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INSTITUTE OF BIOSCIENCES  
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**FOLIAR SECRETORY CAVITIES IN NEOTROPICAL SPECIES OF MYRTACEAE: A  
HISTOCHEMICAL AND ONTOGENETIC APPROACH**

**PORTO ALEGRE**

**2022**

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Dissertation submitted to the Botany Graduate Program of Federal University of Rio Grande do Sul as a partial requirement for obtaining the degree of Master in Science.

Advisor: Prof. Dr. Jorge Ernesto de Araujo Mariath

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## [EN] Foliar secretory cavities in Neotropical species of Myrtaceae: a histochemical and ontogenetic approach

### Abstract

Secretory cavities are a conspicuous characteristic of Myrtaceae and have been known for a long time as essential oil (EO) producers. The mode of development of intercellular secretory reservoirs has been a challenging issue since the 19<sup>th</sup> century, when ontogenetic investigations began. Henceforth, many conflicting reports were made, and consensus on how the cavities develop has not been reached. Besides the controversy concerning the cavities' development, some recent evidence suggests that glands in Myrtaceae are not only oil repositories as was believed before. However, those findings are still limited to a few *Eucalyptus* species. To address those questions, gland development must be cautiously investigated, and gland metabolites must be explored in other species. In this dissertation, an ontogenetic investigation is carried out on Neotropical Myrtaceae, comparing aldehyde-fixed tissue and fresh sections mounted in mineral oil, both under light microscopy. The overall structure of mature glands is also explored. The cavities content of nine Neotropical species was investigated through classical histochemical tests, using NADI test for terpenes; Copper Acetate test for resins (diterpenoids); 2,4-DNPH test to detect carbonyl groups (Aldehydes and Ketones); DPBA for flavonoids; and UV excitation to analyze the fluorescence behavior of secretion. Our results suggest that glands in Myrtaceae develop by schizogeny, and lysigenous appearance is the result of technical artifacts. The mature gland is constituted by epithelium cells and surrounding sheath cells, which are suberized at maturity. The histochemical tests showed that Neotropical species contain a complex mixture of metabolites in the glands, including terpenes, resins, aldehydes or ketones, and flavonoids. Some variations were observed among the analyzed species. These findings, along with additional studies, can better elucidate the metabolites present in Myrtaceae glands and show that tissue processing may result in false impressions of lysigeny.

**Keywords:** artifacts; flavonoids; gland; microscopy; oil; schizogeny; terpenes.

## [PT] Cavidades secretoras foliares em espécies neotropicais de Myrtaceae: uma abordagem histoquímica e ontogenética

### Resumo

Cavidades secretoras são uma característica marcante de Myrtaceae e são conhecidas há muito tempo como produtoras de óleo essencial (OE). O modo de desenvolvimento dos reservatórios secretores intercelulares tem sido uma questão desafiadora desde o século 19, quando começaram as investigações ontogenéticas. A partir de então, muitos relatos conflitantes foram feitos e o consenso sobre como as cavidades se desenvolvem não foi alcançado. Além da controvérsia sobre o desenvolvimento das cavidades, algumas evidências recentes sugerem que as glândulas em Myrtaceae não são apenas depósitos de óleo como se acreditava anteriormente. No entanto, esses achados ainda estão limitados a algumas espécies de *Eucalyptus*. Para abordar essas questões, o desenvolvimento da glândula deve ser cuidadosamente investigado e os metabólitos da glândula devem ser explorados em outras espécies. Nesta dissertação, uma investigação ontogenética é realizada em Myrtaceae Neotropical, comparando tecido fixado em aldeído e cortes frescos montados em óleo mineral, ambos sob microscopia de luz. A estrutura geral das glândulas maduras também é explorada. O conteúdo de cavidades de nove espécies neotropicais foi investigado por meio de testes histoquímicos clássicos, utilizando o teste NADI para terpenos; Teste de acetato de cobre para resinas (diterpenóides); teste de 2,4-DNPH para detecção de grupos carbonila (Aldeídos e Cetonas); DPBA para flavonóides; e excitação UV para analisar o comportamento de fluorescência da secreção. Nossos resultados sugerem que as glândulas em Myrtaceae se desenvolvem por esquizogenia, e a aparência lisígena é resultado de artefatos técnicos. A glândula madura é constituída por células epiteliais e células da bainha circundante, que são suberizadas na maturidade. Os testes histoquímicos mostraram que as espécies neotropicais contêm uma mistura complexa de metabólitos nas glândulas, incluindo terpenos, resinas, aldeídos ou cetonas e flavonóides. Algumas variações foram observadas entre as espécies analisadas. Esses achados, juntamente com estudos adicionais, podem elucidar melhor os metabólitos presentes nas glândulas de Myrtaceae e mostrar que o processamento tecidual pode resultar em falsas impressões de lisigenia.

**Palavras-chave:** artefatos; flavonóides; glândulas; microscopia; óleo; esquizogenia; terpenos.

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## LIST OF ABBREVIATIONS AND SYMBOLS (SI)

DC	de Candolle
DIC	Differential interference contrast
DPBA	Diphenylboric acid-2-aminoethyl ester
EO	Essential oil
HCl	Hydrochloric acid
NADI	1 – Naphthol and N, N-Dimethyl-p-phenylenediamine
UV	Ultraviolet
2, 4-DNPH	2, 4-dinitrophenyl hydrazine
M	molarity
mg	miligrame
ml	milimeter
nm	nanometer
μl	microliter
μm	micrometer

