Instituto de Pesquisas Jardim Botânico do Rio de Janeiro
Escola Nacional de Botânica Tropical
Programa de Pós-graduação em Botânica

Tese de Doutorado

Anatomia foliar e da madeira de Primulaceae e seu significado filogenético

Bruna Nunes de Luna

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Instituto de Pesquisas Jardim Botânico do Rio de Janeiro
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Tese apresentada ao Programa de Pós-Graduação em Botânica da Escola Nacional de Botânica Tropical (Jardim Botânico do Rio de Janeiro) como parte dos requisitos necessários para a obtenção do título de Doutor em botânica.

Orientadora: Dr[a] Claudia Franca Barros
Co-orientadora: Dr[a] Maria de Fátima Freitas

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Dedicatória

À minha família, noivo e amigos.

Um tanto (Rodrigo Suricato)

Se eu fosse como o sol
Eu entraria na tua casa
Pra mostrar e aquecer
O coração que não acredita em nada

Acho que ainda não chorei
Tudo o que ganhei
Mas eu não durmo
Sem antes ter sonhado um tanto

Um tanto

Antes de virar senhor
Queria o mundo viajar
Conhecer histórias que farão de mim
Melhor em qualquer lugar

Acho que ainda não ganhei
Tudo o que rezei
Mas eu não durmo
Sem antes ter sonhado um tanto

Um tanto

Descobri que tudo que aprendi
Foi errando feio
Tanto tempo esperei pra ser feliz
Como agora mais de dentro que pra fora

Se eu fosse como o sol
Eu entraria na tua casa

Acho que ainda não ganhei
Tudo o que rezei
Mas eu não durmo
Sem antes ter sonhado um tanto

Um dia após o outro (Tiago Iorc)

Pra começar
Cada coisa em seu lugar
E nada como um dia após o outro
Por que apressar?
Se nem sabe onde chegar
Correr em vão se o caminho é longo
Quem se soltar, da vida vai gostar
E a vida vai gostar de volta em dobro
E se tropeçar
Do chão não vai passar
Quem sete vezes cai, levanta oito
Quem julga saber
E esquece de aprender
Coitado de quem se interessa pouco
E quando chorar
Tristeza pra lavar
Num ombro cai metade do sufoco
O novo virá
Pra re-harmonizar
A terra, o ar, água e o fogo
E sem se queixar
As peças vão voltar
Pra mesma caixa no final do jogo
Pode esperar
O tempo nos dirá
Que nada como um dia após o outro
O tempo dirá
O tempo é que dirá
E nada como um dia apos o outro

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Introdução geral

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Lista de siglas e abreviaturas

- µm – micrômetro
- BHCB - Herbário do Departamento de Botânica, Universidade Federal de Minas Gerais
- ca. – cerca de
- CEPEC – Herbário André Maurício Vieira de Carvalho, Centro de Pesquisas do cacau
- CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico
- EvoDevo – Evolução e desenvolvimento
- IC – Índice de consistência
- INPA – Herbário do Instituto Nacional de Pesquisas da Amazônia
- IR – Índice de retenção
- LCSM – Laser confocal scanning microscopy
- LM – Light microscopy
- MEV – Microscopia eletrônica de varredura
- mm – milímetros
- mm' – milímetro linear
- mm^2 - milímetros quadrados
- MO – Microscopia óptica
- NGS – Next generation sequencing
- p.ex. – por exemplo
- PCA – Principal components analysis
- RB – Herbário Barbosa Rodrigues, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro
- SEM – Scanning electron microscopy
- v - version

