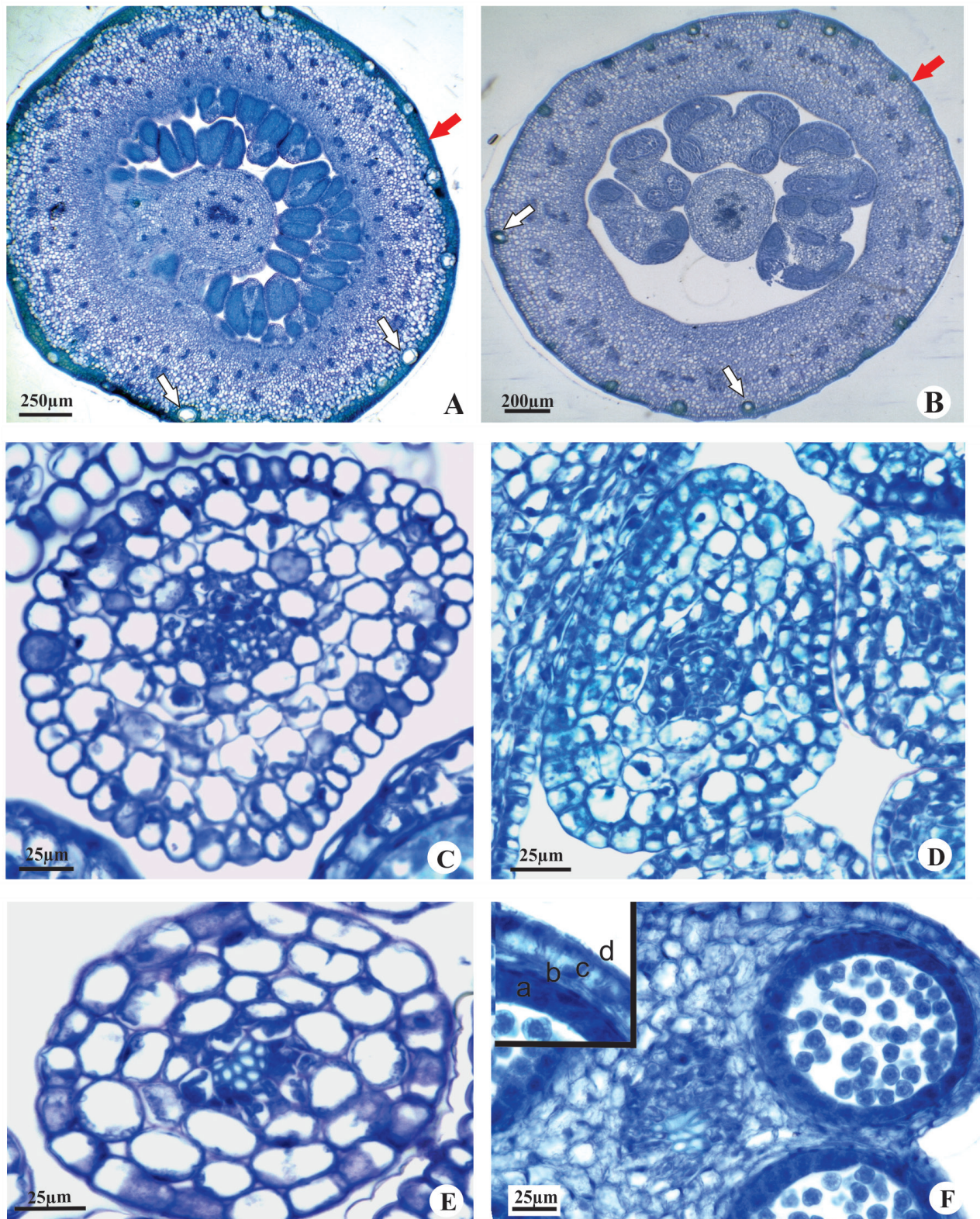
**Figure 1.** Perianth in cross section. (A, E) *Eugenia pitanga*, (B) *Myrcia multiflora*, (C, D, F) *Myrceugenia alpigena*. A. Sepals and petals showing the epidermis, homogeneous mesophyll and vasculature pattern. B-C. Sepals showing epidermis with glandular trichomes and homogeneous mesophyll. D. Detail of the epidermis showing a T-shaped trichome. E. Petals displaying vasculature pattern. F. Perianth showing homogeneous mesophyll with secretory cavities. (ep: epidermis; pe: petals; pink arrows: druse cells; sc: secretory cavities; se: sepals; tt: t-shape trichome; vb: vascular bundle). Please see the PDF version for color reference.

is centrally located (*C. adamantium*); or several vascular bundles (*E. pitanga* and *M. alpigena*) (Fig. 1A and C).