# CHAPTER 1

## 1. GENERAL INTRODUCTION AND LITERATURE REVIEW

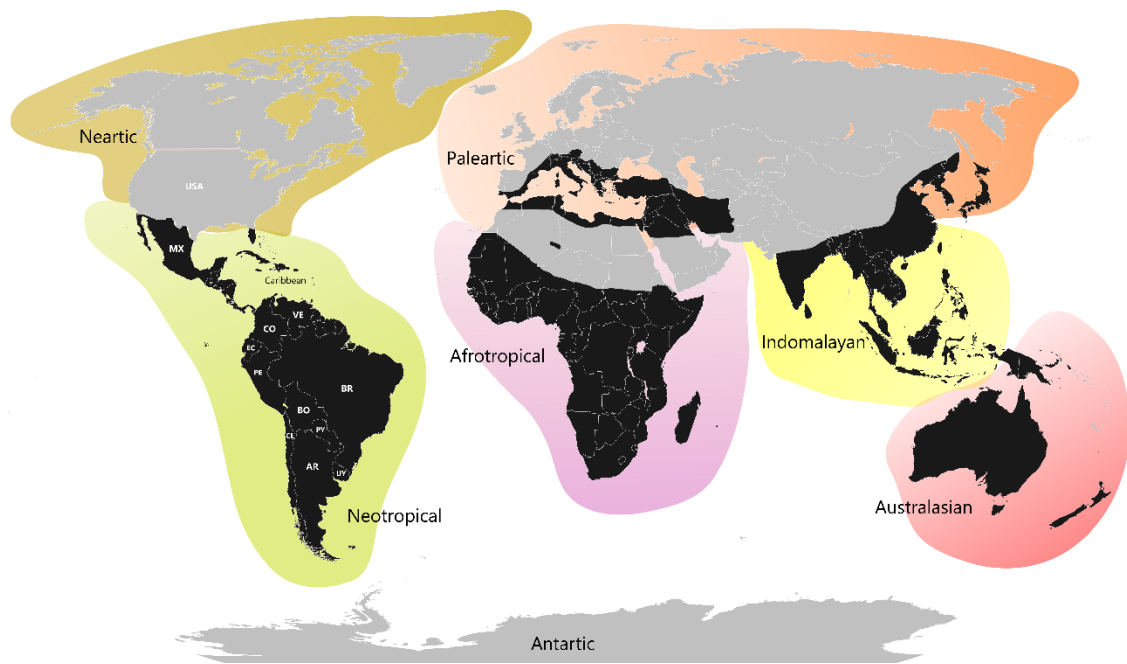
### 1.1 Myrtaceae

Myrtaceae was established in 1789 by Antoine Laurent de Jussieu and currently includes ca. 5950 species and 139 genera (Christenhusz and Byng, 2016). It is the eighth largest family of flowering plants (Grattapaglia *et al.*, 2012), occurring mainly in tropical and southern subtropical regions ([Figure 1](#)), with relatively low representation in Africa (Wilson, 2011). Myrtaceae constitute a significant part of the vegetation in Australia and the Neotropical region and are of high ecological and economic importance (Neto *et al.*, 2022). Fruit-eating fauna in the Americas relies on the abundance of fleshy-fruited species. Some of them are cultivated worldwide and are of great commercial value. In Australia, *Eucalyptus* L'Hér. species include the most important timber trees, which have become one of the most cultivated woody plants in the world. In addition, this family produces several specialized metabolites, including terpenes and phenolic compounds, which have many roles against herbivory, pathogens, competitors, and abiotic stresses (Padovan *et al.*, 2014; Niinemets, 2018). The biological activities make those compounds promising candidates for pharmacological usage (Reddy, 2019).

According to the original description, plants that belong to this family have petals and sepals in equal numbers, many stamens, an inferior ovary, rarely half-superior, a simple style, and leaves that are usually opposite, rarely alternate, with pellucid dots. The fruits are berries (also referred to as drupes) or capsular. The endosperm is absent, and the embryo is straight or curved. The growth habit of Myrtaceae includes trees or shrubs with variable inflorescence, usually axillary.

Initially, Jussieu (1789) placed in Myrtaceae several genera that are still nested within this family (e.g., *Eugenia* L., *Leptospermum* J.R. Forst. & G. Forst., *Melaleuca* L., *Myrtus* L., *Plinia* L., and *Psidium* L.) and others that currently belong to Lecythidaceae (e.g., *Couroupita* Aubl. and *Lecythis* Loefl.). Curiously, Lecythidaceae lack pellucid dots, a universal characteristic of Myrtaceae (Neidenzu, 1893). Since the establishment by Jussieu, the taxonomy and systematics of the family have changed, and now two subfamilies are recognized based on molecular evidence (Wilson *et al.*, 2005): Myrteoideae, with 15 tribes, and Psiloxylloideae, with two tribes ([Figure 2](#)).

The subfamily Psiloxylloideae, a subfamily distributed in southern Africa and Mauritius Island (southeast coast of Africa), includes the tribes Heteropyxidoideae (genus *Heteropyxis* Harv.) and Psiloxylloideae (genus *Psiloxylon* Thouars ex Tul.), the earliest surviving lineages of Myrtaceae. The subfamily Myrtoideae is distributed globally, with centers of diversity in the Neotropics, Australia, and Malesia. It is subdivided into 15 subtribes (Wilson *et al.*, 2005): Backhousieae (2 genera), Chamelaucieae (15 genera), Eucalypteae (7 genera), Kanieae (8 genera), Leptospermeae (8 genera), Lindsayomyrteae (1 genus), Lophostemoneae (4 genera), Melaleuceae (9 genera), Metrosidereae (1 genus), Myrteae (51 genera), Osbornieae (1 genus), Syncarpieae (1 genus), Syzygieae (1 genus), Tristanieae (3 genera) and Xanthostemoneae (3 genera). Except for Myrteae, with a center of diversity in Neotropics, and Syzygieae, with the center in Malaysia, all other tribes lack freshly-fruit.



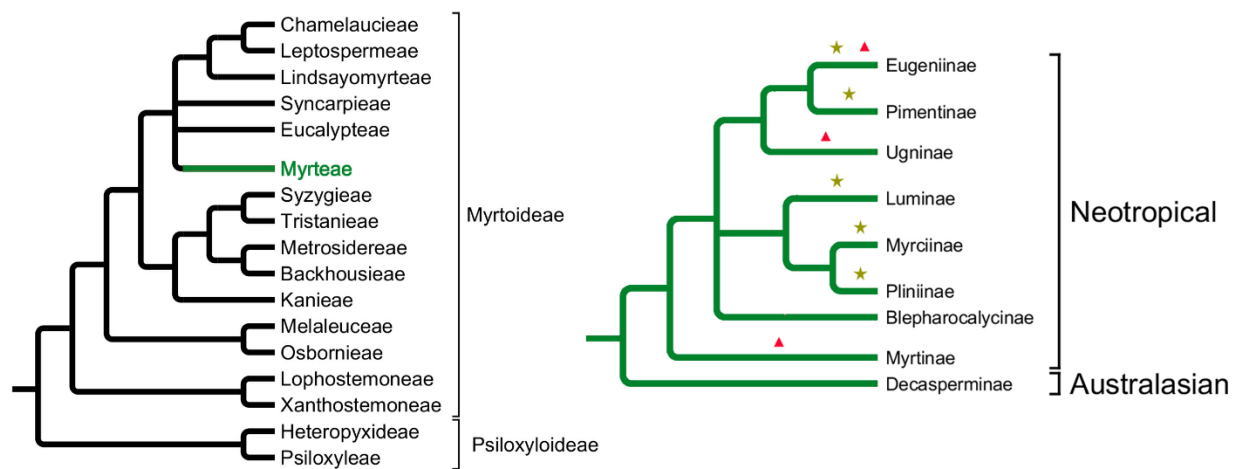
**Figure 1.** Geographical distribution of Myrtaceae (black area). The biogeographic realms are detached in different colors. AR = Argentina; BO = Bolivia; BR= Brazil; CL = Chile; CO = Colombia; CR = Costa Rica; GT = Guatemala; GY= Guyana; MX= Mexico; PE = Peru; USA = United State of America; UY = Uruguay; VE = Venezuela. Myrteae tribe have their center of diversity in Neotropical region, which comprises the South and Central America, Mexico, the Antilles (Caribbean) and South Florida (USA). Based on Wilson *et al.* (2005).