Resumo

Primulaceae, circunscrita na ordem Ericales, é uma família de distribuição pantropical, que abrange cerca de 2.500 espécies. Durante as últimas duas décadas, as relações filogenéticas em Ericales, especialmente no clado formado pelas anteriormente chamadas famílias Myrsinaceae, Theophrastaceae, Maesaceae e Primulaceae (grupo "primulóide"), foram alvo de estudo de muitas pesquisas que utilizaram dados morfológicos e moleculares combinados. Entretanto, as delimitações infragenéricas na família ainda permanecem incertas. Desta maneira, conduzir análises filogenéticas, utilizando dados morfológicos combinados com dados anatômicos é um passo significativo na resolução da delimitação infragenérica da família. Além do interesse em elucidar as relações de parentesco entre os gêneros e espécies, o presente trabalho levanta questões a respeito da morfologia e desenvolvimento de estruturas secretoras que ocorrem na folha e na madeira. Sendo assim, no presente estudo foram descritos e avaliados os caracteres anatômicos foliares e da madeira, indicando se há potencial para a caracterização de clados e táxons em vários níveis da hierarquia taxonômica. Para isto, foi analisada a anatomia foliar (Capítulo 1) e a anatomia da madeira (Capítulo 2) de espécies Neotropicais dos gêneros *Myrsine, Cybianthus, Ardisia* e *Stylogyne*, pertencentes à Myrsinoideae; e *Clavija* e *Jacquinia* de Theophrastoideae. Diante das análises realizadas nos primeiros capítulos, foram descritas e classificadas as estruturas secretoras foliares presentes na família (Capítulo 3). E, a partir do levantamento e classificação destes caracteres, foi elaborada uma hipótese filogenética para as espécies Neotropicais de Primulaceae (Capítulo 4). Quanto à anatomia foliar, a subfamília Myrsinoideae pode ser caracterizada pela presença de cavidades/ductos secretores, enquanto Theophrastoideae destaca-se pela presença de feixes de fibras percorrendo o mesofilo e pela presença de hipoderme. *Cybianthus* diferencia-se dos demais gêneros pela presença de estômatos paracíticos, *Myrsine* pela presença de um feixe adicional sobre o sistema vascular no pecíolo, *Stylogyne* e *Ardisia* pelo mesofilo fracamente dorsiventral e *Jacquinia* pela presença de feixes de fibras ao longo de todo o mesofilo e pela presença de esclerênquima na margem da folha. A anatomia da madeira, embora com diferenças não tão evidentes, também auxiliou a segregação dos gêneros. Em *Cybianthus*, as espécies do subgênero *Weilgetia* se distanciaram das demais pela ausência de raios em *C. densiflorus* e presença de placa de perfuração escalariformes em *C. nemoralis*. Quanto às estruturas secretoras, foram identificadas na folha: cavidades/ductos secretores, hidatódios, tricomas glandulares, idioblastos. O desenvolvimento das cavidades/ductos e tricomas é assincrônico e se inicia antes da diferenciação dos demais tecidos foliares. A

secreção das cavidades/ductos é quimicamente diversa. Apesar do alto grau de homoplasia, os caracteres anatômicos da folha e da madeira foram capazes de recuperar o monofiletismo das subfamílias Neotropicais de Primulaceae, além de conferirem suporte aos ramos e explicar o monofiletismo de *Cybianthus* e seus subgêneros e de *Myrsine*. Os gêneros *Ardisia* e *Stylogyne* não emergiram monofiléticos, o que reflete a sua proximidade morfológica e recente diversificação.

Palavras-chave: anatomia vegetal, estruturas secretoras, ontogenia, filogenia, Myrsinaceae;

Abstract

Primulaceae, order Ericales, is a pantropical family with approximately 2.500 species, with herbs, shrubs and trees. In the past 20 years, many researchers using both morphological and molecular characters extensively studied phylogenetic relationships in Erilcales, especially in the clade formed by Myrsinaceae, Theophrastaceae, Maesaceae and Primulaceae ("primuloid group"). However, the generic boundaries in the family are still incipient. Thus, lead phylogenetic approach combining morphological and anatomical data is a significant step in the infrageneric delimitation of the family. Apart from the interest in understand phylogenetic relationships among genera and species, this work raises questions about morphology and development of secretory structures that occur in both leaves and xylem. In this sense, in the present study there were described and evaluated the leaf and wood anatomical characters, indicating of there is any potential use for clades and taxons classification in many hierarchical taxonomical levels. For this, the leaf anatomy (Chapter 1) and the wood anatomy (Chapter 2) of Neotropical species of the genus *Myrsine*, *Cybianthus*, *Ardisia* and *Stylogyne* (Myrsinoideae); and *Clavija* and *Jacquinia* (Theophrastoideae) were analyzed. From the analyses of the previous chapters, the secretory structures present in the family were described and classified (Chapter 3). And, from the survey and classification of these characters, a phylogenetic hypothesis was proposed for the Neotropical species of Primulaceae (Chapter 4). In relation to the leaf anatomy, Myrsinoideae can be characterized by the presence of secretory cavities/ducts, while Theophrastoideae is distinguished by the presence of extraxylary fibres, hypodermis and marginal sclerenchyma. *Cybianthus* differs from other genera by the presence of paracytic stomata, *Myrsine* by the presence of an additional bundle above the vascular system in the petiole, *Stylogyne* and *Ardisia* by the weakly dorsiventral mesophyll and *Jacquinia* by the presence of bands of extraxylary fibers throughout the mesophyll and by the presence marginal sclerenchyma. In the wood anatomy, although with not so marked differences, also aided the genera segregation. In *Cybianthus*, the species of the subgenus *Weilgetia* distanced themselves from the other species by the absence of rays in *C. densiflorus* and presence of scalariform perforation plates in *C. nemoralis*. In relation to the secretory structures, there were identified in the leaves: secretory cavities/ducts, hydathodes, glandular trichomes and idioblasts; and in the xylem, there were found: breakdown areas in rays. The development of the secretory structures is asynchronous and starts even before the whole maturation of the leaf and the differentiation of the other tissues. Leaf and wood anatomical characters were able to recover the monophyly of the Neotropical Primulaceae