Petals have a glabrous uniseriate epidermis, and spongy parenchymatous mesophyll (Fig. 1E-F), interspersed with secretory cavities. Druse idioblasts occur in the mesophyll of only in *Myrceugenia alpigena* (Fig. 1F). One collateral vascular

bundle (Fig. 1F) runs through the midvein of all species.

**Hypanthium** – The hypanthium consists of glabrous uniseriate outer and inner epidermis, and homogeneous parenchyma with secretory cavities (Fig. 2A-B). One or two layers of parenchyma cells adjacent to the epidermis of *Myrciaria cuspidata* are composed of mostly elongated



**Figure 2.** Structure of the hypanthium and androecium of *Campomanesia adamantium* (A, D, F), *Eugenia pitanga* (B, C) and *Myrceugenia alpigena* (E), in cross sections. A-B. Hypanthium with glabrous epidermis and secretory cavities. C-E. Filaments showing amphicribal, U-shaped and collateral bundles, respectively. F. Anther showing connective, pollen sac, and detail of the wall. (red arrow: epidermis; white arrow: secretory cavities; a: tapetum; b: middle layer; c: endothecium; d: epidermis). Please see the PDF version for color reference.

cells; their secretory cavities are conspicuous and account for almost 2/3 of the thickness of the hypanthium. The hypanthia of *Myrceugenia alpigena* and *Campomanesia adamantium* are much thicker than those of the other species and contain the perianth and vascular traces of the androecium (Fig. 2A). Druse idioblasts are distinguishable in *M. cuspidata* hypanthium.

**Androecium** – Different types of vascular bundles may be recognized in the filaments of the studied species, including an amphicribal bundle in *Eugenia pitanga* (Fig. 2C), a U-shaped bundle in *Campomanesia adamantium* (Fig. 2D) and *Myrciaria cuspidata*, and a collateral bundle in *Myrceugenia alpigena* (Fig. 2E) and *Myrcia multiflora*. Anthers are tetrasporangiate and their immature wall consists of a uniform or interrupted epidermis, an endothecium, a middle layer, and a secretory tapetum (Fig. 2F). Only in *C. adamantium* does the middle layer consists of only one or, in some regions, two cell layers (Fig. 2F). The occurrence of druses and a conspicuous secretory cavity is notable in the connectives of *M. alpigena*, *M. multiflora* and *M. cuspidata*.

**Gynoecium** – Ovary is globose with axillar placentation, and exhibits morphological variation among all species. Two carpels and locules are found in *Eugenia pitanga*, *Myrcia multiflora* and *Myrciaria cuspidata* (Fig. 3A-B), whereas *Campomanesia adamantium* possesses seven to nine (Fig. 3C) and *Myrceugenia alpigena* has three or four (Fig. 3D). The ovules vary in number and position: numerous ovules of each locule can be connected to the same portion of the middle region of the septum (*E. pitanga*) (Fig. 3A); many ovules per locule can be arranged in two longitudinal rows (Fig. 3C) (*M. alpigena* and *C. adamantium*); or two ovules per locule can be connected to the same portion of the basal third of the septum (the other species).

The ovary is characterized by having uniseriate outer epidermis, which consists of squared, rounded to cylindrically-shaped cells in cross-section. The epidermis is glabrous (Fig. 3A-C), but *Myrceugenia alpigena* shows

T-shaped trichomes (Fig. 3D). Mesophyll of *Eugenia pitanga* and *Myrciaria cuspidata* is composed of homogeneous parenchyma, whereas *Myrcia multiflora* has radially elongated cells beneath the epidermis. Collenchyma and parenchyma were observed in the mesophyll of *M. alpigena*, while three tissue regions can be recognized in the mesophyll of *C. adamantium*, including an outer region with tangentially elongated cells near the outer epidermis, a middle region composed of large cells, and an inner region composed of short cells. Secretory cavities are always present in the mesophyll, usually near the outer epidermis (Fig. 3A-C); idioblasts with druses and monocystals are found only in *M. multiflora* and *M. alpigena*.

The septum of the ovary of *Eugenia pitanga*, *Myrcia multiflora* and *Myrciaria cuspidata* consists of glabrous uniseriate epidermis and parenchyma with vascular traces of the ovules, and transmitting tissue. The septum of *Campomanesia adamantium* and *Myrceugenia alpigena* is nearly the same as in the other species, although it is narrower (Fig. 3C-D). Especially notable is the *compitum* (Fig. 3A), which interrupts the septum, thereby communicating the locules.