## 1.2 Tribe Myrteae

The largest tribe within Myrtaceae, Myrteae, includes ca. 2500 species and ca. 51 genera (Vasconcellos *et al.*, 2017). This group is particularly rich in South America and the Caribbean, although it also occurs in Southeast Asia, Northeast Australia, the Pacific Islands, and Africa (Wilson *et al.*, 2001). All species from the Neotropical region, except for *Metrosideros stipularis* (Hook. & Arn.) Hook, restricted to Chile, belong to the Myrteae section (Pillon *et al.*, 2015; Wilson *et al.*, 2005). The tribe is distinguished from the 14 other tribes in Myrtoideae (Wilson *et al.*, 2005) by their indehiscent fleshy fruits, transeptal vascular system, and, commonly, the presence of uni- or multi-cellular hairs (Schmid, 1972; Wilson *et al.*, 2001).

Neotropical Myrtaceae play a key role in South American areas, with significant animal interactions due to the floral resources and fresh fruits that are edible. It is also a key component in the vegetation physiognomy of tropical America, mainly in the eastern part (Atlantic Rainforest and Cerrado), Guayanas Highlands, and Caribbean (McVaugh, 1968), especially *Eugenia* L. and *Myrcia* DC., which are the more expressive genera. Myrteae is a taxonomically complicated group, and the species are often misidentified.

Myrteae is divided into ninth subtribes according to Lucas *et al.* (2019): Myrciinae, Eugeniinae, Myrtinae, Pimentinae, Blepharocalycinae, Decasperminae, Luminae, Pliniinae and Ugninae (Figure 2).



**Figure 2.** Phylogeny of Myrtaceae, showing the placement of the Myrteae tribe. Myrteae is divided in nine subtribes, distributed mainly in the Neotropical region. The subtribes marked with stars contain species analyzed in this research, and those marked with triangle include species occurring outside of the Neotropics. Based on Wilson *et al.* (2005) and Lucas *et al.* (2019). Created with TreeGraph 2.

The earliest divergent clade within Myrteae is the subtribe Decasperminae, which appears as the sister group of all other subtribes. Their species occur only in the Australasian and Pacific regions (Lucas *et al.*, 2019). This subtribe includes the genera *Archirhodomertus* Burret (5 spp.), *Austromyrtus* (Nied.) Burret (8 spp.), *Decaspermum* J.R. Forst. & G. Forst. (34 spp.), *Gossia* N. Snow & Guymmer (37 spp.), *Kanakomyrtus* N. Snow (6 spp.), *Lithomyrtus* F. Muell. (11 spp.), *Myrtella* F. Muell. (2 spp.), *Octamyrtus* Diels (6 spp.), *Pilidiostigma* Burret (6 spp.), *Rhodamnia* Jack (35 spp.), *Rhodomertus* (DC.) Rchb. (21 spp.), and *Uromyrtus* Burret (23 spp.) (Lucas *et al.* 2019). The remaining subtribes occur solely on the American continent, except Myrtinae, Eugeniinae, and Ugninae, which have some representatives outside of the Neotropical region. **Myrtinae** is a disjunctive group that includes the genus *Accara* Landrum (1 sp.), *Calycolpus* O. Berg (15 spp.), *Chamguava* Landrum (3 spp.), and *Myrtus* L. (2 spp.). *Accara* occurs only in the rock outcrops of Minas Gerais (Brazil), while *Calycolpus* is distributed mainly in northern South America (Landrum, 2010). *Chamguava* occurs in Central America, and *Myrtus* is endemic to the Mediterranean region and includes the *typus* of Myrtaceae, *Myrtus communis*.

**Eugeniinae** comprises *Eugenia* L. (c. 1000 species), a pantropical distributed genus, with some species in Africa, Southeast Asia, and the Pacific. *Eugenia* has high diversity in the Neotropics. The genera *Calyptrogenia* Burret (6 spp.) and *Hottea* Urb. (9 spp.) from the Caribbean, and *Pseudanamomis* Kausel (1 sp.) from Hispaniola and northern South America (Liogier, 1994), were recently transferred to *Eugenia* based on molecular evidence (Flickinger *et al.*, 2020). Another genus nested in Eugeniinae is *Myrcianthes* O. Berg (37 spp.), which occurs mainly in the Andean region (Griffo, 1992). **Ugninae** comprises the Andean genera *Ugni* Turcz. (4 spp.) and *Myrteola* O. Berg (3 spp.), distributed from the Falkland Islands and the southern tip of South America to the paramos of high mountains in northern South America (Landrum, 1988). Outside of the Neotropics, *Neomyrtus* Burret and *Lophomyrtus* Burret (2 spp.) are endemic to New Zealand, while *Lenwebbia* N. Snow & Guymmer (2 spp.) is endemic to northeastern Australia (Lucas *et al.*, 2019).

Considering the solely Neotropical groups, **Myrciinae** is the richest subtribe, encompassing the genus *Myrcia* DC. and its synonyms *Calyptranthes* Sw., *Gomidesia* O. Berg, and *Marlierea* Cambess., according to recent phylogenies (Lucas *et al.*, 2011). Myrciinae is widespread from the United States (Florida) and Mexico to Argentina and *Myrcia* is the largest

exclusively Neotropical genus with c. 770 species. The **Blepharocalycinae** section is a small clade containing three species that belong to the genus *Blepharocalyx* O. Berg. This subtribe occurs from the Caribbean to southern Chile. *Algrizea* Proença & Nic Lugh. (2 spp.), *Myrciaria* O. Berg (26 spp.), *Neomitranthes* D. Legrand (15 spp.), *Siphoneugena* O. Berg (11 spp.), and *Plinia* L. (69 spp.) are all members of the **Pliniinae** subtribe. *Myrciaria* species range from Mexico and the Caribbean to northern Argentina; *Plinia* species range from Central America and the Caribbean to Brazil; *Neomitranthes* and *Algrizea* are found only in Brazil (Landrum and Kawasaki, 1997); and *Siphoneugena* occurs from Puerto Rico to Northern Argentina (Proença, 1990). **Luminae** includes the genus *Luma* A. Gray (1 sp.) and *Myrceugenia* O. Berg (c. 50 spp.), which occur in tropical to sub-temperate parts of South America (Lucas *et al.*, 2019); *Nothomyrcia* Kausel (1), which is endemic to the Juan Fernández Islands (Chile); and *Temu* O. Berg (1 sp.), which is also endemic to Chile (Murillo-Aldana and Ruiz, 2011).

The **Pimentinae** subtribe ranges from the Greater Antilles and Central America to Uruguay and southern Chile in South America and includes the following genus (Lucas *et al.*, 2019): *Acca* O. Berg (2 spp.), occurring in the Andean region. *Curitiba* Salywon & Landrum (1 sp.), native to southern Brazil. *Feijoa* O. Berg (1 sp.) native to Uruguay, Argentina, Paraguay and southern Brazil. *Legrandia* Kausel (one species), native to Chile. *Mosiera* Small (33 spp.) which occurs mainly in the Caribbean. *Myrrhinium* Schott (1 sp.) which grow from Rio de Janeiro (Brazil) to Uruguay and northwestern Argentina north to Colombia (Landrum, 1986). *Pimenta* Lindl. (16 spp.) native to Caribbean and Central America, with one species in southern Brazil. *Psidium* L. (112 spp.), native from Mexico and the Caribbean to northern Argentina (Landrum, 1986; Wilson, 2010; Vasconcelos *et al.*, 2019); and *Campomanesia* Ruiz & Pav. (38 spp.) which occurs from north of Argentina to Trinidad, and from the coast of Brazil to the Andes (Peru, Ecuador, and Colombia).