subfamilies, and despite the high degree of homoplasy of these characters, they gives support to the monophyly of *Myrsine* and *Cybianthus* and its subgenera. *Ardisia* and *Stylogyne* did not emerge monophyletic, which probably reflects their recent diversification and morphological sobreposition.

Key words: Plant anatomy; secretory structures; phylogeny; ontogeny; Myrsinaceae.

PREFÁCIO

A presente tese encontra-se dividida em uma introdução geral, quatro capítulos, conclusões e anexos. A introdução geral abrange o tema de estudo e as diferentes abordagens e conceitos que permeiam a discussão dos resultados, enfatizando a relevância do trabalho.

O primeiro capítulo corresponde ao primeiro artigo, aceito para publicação no periódico *International Journal of Plant Sciences*, e discorre sobre a anatomia foliar de 5 gêneros neotropicais de Primulaceae através de um viés sistemático, taxonômico e filogenético.

O segundo capítulo e artigo fornece a descrição anatômica da madeira de espécies de Primulaceae, com discussões a respeito do significado ecológico, taxonômico e filogenético dos caracteres anatômicos. Este trabalho será submetido ao periódico *Botanical Journal of the Linnean Society*.

O terceiro artigo apresenta os resultados da descrição das estruturas secretoras foliares e ontogenia das cavidades e tricomas secretores das espécies estudadas. Este trabalho será submetido ao periódico *Plant Biology*.

O quarto capítulo apresenta a hipótese filogenética baseada nos dados morfo-anatômicos levantados nos capítulos anteriores. Também foi realizado o mapeamento das estruturas anatômicas da folha e madeira. Nas conclusões gerais são sumarizados todos os dados levantados nos quatro capítulos.

Por fim, são apresentados os anexos, que abrangem: 1) as coletas realizadas durante os anos de 2013 a 2016; 2) os artigos publicados durante o doutorado, que tem relação com o tema de estudo; 3) os artigos aceitos para publicação; e 4) os trabalhos de conclusão de curso que foram desenvolvidos em parceria com alunas de graduação da Universidade Federal do Estado do Rio de Janeiro (UNIRIO), sob supervisão da Profa. Dra. Alice Sato.

INTRODUÇÃO GERAL

Ontogenia e filogenia

A biologia evolutiva do desenvolvimento (EvoDevo) busca compreender a evolução da diversidade morfológica dos organismos. Sendo assim, uma das principais ferramentas utilizadas em estudos evolutivos é a comparação de eventos ontogenéticos entre espécies filogeneticamente relacionadas (Gould 1977; McKinney & McNamara 1991; Friedman *et al.* 2004). A evolução morfológica em vegetais é resultado de alterações que ocorrem durante o seu processo de desenvolvimento, sendo a heterocronia - a mudança na taxa ou tempo do processo de desenvolvimento de um descendente em relação ao seu ancestral – uma das possíveis maneiras de caracterizar estas modificações (Gould 1977, 1992; Li & Johnston 2000).

Define-se ontogenia, como a sequência de estágios ou eventos que ocorrem durante o desenvolvimento do organismo (Gould 1977), ou ainda, como o desenvolvimento de células e tecidos compreendido desde o estágio embrionário, até a maturidade (Gifford & Foster 1989). Uma vez que as diferenças entre as características morfológicas de cada organismo estão relacionadas à sua ontogenia, análises comparativas sobre esse aspecto, tendo como plano de fundo o contexto filogenético, permitem que sejam inferidos os possíveis mecanismos que geram a diversidade morfológica entre os organismos (Li & Johnston 2000).