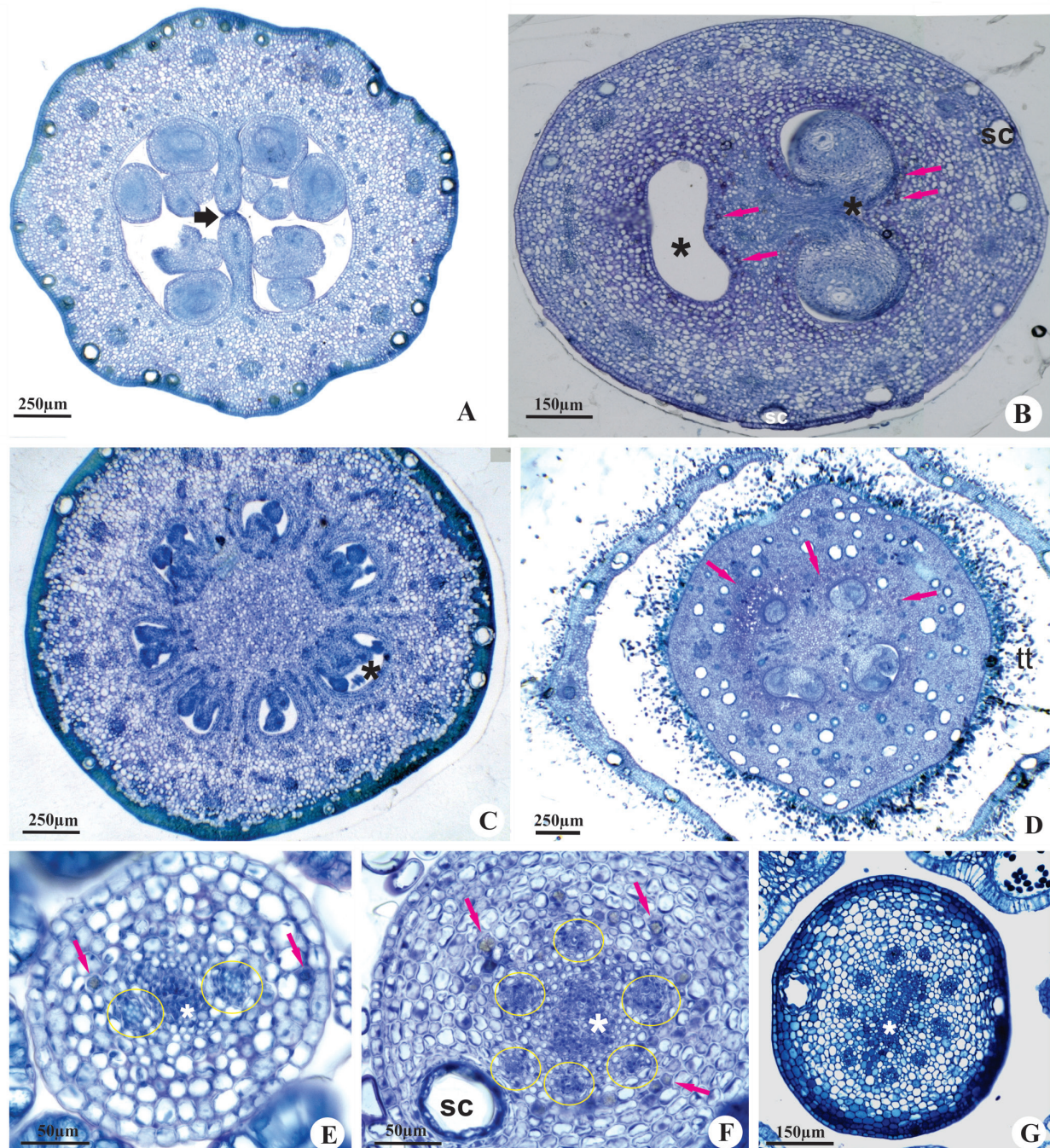
A more detailed analysis of the serial sections of the ovaries of the studied species indicates that in all species the *compitum* (Fig. 3A), characterized by the interruption of the septum, consists of a slit communicating the locules and having a aperture of variable size. The position in which the cleft becomes evident is related to the distribution of ova in the ovary, being median in *Eugenia*, from the median region to the apex in *Campomanesia* and *Myrceugenia* and basal in *Myrcia* and *Myrciaria* (Tab. 2).