### 1.3 A brief overview of secretory cavities knowledge

Secretory cavities are specialized internal structures responsible for the plant mucilage, resin, and essential oils synthesis (Esau, 1974; Fahn, 1988; Turner, 1999). They consisting of multiple secretory cells ([Figure 3, n°1](#)) arranged at the border of a more or less spherical space, forming a uni- or multiseriate epithelium, which produces such compounds and secret them into the center of this sphere (Picherski and Dudareva, 2020). Additionally, parenchymatous cells may enclose the epithelial cells to form a sheath ([Figure 3, n°2](#)), with casual reports of suberization

(Lutz, 1895). The suberin impregnation in secretory structures is commonly present in idioblasts (oil cells and mucilage cells) or trichomes (Zacharias, 1879; Thompson, 1979; Sadala-Castilho *et al.*, 2016) but scarcely described to cavities. In some instances, the cavities might be in direct contact with adjacent cells of the epidermis (cover cells) or connected to it through a series of cells ([Figure 3, n° 3](#)) called neck cells (Carr and Carr, 1970).

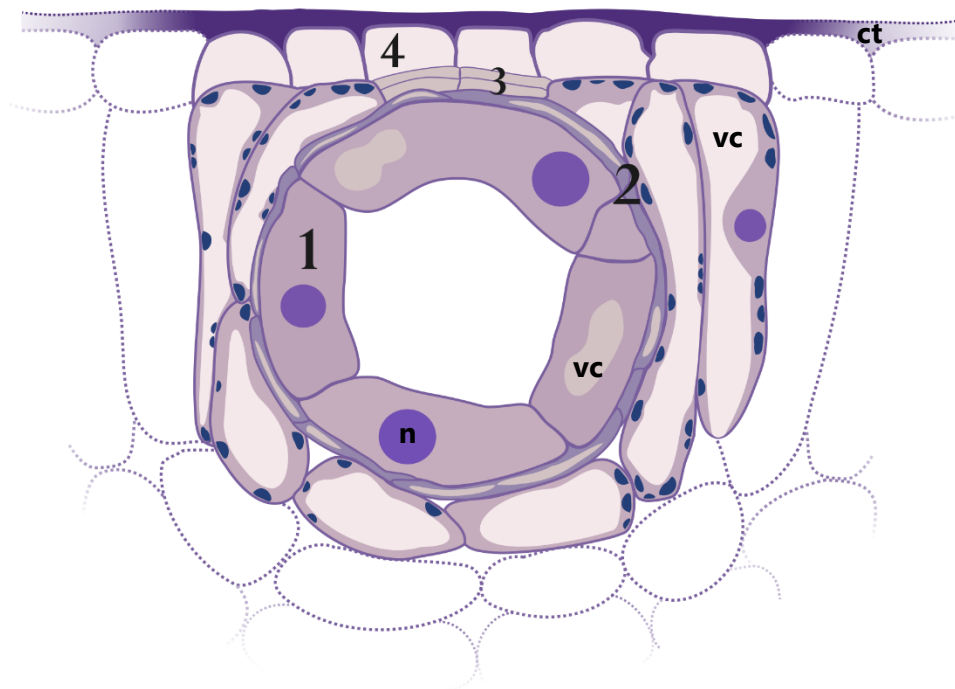
The oldest plant anatomists employed the term glands to refer to the secretory cavities, an imprecise term used to many other structures (Frank, 1868), including stomata. For many years, little was known about their true nature. Mirbel (1808) was the first to describe those structures as holes in *Ptelea* L. (Rutaceae), but the investigations of how the intercellular space develops started only with Frank in 1868. Currently, three developmental processes are recognized (Fahn, 1988; Turner, 1999): (1) Lysigeny, with intercellular space arising by lysis of the secretory cell; (2) Schizogeny, arising by separation of secretory cells; and (3) Schizolysigeny, by a combination of both processes. The cells separate and then undergo autolysis. The terminology “schizogeny” (*schizo*: split; *genes*: originated) and “lysigeny” (*lysis*: rupture; *genes*: originated) was established by De Bary (1877), while Tschirsch proposed the term “schizolysigeny” in 1889. However, those processes were reported long before.

The schizogeny was conceived by the earliest anatomists to resin canals (Carr and Carr, 1970). At that time, the cell theory was not established, and consequently, the comprehension of those authors was somewhat different from our modern vision of cells and tissues. Nehemiah Grews in *The Anatomy of Plants* (1682) suggested that resin canals of Pine arose from tissue separation, while Duhamel du Monceau (1764) believed that those intercellular spaces arose from the pressure of the secreted resin, preventing the cells from growing together, creating a prismatic space. The lysigeny idea was conceived originally by Karsten (1857), who postulated that the walls of secretory cells underwent liquefaction and resorption, allowing the secretion to be released. The term “resorption” achieved wide popularity in scientific literature and was applied immediately by many authors until its replacement by lysigeny, which designates the cellular spaces formed through disorganization, disintegration, or rupture (De Bary, 1877). However, the origin of the lysigeny idea is even older. Mirbel hypothesized, already in 1808 that the cavity of glands might result from the destruction of a delicate tissue that initially occupied its place.

Many controversies concerning the development of secretory structures, including cavities, remain unresolved owing to the contradicting observations. Since early times plant anatomists



have had divergent interpretations. Mirbel (1808) and Moldenhauer (1812), for example, reported having seen a delicate epithelium lining the resin ducts in *Pinus* L., while Meyen (1837) could never clearly see the cells bordering the canal. Until today, the presence or absence of sharply secretory cells at the fully developed stages is considered a decisive characteristic to interpret the development process, to both light and electron microscopy. Some authors (Turner *et al.*, 1998; Turner, 1999) judge that non-identifiable cells bordering the secretory cavities are artifacts. They show that the epithelial secretory cells may suffer changes during fixation or by mounting media used for sections made from fresh material. Both methods may cause cellular swelling and bursting, leading to a misinterpretation of results. Osmotic shocks associated with thin cell walls or even poor fixation are probably the cause of this damage. Hence, characteristics of the lysigenous process, as the presence of cell wall residues in the secretory space or absence of epithelial cells (Höhnel, 1881; Fahn, 1979) may be a false impression of cell death. Thus, the lysigeny hypothesis is still hotly debated among plant anatomists, and previous reports merits reexamination (Evert, 2006).



**Figure 3.** Secretory cavity structure. (1) Secretory cells; (2) sheath cells; (3) neck cells; and (4) cover or lid cells. Illustration based on Carr and Carr (1970), and Brocheriou and Belin-Depoux (1974). n: nuclei; vc: vacuole; ct: cuticle. Created with Inkscape (free software).