Essas premissas têm conduzido estudos evolutivos de diferentes estruturas vegetais (reprodutivas e vegetativas), como as das partes florais, os tipos de inflorescência (p.ex.: Otegui & Cocucci 1999, Anderberg *et al.* 2007, Schönenberger *et al.* 2005, Burke *et al.* 2010, Simon *et al*. 2011), os nectários florais (p.ex.: Simon *et al.* 2011), os frutos, as sementes (p.ex.: Jacobs *et al.* 2009, Pabón-Mora & Litt 2011), o padrão de nervação foliar (p.ex.: Roth-Nebelsick *et al.* 2001), as estruturas secretoras: tricomas glandulares (Nogueira *et al.* 2013), idioblastos, laticíferos e coléteres (Vitarelli *et al.* 2015; Feio *et al.* 2016); e as estruturas anatômicas da madeira (p.ex.: Olson 2007, Pace *et al.* 2009).

Estruturas secretoras e Primulaceae

Estruturas secretoras vegetais são sítios de secreção e/ou armazenamento de substâncias provenientes do metabolismo secundário das células vegetais (Fahn 1979). Toda célula vegetal é potencialmente secretora, uma vez que os componentes da parede celular vegetal, ceras, cutícula, lignina e suberina resultam de um processo de secreção.

A própria formação da parede celular envolve um processo de secreção, com participação de uma maquinaria celular específica ao processo secretor (Mauseth 1988; Evert

2006). Entretanto, muitas plantas desenvolveram estruturas específicas de secreção. Essas estruturas variam quanto à localização, morfologia e função e o principal sistema de classificação, proposto por Fahn (1988), leva em consideração esses parâmetros. Desta maneira, as estruturas secretoras, quanto à localização, podem ser: (*a*) internas ou (*b*) externas; quanto à função, podem ser (*a*) atrativas ou (*b*) de defesa (repelentes); e quanto à morfologia podem possuir as mais variadas formas, como os tricomas (peltados ou pluricelulares), cavidades e ductos secretores, laticíferos (articulados ou não articulados), coléteres, nectários (florais ou extraflorais) e hidatódios (Fahn 1988, Evert 2006).

A variabilidade morfológica reflete a diversidade de metabólitos secretados por estes tecidos. Substâncias da mesma natureza química podem ser secretadas por estruturas distintas, por exemplo, tricomas glandulares e cavidades secretoras como sítios de produção de óleos essenciais. A secreção pode não sofrer muitas modificações ao longo do processo secretor, p.ex. em hidatódios, estruturas que secretam água e solutos provenientes do tecido xilemático, ou pode ser inteiramente sintetizada por células ou tecidos secretores (Fahn 1979). Neste último caso de secreção de substâncias complexas, podem-se citar as resinas, gomas, mucilagens, óleos e látex (Langenheim 2003). Esses metabólitos secundários, produtos do metabolismo vegetal, desempenham diferentes funções no organismo.

Todas as 32 ordens reconhecidas de eudicotiledôneas (APG III 2009, APG IV 2016) circunscrevem espécies, famílias ou clados, que apresentam algum tipo de estrutura secretora (p.ex.: Bottega & Corsi 2000, Cicarelli *et al.* 2001, Cury & Appezzato-da-Glória 2009, Corsi & Biasci 1998, Gomes & Lombardi 2010, Klein *et al.* 2004, Kalachanis & Psaras 2005, Kim & Mahlberg 2000, Liang *et al.* 2009, Machado *et al.* 2014, Silva *et al.* 2016, Miguel *et al*. 2016, Otegui & Maldonado 1998, Rocha *et al.* 2011, Sadala-Castilho *et al.* 2016, Teixeira & Rocha 2009, Thadeo *et al.* 2009, Tolera *et al*. 2013). Sabe-se, portanto, que um mesmo tipo de estrutura ocorre em ordens distintas, que não apresentam relação filogenética direta. Como por exemplo, as cavidades secretoras encontradas em *Copaifera langsdorffii* Desf. e *Copaifera trapezifolia* Hayne (Leguminosae-Caesalpinoideae- Fabales, respectivamente, Rodrigues *et al.* 2011 e Milani *et al.* 2012), e as de *Myrsine laetevirens* (Mez) Arechav. (Primulaceae-Myrsinoideae- Ericales, Otegui *et al.* 1998). Em ambos os casos o processo de formação das cavidades e a morfologia dessas estruturas são semelhantes, entretanto, as espécies circunscritas em Fabales estão classificadas hierarquicamente no cladograma das relações filogenéticas entre as Rosídeas, enquanto as de Ericales estão agrupadas entre as Asterídeas (Judd *et al.* 2009).