Ovary vasculature of *E. pitanga*, *M. alpigena* and *M. cuspidata* consists of two bundle rings, the outer one with comprised of collateral and/or bicollateral bundles interspersed with smaller bundles, and the inner ring comprised of small bundles (Fig. 3A, D). In *C. adamantium* there are three rings of collateral vascular bundles, in which

**Table 2.** Comparatives structural characters of flowers of *Eugenia pitanga*, *Campomanesia adamantium*, *Myrceugenia alpigena*, *Myrcia multiflora* and *Myrciaria cuspidata*.

Species/ Characters	Sepal vasculature	Petal vasculature	Number of carpels	Ovary vasculature	Number of ovules/locule	Degree of rift opening in <i>compitum</i>	Secretory cavity in the connective
<i>E. pitanga</i>	>1	1	2-carpels	Two rings of bundles (the outer with greater caliber)	>2 ovules/locule	Septum filled by transmitting tissue	Absent
<i>C. adamantium</i>	>1	1	>2-carpels	Three rings of bundles (the central with greater caliber)	>2 ovules/locule	Reduced rift	Absent
<i>M. alpigena</i>	>1	1	>2-carpels	Two rings of bundles (the outer with greater caliber)	>2 ovules/locule	Reduced rift	Present
<i>M. multiflora</i>	1	1	2-carpels	One ring of bundle	2 ovules/locule	Wide rift	Present
<i>M. cuspidata</i>	1	1	2-carpels	Two rings of bundles (the outer with greater caliber)	2 ovules/locule	Wide rift	Present





**Figure 3.** Ovary and style of *Eugenia pitanga* (A), *Myrcia multiflora* (B), *Campomanesia adamantium* (C) and *Myrceugenia alpigena* (D), in cross sections. A-D. Ovaries showing wall with vascular bundles and secretory cavities, compitum (black arrow), locules (black asterisk), and placentation. E-G. Styles of *Myrcia multiflora*, *Myrceugenia alpigena* and *Campomanesia adamantium* showing central transmitting tissue (white asterisk) and vascularization by two, six and nine bundles (yellow circles), respectively. (sc: secretory cavities; pink arrows indicate druse cells). Please see the PDF version for color reference.

the main bundles run into the middle region of the ovary wall (Fig. 3C). The *M. multiflora* ovary shows a single ring of collateral vascular bundles (Fig. 3B).

The style is solid with a single strand of transmitting tissue, (Fig. 3C, G) although a central rift or intercellular spaces in the tissue of *M. multiflora* (Fig. 3E) has been

observed. Beneath the uniseriate epidermis of the style (Fig. 3E-G) there is parenchyma with secretory cavities (Fig. 3F-G) and druse idioblasts (Fig. 3E-F). The style of *M. alpigena* resembles that of the other species with the exception that it also exhibits collenchyma near the surface. The most remarkable histological distinction among the

styles is the number and type of vascular bundles: *E. pitanga*, *M. multiflora* and *M. cuspidata* have two collateral bundles (Fig. 3E), *M. alpigena* has six to eight collateral bundles (Fig. 3F), whereas *C. adamantium* has seven or nine collateral, amphicribal or U-shaped bundles (Fig. 3G).

## Discussion

Green sepals may be structurally similar to foliage leaves (Fahn 1990; Endress 1991). The latter are isobilateral or centric in Myrtaceae (Metcalf & Chalk 1957), but contrary to interpretation of the green calyx by Fahn (1990) and Endress (1991), the sepals analyzed here have homogeneous mesophyll. Based on the literature, Endress (1991) refers to a widely accepted hypothesis that the sepals of angiosperms are evolutionarily derived from bracts and the petals from stamens, with the sepals being vascularized by three traces and the petals by one.

Compared with the results of previous studies of Myrteae (Volgin & Stepanova 2001; 2002a; b; 2004; Lopes 2008; Moreira-Coneglian 2011; Pires & Souza 2011), the sepal vasculature of the species investigated here exhibit a tendency for reduction to just one vascular bundle, based on the phylogeny of Lucas *et al.* (2007). The petal vasculature of all of the species analyzed herein have one vascular bundle, which is very different from the 11 vascular bundles found in petals of *Eugenia uniflora* (Lopes 2008). If considered exclusively, perianth vasculature is insufficient for forming phylogenetic hypotheses.

Davis (1966) defined four types of anther wall formation, with the Dicotyledoneous and Monocotyledoneous types possessing four layers, both derived from the Basic type by suppression of the periclinal divisions in the inner and the outer secondary parietal layers, respectively. Dahlgren (1991) reported the Dicotyledoneous type for Myrtales (Myrtaceae), but ontogenetic studies are needed to determine the type of anther wall formation in the species investigated species here.