#### 1.4 Historical revision about secretory cavities in Myrtaceae

The interpretation of the structure and origin of secretory cavities in Myrtaceae has been historically contradictory, and remains a question to be solved.

Secretory cavities are widely present in several organs of Myrtaceae, such as leaves, floral structures (ovary, anther, sepals, and petals), stem cortex, and roots (Neish, 1995). Contrary to some authors, Van Tieghem (1885) did not observe oil cavities in roots. In the leaf, those structures are a diagnostic feature of Myrtaceae (Niedenzu, 1893) and are known as “pellucid dots”, “translucid dots/spots”, or “oil glands”. Against the light, the oil cavities are occasionally visible as bright spots to the naked eye, and historically this feature was used to segregate Lecythidaceae from Myrtaceae. The density of secretory cavities in leaves and other tissues varies markedly.

According to Lignier (1887), the translucent punctuations present in the leaves of Myrtaceae were responsible for the earliest anatomical research in this family. Guettard (1745) made the first more significant contribution to oil cavities knowledge on Myrtaceae and named leaf punctuations as vesicular glands, describing them as transparent bladders. He supposed at the time that the aromatic character of the Myrtle family was derived from those bladder-like glands. Although leaf punctuations were already known previously, they had not received much attention until then. Later authors (Schrank, 1794; Mirbel, 1815) brought neither terminology changes nor additional data to the knowledge of those structures until Schultz (1823). He analyzed glands in *Melaleuca salicifolia* Andrews and named them “oil sacks” (*Oelsäcke*). Macerating the leaves, Schultz isolated the glands in *Metrosideros* Banks ex Gaertn. and *Melaleuca* Vest, revealing that those structures formed independent units delimited by a delicate cell tissue in which the essential oil is found ([Figure 4, A and B](#)). Nothing else could be understood about single cells, from which the entire gland is composed. Afterward, De Candolle (1827) renamed those glands as vesicular reservoirs, defining them as more or less spherical vesicles containing oily, volatile, and aromatic juices. Meyen (1837) described them as glands situated immediately below the epidermis, with a central cavity filled with essential oil.

Starting the ontogenetic studies of Myrtaceae, Frank (1868) described schizogenous development for oil cavities of *Myrtus communis* L., stating that the origin of the cavity lay in a

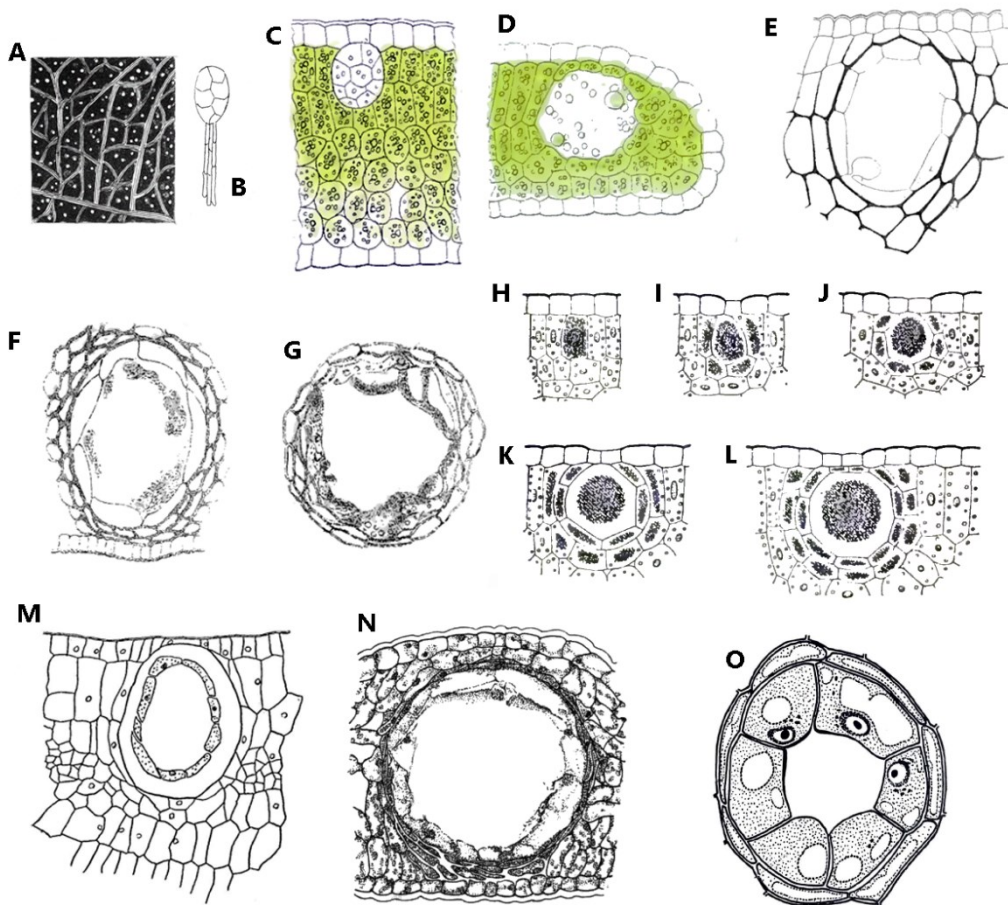
hypodermal chlorophyll-less cell with a large diameter and thin wall. This cell then undergoes successive divisions to form the secretory cells, which separate at their central point of contact to create an intercellular space filled with oil. Frank (1868) also found that the cavities arise continually one after the other in the early stages of leaf growth. When fully developed, they are positioned just beneath the epidermis, along both the abaxial and adaxial sides. Martinet (1872) remarked that the secretory epithelium of glands of the Myrtaceae undergoes resorption and classified it as lysigenous. Chatin (1875) concluded that the glands arise from protodermal cells: the initial cell divides to form the glandular cells that suffer disintegration over the ontogeny, forming the intercellular space ([Figure 4, C and D](#)). Some years later, Von Höhnelt (1881) found schizogenic glands, which are originated either from protodermal cells and divide several times into different planes creating the secretory cavity cells, or from ground meristem.

Bokorny (1882) studied several genera of Myrtaceae, and observed in leaf cross-sections a colorless epithelium lining the cavities, constituted by a single layer of flat and very delicate cells. Also studying the glands ontogeny of *Myrtus communis* L., this author confirmed the information provided by Frank (1868), and classified them as schizogenous. Two years later, De Bary (1884), without examining the development of any cavities and based on the resemblance of mature glands of Myrtaceae with Rutaceae, agreed with the lysigenous origin reported by Martinet (1872). Van Tieghem (1885) established that oil cavities in Myrtaceae are schizogenous, affirming that they are the result of dissociation, not of destruction. Leblois (1887), under the guidance of Van Tieghem, found schizogenous glands ([Figure 4, E](#)), which arise from cells of protoderm or ground meristem.