Na maior compilação de dados sobre estas estruturas, Fahn (1979) reúne algumas considerações evolutivas acerca de nectários, ductos e tricomas glandulares. Contudo, não se sabe como a evolução tem atuado na formação de estruturas com alto grau de diferenciação morfológica.

Ericales está inserida no clado das asterídeas e apresenta monofiletismo bem suportado (Bremer *et al.* 2002; Schönenberger *et al.* 2005). A ordem circunscreve 22 famílias e 346 gêneros (Fig. 1; APG IV 2016) e agrupa famílias que apresentam grande diversidade de estruturas secretoras, o que a torna um bom modelo de estudo. Roridulaceae, por exemplo, engloba espécies insetívoras, que apresentam adaptações estruturais para a captura de pequenos organismos. Dentre essas, destacam-se os tricomas que secretam substâncias mucilaginosas para a apreensão das presas e os nectários extraflorais que atraem as presas (Anderson 2005). Sapotaceae, agrega representantes que contêm laticíferos (Farrel *et al.* 1991), enquanto Primulaceae circunscreve as espécies que apresentam a maior diversidade de estruturas secretoras (Otegui *et al.* 1998).

Figura 1 - Famílias subordinadas à ordem Ericales. Primulaceae. Os ramos marcados são os que apresentam 100% de suporte Bayesiano. Extraído do *Angiosperm Phylogeny Website* [http://www.mobot.org/MOBOT/research/APweb/. Acessado em 17/08/2015z]

Filogenia, anatomia e Primulaceae

Primulaceae, subordinada à ordem Ericales (APG III 2009 e APG IV 2016), apresenta distribuição pantropical. Seus representantes, cerca de 2500 espécies agrupadas em 58 gêneros, apresentam porte arbóreo (Fig. 3A), arbustivo (Fig. 3B-C), sub-arbustivo (Fig. 3D), ou herbáceo. No Brasil ocorrem 140 espécies e 12 gêneros (BFG 2015, Freitas *et al.* 2017). As relações filogenéticas na família têm sido foco de estudo de muitos grupos de pesquisa, que utilizam dados morfológicos e/ou moleculares, destes, sequências de genes plastidiais (*atpB*, *ndhF*, *rbcl* e t*rnL-F*), nucleares (ITS) e mitocondriais (*atp1* e *matR*) (Anderberg & Stahl 1995, Morton *et al.* 1996, Anderberg *et al.* 1998, 2000, 2002; 2007; Caris & Smets 2004, Oh *et al.* 2008); e, atualmente, os sequenciamentos por próxima geração (NGS – Zhang *et al.* 2016).

A partir do APG III (2009) as famílias Myrsinaceae, Theophrastaceae e Maesaceae foram subordinadas à Primulaceae, com as subfamílias: Maesoideae, Myrsinoideae, Primuloideae e Theophrastoideae, formando um clado monofilético (Fig. 1). Maesoideae apresenta distribuição paleotropical, Theophrastoideae é neotropical, Primuloideae ocorre na região holoártica e Myrsinoideae, a mais amplamente distribuída, com ocorrência pantropical (APG III – Figura 2).