Secretory cavities of the anther wall do not occur in *Eugenia pitanga* (Eugenia Group), are absent or, if present, their number can vary in the species belonging to the Pimenta Group, as observed for *Campomanesia adamantium* in the present work and by Landrum & Bonilla (1996) for this species and 298 other species of Myrtinae. In *Myrceugenia*, *Myrcia* and *Myrciaria*, secretory cavities were always present, as observed in *Myrceugenia alpigena*, *Myrcia multiflora* and *Myrciaria cuspidata*. The results of the present study, combined with data from other studies such as Landrum & Bonilla (1996), suggest a tendency towards the maintenance of secretory cavities in the anther in the most derived clades of Myrteae *sensu* Lucas *et al.* (2007).

Most species of Myrtaceae have flowers with mostly inferior ovaries (Schmid 1972a), but Wilson *et al.* (2005) describe species with half-inferior and superior ovaries. Half-inferior ovaries are present in Myrteae, particularly in

*Luma apiculata* (Belsham & Orlovich 2003), *Myrcia rosangelae* (Lughadha *et al.* 2012) and *Myrciaria floribunda* (Pimentel *et al.* 2014). On the other hand, the species studied here along with other species of Myrteae (Lopes 2008; Pimentel 2010; Moreira-Coneglian 2011) have inferior ovaries. In contrast to *M. floribunda* (Pimentel *et al.* 2014), which has half-inferior ovaries, the ovaries of *M. cuspidata* are inferior, indicating variation in ovary position within the genus.

Bicarpellate and biloculate ovaries are present in *Eugenia pitanga*, *Myrcia multiflora* and *Myrciaria cuspidata*, while there is a variation in the locule/carpel number in the other studied species: three to four in *Myrceugenia alpigena* and seven to nine in *Campomanesia adamantium*. The range of diversity in locule/carpel number is especially pronounced in the gynoecium of other species of the same genera, such as having two locules (*Eugenia puniceifolia*) (Silva & Pinheiro 2006), three locules (*Myrcia venulosa*), six to seven locules (*Campomanesia pubescens*) and two to six locules (*Psidium*) (Bünger *et al.* 2012).

Pimentel *et al.* (2014) found that most South American species have two carpels and an apical septum in the ovary, whereas exceptions regarding the number of carpels have been documented in *Myrceugenia myrcioides* (three), *Ugni molinae* (three) and *Psidium* (four). In agreement with these data, it is suggested that the evolution of South American clades involved a decrease in carpel number. Considering the condition of three carpels to be a plesiomorphic feature; one carpel could have been lost in *Blepharocalyx salicifolius*, the “*Eugenia*” group and the common ancestor of the “*Myrcia*” and “*Plinia*” groups. The presence of more than three carpels was only observed in the “*Pimenta*” group.

The reduced number of ovules per locule in the species investigated here reinforces the hypothesis of an evolutionary trend towards a reduction in the number of ovules. This was suggested by Lucas *et al.* (2007) in explaining variation in the number of locules in Myrteae, from unilocular locules in *Rhodamnia* to up to 20 locular in *Campomanesia*, while in the “*Myrcia* group” and some genera of the “*Plinia* group” two ovules/locule are found. Based on this, Lucas *et al.* (2007) concluded that the optimization of this character indicates that ancestral species of Myrteae had between 12 and 70 ovules.

Another interpretation of ovule number is that the report of up to six ovules in the *Neomitranthes* / “*Plinia*” group (Landrum & Kawasaki 1997) constitutes strong evidence in support of the statement by Lucas *et al.* (2007) that: the ovule number is either a polymorphic character or clearly represents a reversal of this character within the group “*Plinia*”, because this genus has a terminal phylogenetic position. Pimentel *et al.* (2014) provided support for the close relationship of the “*Plinia*” and “*Myrcia*” groups, while Vasconcelos *et al.* (2015) noted the same strongly incurved pre-anthetic stamen position.

All five species of Myrteae have a compitum in the ovary, which is a frequently used character among species



of Myrtaceae that have axile placentation (Lughadha & Proença 1996). Carr & Carr (1961) considered the compitum (eu-syncarpous gynoecium) as a connection between the carpels that allows pollen tubes from grains germinating on any stigma or part of the stigma to fertilize ovules belonging to more than one carpel. Armbruster *et al.* (2002) found eu-syncarpous gynoecia for all species of Myrtaceae they analyzed, however, there is variation in the degree of development of the *compitum*.