Brioso (1892) described the glands as lysigenous. According to this author, the epithelium seen in other works were protoplasmic remnants without cellulosic walls. Ontogenetically, its origin was attributed to two cells, an epidermal and a subepidermal, or from ground tissue. In the following year, Tschirch (1893) described the oil glands of cloves (*Syzygium aromaticum* (L.) Merr. & L.M. Perry) as schizogenous. He also believed that the synthesis of resinous secretion in plants did not occur in the cytoplasm of secretory cells, but in their walls, from a mucilaginous stratum (resinogenic layer). Tschirch (1893), however, has never seen intracellular contents of secretion. The resinogenic hypothesis was refuted years later. He also observed a positive reaction with HCl-phloroglucinol in epithelial cell walls and concluded that they were, if not lignified, infiltrated with aromatic aldehydes.

Haberland (1896) affirmed that the glands develop through the separation of the secretory cell, which in the mature state line the walls of schizogenous space as a coherent cell layer. In further studies by this author, little was added to the knowledge about cavities development. Niedenzu (1897) affirmed that those glands always develop by lysigeny (Figure 4, H-L) and are derived from epidermal or cells of the ground meristem. The cavities are not only oil-filled but also contain protoplasmic remains left by the dissolved cells. Lutz (1895) considered the nomenclature “glands” incorrect to Myrtaceae since those structures do not always arise from epidermal cells. The use of the term “gland” was previously discussed by De Bary (1884). Conforming to Lutz (1895), the cavities develop schizogenously, and the epithelial cells are obliterated and suberized at the end of the cavities’ development since the walls of secretory cells are resistant to acid hydrolysis and gave no result with Phloroglucinol. According to Mielke (1892) in older leaves of *Eucalyptus* L’Hér., the oil cavities become obliterated by the destruction of epithelial cells and casing cells. Discussing taxonomical questions, Engler (1897) stated that cavities in *Heteropyxis* Harv. leaves are lysigenous like Rutaceae, although he shows no evidence. Welch (1920) studying *Eucalyptus* L’Hér. recognized the oil cavities in Myrtaceae as schizolysigenous. In his analysis, he found that collapsed cells of the epithelium are separated from the wall of the cavity (Figure 4, M). Later, revising the incongruences concerning the development of oil cavities, Fohn (1935) described them as lysigenous in *Eucalyptus*. During the ontogeny of cavities, this author noted a core of cells with an inconspicuous wall that undergoes lysis, a process that gradually spreads to the surrounding cells, leaving protoplasmic remains visible in the mature stage (Figure 4, N). Carr and Carr (1970) reported schizogenous cavities in *Eucalyptus* L’Hér, which arise from epidermal cells. Those authors noted that in the early stages of ontogeny the precursors cells of epithelial cells have undetectable walls. Also, the mature casing cells have walls impregnated with substances such as suberin or lignin which resist acid hydrolysis and give a positive reaction to phloroglucinol-hydrochloric acid. Brocheriou and Belin-Depoux (1974) investigated the origin and development of secretory cavities in various species and found schizogenous types (Figure 4, O). Fahn (1979) presented in the book “Secretory Tissues in Plants” images of ultrastructure provided by Lazerovitz (1978), indicating a schizogenous development of oil cavities. Those results showed living epithelial cells in a mature state with osmiophilic content. Johnson (1980) observed in *Leptospermum* a schizogenous origin of cavities, which originate from protodermal cells. In the mature state, the epithelial cells are thick-walled and line the cavities forming a single

continuous layer. According to List *et al.* (1995), the cavities are formed schizogenously or schizolysigenous (inconclusive). The epithelial cells lack the inner tangential wall. Cicarelli (2003) affirmed that ontogenesis of secretory cavities is schizolysigenous in *Myrtus communis* L. and the cells degenerate close to maturity. On the other hand, Kalachanis (2005) described schizogenous development to this same species. Goodger *et al.* (2010) isolated intact sub-epidermal oil-cavities from *Eucalyptus* L'Hér by partial enzymatic digestion and observed intact nuclei with Hoechst staining (a fluorescent dye used to stain DNA). Retamales (2014) analyzed *Ugni molinae* Turcz. and related schizogenous oil cavities, based on the presence of epithelial cells lining the cavity at the mature state. According to this author, the epithelial cells almost degenerate, and he found that those cavities arise from both protodermal and ground meristem cells. Finally, more recently, Costa *et al.* (2020) found schizolysigenous development in *Myrcia splendens* (Sw.) DC., and Ribeiro *et al.* (2021) reported a schizolysigenous origin to eleven species of Neotropical species.



**Figure 4.** Illustrations of selected works about secretory cavities in Myrtaceae. (A) and (B) *Tristania neriifolia* R. Br. (syn. *Melaleuca salicifolia* Andrews). (A) Glands are scattered in the leaf parenchyma. (B) Isolated gland through maceration, associated with vessels. Drawings by Schultz (1823). (C) *Psidium montanum* Sw. and (D) *Myrtus communis* L. (from Chatin, 1875). (E) *Eucalyptus callosa* (non-valid name) by Leblois (1888). (F) *Pimenta dioica* (L.) Merr. (syn. *Eugenia pimenta* (L.) DC.) and (G) *Leptospermum uncinatum* (non-valid name) from Lutz (1895). H-L Ontogeny of *Myrtus communis* L. (from Niedenzu, 1898). (M) *Eucalyptus smithii* R.T. Baker by Welch (1920). (N) *Eucalyptus globulus* Labill. by Fohn (1935). (O) *Callistemon citrinus* (Curtis) Skeels (from Brocheriou and Belin-Depoux, 1974).

### 1.5 The Secretory cavities and Myrtaceae phytochemistry

Secretory cavities in Myrtaceae are secularly known as producers of “essential oils,” which are scented liquids obtained from plants through steam or pressure. Some discussions exist concerning the validity of using this term to refer to those compounds *in natura*. In any case, essential oils present a complex composition. The most obvious compounds are terpenes, molecules derived from isoprene building blocks ([Figure 5, A](#)), which contain carbon and



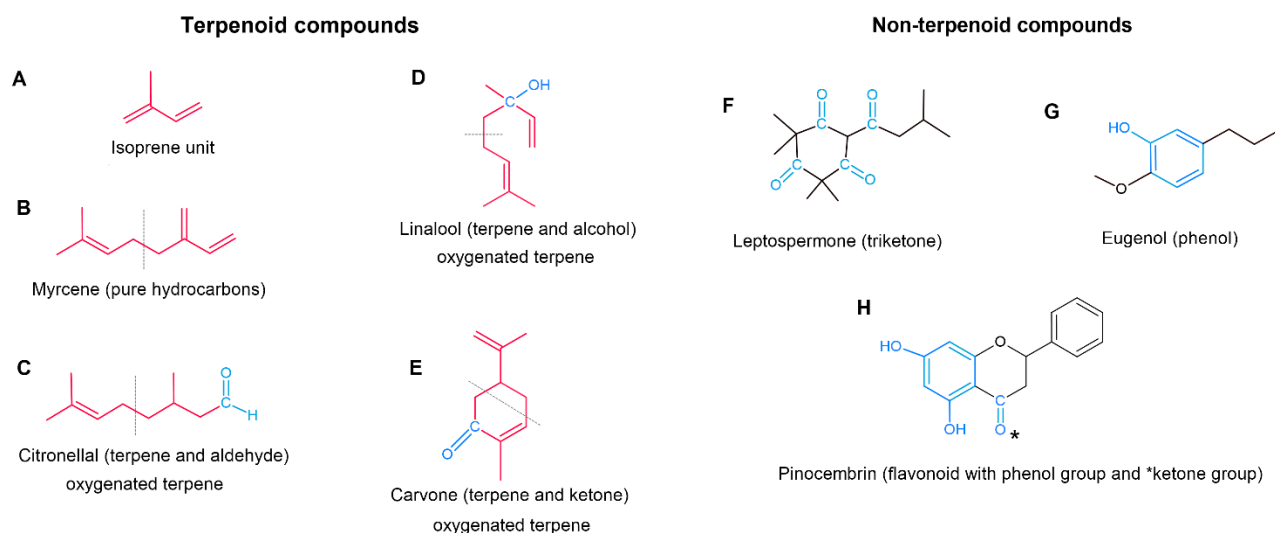
hydrogen atoms (Croteau *et al.*, 2000). Monoterpenes (two isoprene units), sesquiterpenes (three isoprene units), and diterpenes (four isoprene units) are the pure hydrocarbons found in oils. Each type of terpene may also contain functional groups such as aldehydes (Figure 5, C), ketones (Figure 5, E), alcohols (Figure 5, D), ethers, esters, or oxides (Croteau *et al.*, 2000). All those contain oxygen, and thus terpenes carrying this element are known as oxygenated terpenes or “terpenoids”. Nevertheless, some of those functions may occur in non-terpenoid compounds (Figure 5, F), and others, such as phenolics (Figure 5, H), may be present in the mixture.

According to Hegnauer (1969), monoterpenes are the main component of the oils in many Myrtaceae, whereas sesquiterpenes are frequently present in significant quantities and are the compound generally predominant in Myrteae (Padovan *et al.*, 2014). Those volatile compounds are responsible for the aromatic character of the family. The other terpenes (diterpenes, triterpenes, and polyterpenes) are little or not at all volatile and have received less attention. Those groups are generally constituents of “resins” (Stahl and Jork, 1965). Although terpene biosynthesis is well established in this family, the early-divergent group Psiloxylloideae, comprising the genus *Psiloxylon*, a gland-dotted taxon (Schimid, 1980), does not produce essential oil (Mohammed *et al.*, 2009), unlike the remaining taxa.

Surprisingly, in recent years, the isolation of intact glands has been demystifying the well-accepted condition that secretory cavities are predominantly essential oil repositories (Goodger *et al.*, 2010). Using gland isolation and chromatography, some studies found non-volatile compounds in *Eucalyptus* glands, such as flavonoids, free  $\beta$ -triketone, flavanone O-glucosides and flavanone- $\beta$ -triketone conjugates (Goodger *et al.*, 2009; Goodger and Woodrow 2011; Goodger *et al.*, 2016). The presence of  $\beta$ -triketones in oil of some Australian Myrtaceae such as *Leptospermum* J.R. Forst. & G. Forst., *Eucalyptus* L’Hér. and *Xanthostemon* F. Muell. has been long recognized (Briggs *et al.*, 1938; Birch and Elliot 1956, Hegnauer 1969; Goodger *et al.*, 2016), and new records have been made to other *taxa* (Osório *et al.*, 2016). Recently Killen *et al.*, 2015 reinforced the evidence of localization of triketones in the cavities by RAMAN microscopy. B-triketones are found mainly in Myrtaceae and have important antimicrobial, antibacterial, antifungal, and insecticidal properties.

Considering the new horizons about metabolites that have precise locations in glands, their contents need to be more widely explored, especially in neglected species, such as Neotropical Myrtaceae. Since previously recorded metabolites have various biological effects, those

compounds might be of great importance for applications in the pharmaceutical, cosmetic, and food industries.



**Figure 5.** Some compounds of Myrtaceae. (A) The basic building block of terpenoid (the isoprene unit). (B) A pure hydrocarbon monoterpene; (C) An oxygenated terpenoid carrying an aldehyde functional group (in blue); (D) An oxygenated terpenoid carrying an alcohol functional group (in blue). (E) An oxygenated terpenoid carrying a ketone functional group (in blue). The dashed line delimits the isoprene units of molecules. (F) a non-terpenoid ketone. The ketone functional groups are detached in blue. (G) Eugenol is a non-terpenoid molecule present in essential oils of some Myrtaceae. The phenol functional group is detached in blue. (H) Flavonoids are polyphenolic metabolites. Pinocembrin contains besides the phenol functional group (in blue), a ketone group (in blue and marked with \*). Based on Guenther and Darrel (1949) and Goodger *et al.* (2016). Created with ACD/ChemSketch software.

## 1.6 Analyzed species

### Subtribe Eugeniinae

*Eugenia pyriformis* Cambess: Uvaia† ([Figure 6, A](#)) can be found in the Atlantic Forest and the Cerrado savannas. It occurs in Argentina, Paraguay, Uruguay, and South Brazil (Paraná, Santa Catarina, and the Rio Grande do Sul), (Legrand and Klein, 1969). The fruit is berry-type with yellow pulp and is usually consumed by local populations.

*Myrcianthes pungens* (O. Berg) D. Legrand: Guabiju† ([Figure 6, B](#)) has black and sweetly edible fruits. It is widely cultivated for its tasty fruit and as an ornamental plant. It occurs in Argentina,



Paraguay, Uruguay, and parts of southern and eastern Brazil and Bolivia (Grandtner & Chevrette, 2013). The leaf pellucid dots (secretory cavities) are visible to the naked eye.

### **Subtribe Myrciinae**

*Myrcia oblongata* DC.: Guamirim† ([Figure 6, C](#)) occurs in Paraguay, northern Argentina, and Brazil (Paraná, Santa Catarina, and the Rio Grande do Sul), where it is often associated with the Araucaria Moist Forest (Sobral, 2003). The leaf pellucid dots (secretory cavities) are easily visible to the naked eye.

### **Subtribe Pliniinae**

*Plinia peruviana* (Poir) Govaerts: Jaboticaba† ([Figure 6, D](#)) is a tree with cauliflorous inflorescence and edible fruits. The distribution included Peru, Brazil (from Minas Gerais to the Rio Grande do Sul), Paraguay, northern Argentina, and Uruguay (Grandtner and Chevrette, 2013). The leaf pellucid dots (secretory cavities) are not visible to the naked eye.

*Siphoneugena reitzii* D. Legrand: Camboim† ([Figure 6, E](#)) is a tree often associated with the Araucaria Moist Forest in high elevations. It is endemic to Brazil, occurring from Minas Gerais to the Rio Grande do Sul in the coldest climates (Proença, 1990). The leaf pellucid dots (secretory cavities) are visible to the naked eye. The genus is adapted to wet montane forests, generally growing at an elevation of more than 900 m (Proença, 1990).