Harthman (2016) proposed a classification of *compitum* types for the 21 species investigated by her based on the degree of its development, which varied from the presence of a slit to its absence (filled by transmitter tissue), not observing a standard on the phylogeny of Lucas *et al.* (2007). However, the results of the present study demonstrate that all species have a *compitum* consisting of a crevice, and what varies is their degree of opening. Furthermore, there seems to be a relationship between the position of the crevice and the distribution of the ovules in the ovary. These differences in the interpretation of these characters highlight the need for more careful investigation.

The origin of the inferior ovary, which was found in the species of Myrteae analyzed here, is a very important question from a phylogenetic point of view, with the established theories in the literature being the appendicular or receptacle theories (Dickison 2000). The vascular supply of the inferior ovary may be a good tool for the morphological interpretation of the inferior ovary, with the presence of recurrent vascular bundles strongly supporting the receptacle origin of the inferior ovary (Roth 1977). The present study suggests an appendicular origin of the inferior ovary for *Myrceugenia alpigena*, *Myrcia multiflora* and *Myrciaria cuspidata*. Pimentel *et al.* (2014) proposed the appendicular origin of the inferior ovary for *Syzygium*, *Blepharocalyx salicifolius* and the “*Myrcia*” and “*Plinia*” groups, and also suggested that the common ancestor of Myrteae had an inferior ovary of receptacular origin. Harthman (2016) also reported the appendicular origin of the inferior ovary in another 10 species belonging to the *Eugenia*, *Myrcia*, *Myrceugenia*, *Plinia* and *Pimenta* groups proposed by Lucas *et al.* (2007).

Solid styles were found in four of the species of Myrteae studied here, while *Myrcia multiflora* was found to possess a reduced stylar canal. Endress (1991) introduced a very detailed classification of the pollen tube transmitting tract for Angiosperms, according to which the tract of the four species of Myrteae may be considered to be of the type defined as “Epidermis weakly secretory, path of pollen tubes internal in cell walls of the transmitting tissue (transmitting tissue several cell layers thick)”.

On the other hand, according to Endress (1991), *M. multiflora* may be characterized as having the type described as “Stylar canal with small lumen and small inner surface”. Endress (1991) also comments that a gynoecium with many ovules, (observed in *Eugenia pitanga*, *Myrceugenia alpigena*

and *Campomanesia adamantium*) tends to have a several-layered tract.

Comparative analysis of the flowers of the species of Myrteae examined and described here (Tab. 2) point to characters, such as the histology of sepal, petal and floral vasculature, and the degree of *compitum* opening, that if investigated in a large number of species of the tribe can reveal evolutionary patterns.

Considering the contribution of morphology to the phylogenetic study of Myrtaceae, a notable character is the floral vascular supply, which may be of great utility for the morphological interpretation of the inferior ovary since the main argument in favor of this character is the presence of recurrent vascular bundles. This seems to be a promising character, worthy of further exploration for a better understanding of the relationships among the different clades/groups of Myrtaceae.

This work also demonstrated the presence of a compitum consisting of a crevice in the five studied species, however, the variable degree of opening seems to be related to the position of the crevice, as well as the distribution of ovules in the ovary.

## Acknowledgements

We acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) for the support granted to achieve this study and Araucaria Foundation/SETI through the productivity scholarship granted to Káthia Socorro Mathias Mourão. We also thank Mariza Barion Romagnolo, Marcos Eduardo Guerra Sobral (identification of the voucher materials) and IAP (Instituto Ambiental do Paraná) (authorization of plant collection in the State of Paraná, Brazil).

## References

- Armbruster WS, Debevec EM, Willson MF. 2002. Evolution of syncarpy in angiosperms: theoretical and phylogenetic analyses of the effects of carpel fusion on offspring quantity and quality. *Journal of Evolutionary Biology* 15: 657-672.
- Barroso GM, Peron MV. 1994. Myrtaceae. In: Lima MPM, Guedes-Bruni RR. (eds.) Reserva ecológica de Macaé de Cima, Nova Friburgo: RJ. Aspectos florísticos das espécies vasculares. Vol. 1. Rio de Janeiro, Jardim Botânico. p. 261-302.
- Belsham SR, Orlovich DA. 2003. Development of the hypanthium and androecium in *Acmena smithii* and *Syzygium austral* (*Acmena alliance*, Myrtaceae). *Australian Systematic Botany* 16: 621-628.
- Berg O. 1855-1856. Revisio Myrtacearum Americae. *Linnaea* 27: 1-472.
- Berg O. 1857-1859. Myrtaceae. In: Martius CFP. (ed.) *Flora Brasiliensis* 14: 1-655.
- Biffin E, Harrington MG, Crisp MD, Craven LA, Gadek PA. 2007. Structural partitioning, paired-sites models and evolution of the ITS transcript in *Syzygium* and Myrtaceae. *Molecular Phylogeny and Evolution* 43: 124-139.