### **Subtribe Pimentinae**

*Feijoa sellowiana* (O. Berg) O. Berg: A widely cultivated plant around the world, Feijoa† ([Figure 6, F](#)) is native to Paraguay, Northern Argentina, Brazil (Minas Gerais to the Rio Grande do Sul) in the highlands, and Uruguay (Landrum, 1986). Other countries, besides Brazil, value their edible fruits. The flowers are ornamental and edible, with a sweet taste. The leaf pellucid dots (secretory cavities) are not visible to the naked eye.

*Campomanesia xanthocarpa* O. Berg: Popularly known as gabirolba† ([Figure 6, G](#)), this tree occurs in Bolivia, Brazil (Mato Grosso do Sul and Minas Gerais to the Rio Grande do Sul), Paraguay, and northern Argentina (Grandtner and Chevrette, 2013). Its fruits are edible and appreciated by local populations. The leaf pellucid dots (secretory cavities) are not visible to the naked eye.

*Psidium cattleianum* Sabine: The araçá† ([Figure 6, H](#)) is native to Northeast, South, and Southeast Brazil, and Uruguay (Legrand and Klein, 1977). It was widely introduced around the world and is invasive in many places. It is used as a medicine, has environmental uses, and for food.

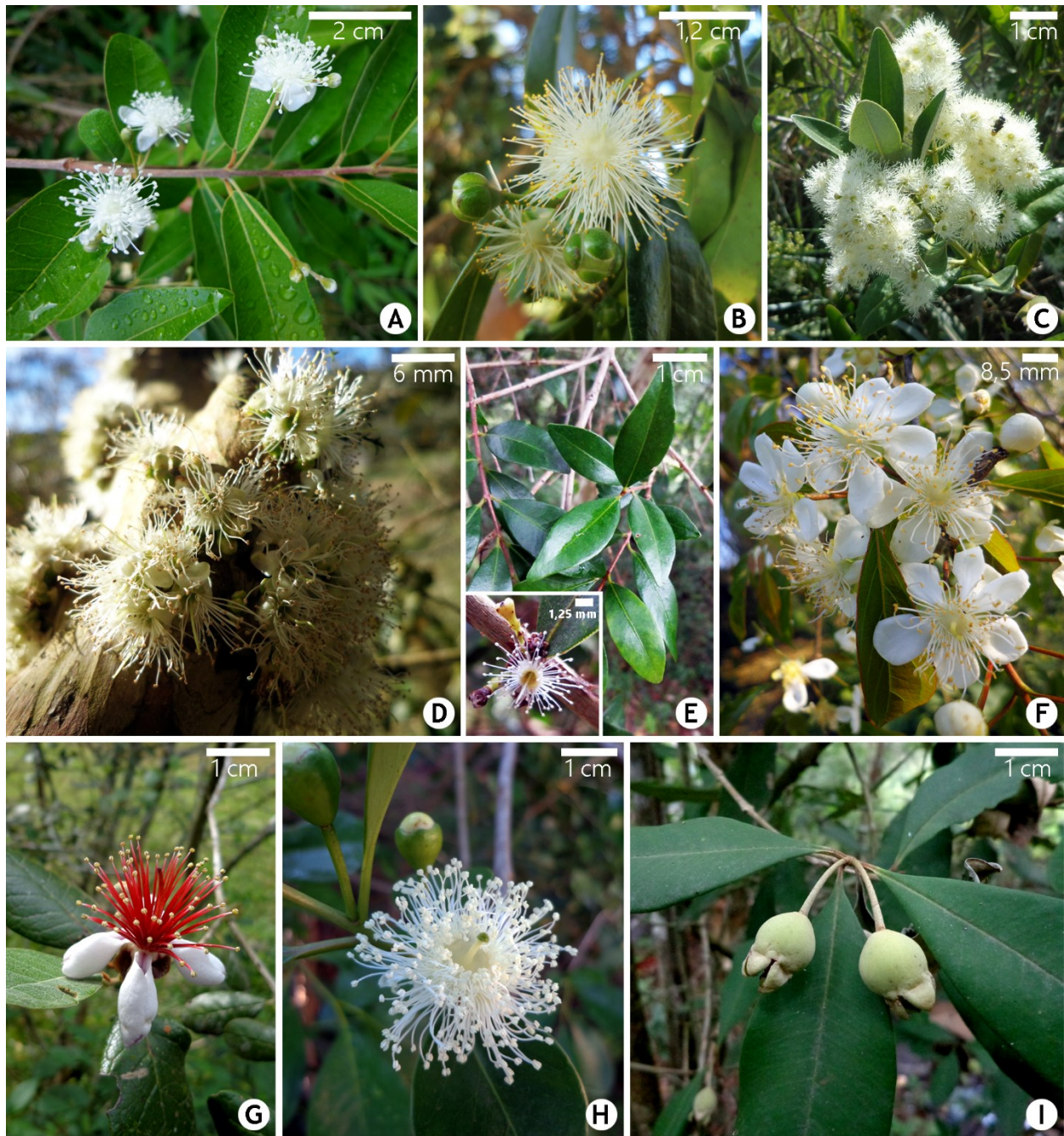
### **Subtribe Luminae**

*Myrceugenia myrcioides* (Cambess) O. Berg: Araçarana† ([Figure 6, I](#)) is a small tree that grows in the shade of the forest canopy (Legrand and Klein, 1970; Wagner and Fiaschi, 2020) and is associated with small creeks. It is endemic to Brazil, occurring from Rio de Janeiro to the Rio Grande do Sul in the Atlantic Rainforest (Legrand and Klein, 1971). The leaf pellucid dots (secretory cavities) are small and visible to the naked eye.

† Common name in Brazil.

## **1.7 Research Aim**

To describe the development and structure of secretory cavities in Neotropical Myrtaceae and investigate their metabolites.



**Figure 6.** Investigated species. (A) *Eugenia pyriformis* Cambess, (B) *Myrcianthes pungens* (O.Berg) D.Legrand., (C) *Myrcia oblongata* DC., (D) *Plinia peruviana* (Poir.) Govaerts, (E) *Siphoneugena reitzii* D. Legrand, (F) *Campomanesia xanthocarpa* (Mart.) O.Berg., (G) *Feijoa sellowiana* (O.Berg) O.Berg, (H) *Psidium cattleianum* Sabine, (I) *Myrceugenia myrcioides* (Cambess.) O.Berg. All pictures were taken in the field during the collecting expeditions.

## 1.8 Research Questions

1. In Myrtaceae, what kind of development occurs during the ontogeny of secretory cavities?
2. Is lignin or suberin present in specialized cell walls of secretory cavities?
3. Do Myrteae secretory cavities store resins (diterpenes) besides low molecular weight terpenes (mono and sesquiterpenes)?
4. Have the secretory cavities of Myrteae flavonoids in their content, as proved recently to some *Eucalyptus* species?
5. Are carbonyl functional groups present in the contents of secretory cavities of Myrteae as evidence of ketones and aldehydes?

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