- Biffin E, Lucas EJ, Craven LA, Costa IR, Harrington MG, Crisp MD. 2010. Evolution of exceptional species richness among lineages of flesh-fruited Myrtaceae. *Annals of Botany* 106: 79-93.
- Briggs BG, Johnson LAS. 1979. Evolution in the Myrtaceae – evidence from inflorescence structure. *Proceedings of the Linnean Society of New South Wales* 102: 157-256.
- Bünger MO, Scaloni VR, Sobral M, Stehmann JR. 2012. Myrtaceae no Parque Estadual de Itacolomi, Minas Gerais, Brasil. *Rodriguésia* 63: 857-881.
- Candolle AP. 1828. Myrtaceae. *Prodromus systematis naturalis regni vegetabilis*. Vol. 3. Paris, Treuttel and Würtz.
- Carr SG, Carr DJ. 1961. The functional significance of syncarpy. *Phytomorphology* 11: 249-256.
- Costa MPF, Fernandes LDRS, Pimentel RR. 2010. Análise da Anatomia Floral da *Eugenia puniceifolia* (Humb., Bonpl. & Kunth) Dc. *Saúde & Ambiente em Revista* 5: 12-17.
- Dahlgren G. 1991. Steps toward a natural system of the dicotyledons: embryological characters. *Aliso* 13: 107-165.
- Davis GL. 1966. *Systematic embryology of the angiosperms*. New York, Wiley.
- Dickson WC. 2000. *Integrative plant anatomy*. San Diego, Harcourt Academic Press.
- Endress PK. 1991. *Diversity and evolutionary biology of tropical flowers*. Cambridge, Cambridge University Press.
- Fahn A. 1990. *Plant anatomy*. 4th. edn. Oxford, Pergamon Press.
- Guerrits PO. 1991. *The application of glycol methacrylate in histotechnology: Some fundamental principles*. Netherlands, State University Groningen.
- Harthman VC. 2016. *Ovário infero de Myrtaceae e sua implicação na taxonomia e filogenia da família*. PhD Thesis, Universidade Estadual de Maringá, Maringá.
- Johansen DA. 1940. *Plant microtechnique*. New York, McGraw-Hill Book Company.
- Johnson LAS, Briggs BG. 1984. Myrtales and Myrtaceae - a phylogenetic analysis. *Annals of the Missouri Botanical Garden* 71: 700-756.
- Kausel E. 1966. *Lista de las Myrtaceas y Leptospermeas argentinas*. *Lilloa* 32: 323-368.
- Landrum LM, Bonilla J. 1996. Anther glandularity in the American Myrtinae (Myrtaceae). *Madroño* 43: 58-68.
- Landrum LR, Kawasaki ML. 1997. The genera of Myrtaceae in Brazil: an illustrated synoptic treatment and identification keys. *Brittonia* 49: 508-536.
- Lopes BC. 2008. *Desenvolvimento floral e estudo palinológico de Eugenia uniflora L.* PhD Thesis. Universidade Federal do Rio de Janeiro, Rio de Janeiro.
- Lucas EJ, Belsham SR, NicLughadha EM, et al. 2005. Phylogenetic patterns in the fleshy-fruited Myrtaceae – preliminary molecular evidence. *Plant Systematics and Evolution* 251: 35-51.
- Lucas EJ, Harris SA, Mazine FF, et al. 2007. Suprageneric phylogenetics of Myrteae, the generically richest tribe in Myrtaceae (Myrtales). *Taxon* 56: 1105-1128.
- Lughadha EMN, Lucas EJ, Sobral M, Woodgyer EM. 2012. A new species and a replacement name in *Myrcia* (*Gomidesia*; Myrtaceae) from north-eastern Brazil. *Kew Bulletin* 67: 19-24.
- Lughadha EN, Proença C. 1996. A survey of the reproductive biology of the Myrtoideae (Myrtaceae). *Annals of the Missouri Botanical Garden* 83: 480-503.
- McVaugh R. 1968. The genera of American Myrtaceae – an interim report. *Taxon* 17: 354-418.
- Metcalfe CR, Chalk L. 1957. *Anatomy of the dicotyledons (leaves, stem, and wood in relation to taxonomy with notes on economic uses)*. Oxford, Clarendon Press.
- Moreira-Coneglian IR. 2011. *Morfoanatomia de ovário, pericarpo e semente de sete espécies de Myrteae DC (Myrtaceae)*. PhD Thesis, Universidade Estadual Paulista, Botucatu.
- Murillo-A, J, Stuessy TF, Ruiz E. 2013. Phylogenetic relationships among *Myrcogenia*, *Blepharocalyx* and *Luma* (Myrtaceae) based on paired-sites models and the secondary structures of ITS and ETS sequences. *Plant Systematics and Evolution* 299: 713-729.
- Niedenau F. 1893. Myrtaceae. In: Engler A, Prantl K. (eds.) *Die Natürlichen Pflanzenfamilien*. Leipzig, Verlag von Wilhelm Engelmann. p. 57-105.
- O'Brien TP, Feder N, McCully ME. 1964. Polychromatic staining of plant cell walls by toluidine blue. *Protoplasma* 59: 368-373.
- Pimentel RR. 2010. *Anatomia e Desenvolvimento da Flor de Eugenia neonitida Sobral (Myrtaceae)*. PhD Thesis, Universidade Federal do Rio de Janeiro, Rio de Janeiro.
- Pimentel RR, Barreira NP, Sapala DP, et al. 2014. Development and evolution of the gynoecium in Myrteae (Myrtaceae). *Australian Journal of Botany* 62: 335-346.
- Pires MMY, Souza LA. 2011. *Morfoanatomia e aspectos da biologia floral de Myrcia guianensis (Aublet) A. P. de Candolle e de Myrcia laruooteana Cambesse (Myrtaceae)*. *Acta Scientiarum* 33: 325-331.
- Roth I. 1977. Fruits of angiosperms. In: Linsbauer K. (ed.) *Encyclopedia of plant anatomy*. Berlin, Gebrüder Boerntraeger.
- Schauer JC. 1841. *Monographia Myrtacearum xerocarpicum, sectio I. Chamaelauciarum: hucusque cognitarum genera et species illustrans*. Bonn, Breslow.
- Schmid R. 1972a. A resolution of the *Eugenia-Syzygium* controversy (Myrtaceae). *American Journal of Botany* 59: 423-436.
- Schmid R. 1972b. Floral anatomy of Myrtaceae. I. *Syzygium*. *Botanische Jahrbücher für Systematik* 92: 433-489.
- Schmid R. 1972c. Floral anatomy of Myrtaceae. II. *Eugenia*. *Journal of the Arnold Arboretum* 53: 336-363.
- Schmid R. 1980. Comparative anatomy and morphology of *Psiloxylon* and *Heteropyxis*, and the subfamilial and tribal classification of Myrtaceae. *Taxon* 29: 559-595.
- Silva ALG, Pinheiro MCB. 2007. *Biologia Floral e da polinização de quatro espécies de Eugenia L. (Myrtaceae)*. *Acta Botanica Brasilica* 21: 235-247.
- Vasconcelos TNC, Prenner G, Bünger MO, De-Carvalho PS, Wingler A, Lucas EJ. 2015. Systematic and evolutionary implications of stamen position in Myrteae (Myrtaceae). *Botanical Journal of the Linnean Society* 179: 388-402.
- Volgin S, Stepanova A. 2001. Morphology and vascular anatomy of the flower in *Myrtus communis* L. and *M. macrophylla* Ehrh. (Myrtaceae). *Visnyk of L'viv University Biological Series* 27: 61-69.
- Volgin S, Stepanova A. 2002a. Morphology and vascular anatomy of the flower in *Melaleuca fulgens* R.Br. and *M. nesophila* F. Muell. (Myrtaceae). *Visnyk of L'viv University Biological Series* 28: 70-79.
- Volgin S, Stepanova A. 2002b. Morphology and vascular anatomy of the flower in *Feijoa sellowiana* Berg and *Psidium cattleianum* Sabine (Myrtaceae Juss. – Myrtoideae). *Visnyk of L'viv University Biological Series* 31: 66-76.
- Volgin S, Stepanova A. 2004. Morphology and vascular anatomy of the flower of certain species of *Psidium* L. (Myrtaceae Juss.). *Visnyk of L'viv University Biological Series* 37: 90-99.
- Wilson PG. 2011. Myrtaceae. In: Kubitzki K. (ed.) *The families and genera of vascular plants*. Vol. 10. Flowering Plants Eudicots. p. 212-271.
- Wilson PG, O'Brien MM, Gadek PA, Quinn CJ. 2001. Myrtaceae revisited: a reassessment of infrafamilial groups. *American Journal of Botany* 88: 2013-2025.
- Wilson PG, O'Brien MM, Heslewood MM, Quinn CJ. 2005. Relationships within Myrtaceae sensu lato based on a *matK* phylogeny. *Plant Systematics and Evolution* 251: 3-19.