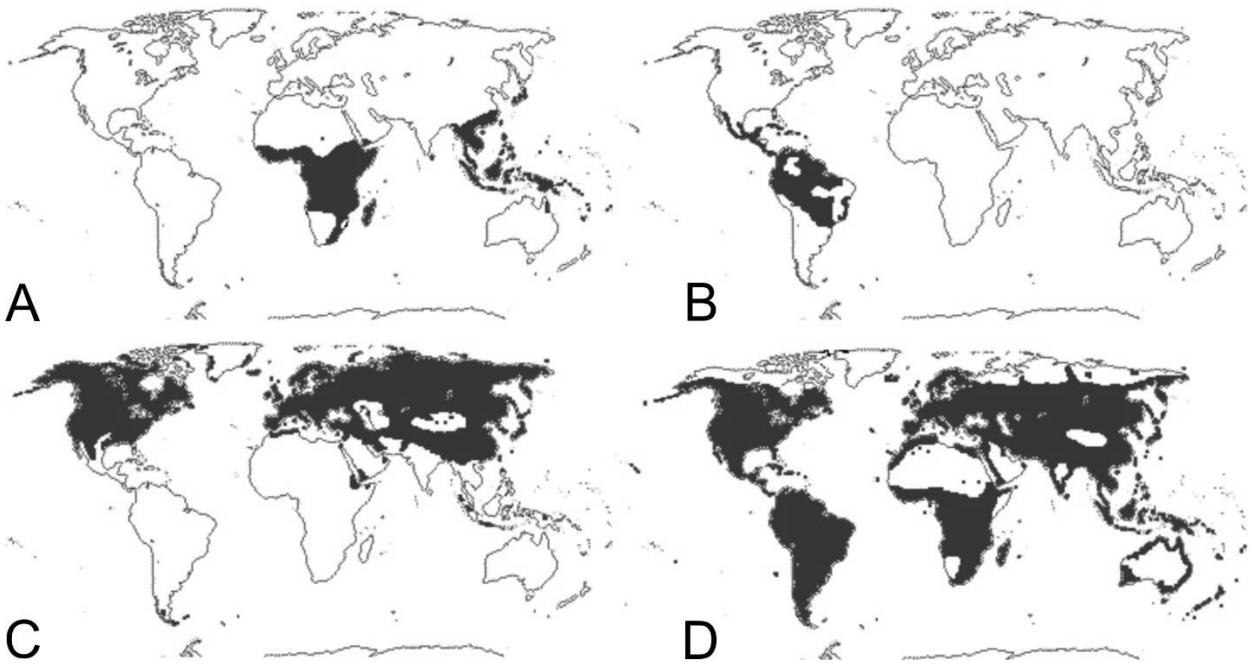

Figura 2 – Mapas de distribuição das subfamílias subordinadas à Primulaceae. A – Maesoideae. B – Theophrastoideae: Theophrasteae. C – Primuloideae. D – Myrsinoideae. [Extraído do *Angiosperm Phylogeny Website* [http://www.mobot.org/MOBOT/research/APweb/].

O monofiletismo desta família é sustentado, dentre outros caracteres, pela presença de cavidades ou canais secretores (Fig. 3E-G) e pela placentação central-livre com um eixo espesso, geralmente globoso. O clado monofilético formado por *Primula* L. + *Lysimachia* L. +

Myrsine L. pode ser subdividido em dois subclados: Primuloideae, que são plantas herbáceas sem cavidades secretoras e Myrsinoideae, que compreende os gêneros que apresentam cavidades secretoras (APG IV 2016). O subclado Primuloideae tem distribuição restrita às regiões temperadas, enquanto o subclado Myrsinoideae tem distribuição pantropical (Källersjö *et al.* 2000).

As substâncias secretadas pelas estruturas secretoras em Primulaceae apresentam interesse farmacológico, devido, por exemplo, à comprovada ação antinociceptiva (Hess *et al*. 2010), anti-inflamatória (Hirota *et al*. 2002, Makabe *et al*. 2003), anti-leishmaniose (Germonprez *et al.* 2004, Vermeersch *et al.* 2009), anti-microbial e anti-inflamatório (López *et al.* 2011), anti-helmíntica (Challam *et al.* 2010), anti-inflamatório e anti-espasmódico (Ahmad *et al.* 2011, Azam *et al.* 2011, Mizushina *et al.* 2000), ao combate à linhagens de células de câncer (Ndonsta *et al.* 2011).

Apesar da importância taxonômica e farmacêutica, o estudo de estruturas secretoras em Primulaceae ainda é escasso. Otegui & Maldonado (1998) elencaram características anatômicas foliares diagnósticas, incluindo tricomas glandulares, para espécies de *Myrsine* da América do Sul. Otegui *et al.* (1997) analisaram as cavidades secretoras observando os seus aspectos ultraestruturais, associados à secreção de hidroxibenzoquinonas. Os mesmos autores atentam para a necessidade de investigação em outras espécies de Primulaceae para a elucidação do processo de formação das cavidades secretoras. Luna *et al.* (2014) analisaram o desenvolvimento das estruturas secretoras e a composição do óleo essencial das folhas de *M. coriacea* e *M. venosa* A. DC.

Os estudos anatômicos têm proporcionado relevantes informações às pesquisas taxonômicas, filogenéticas e ecológicas, por exemplo, Macedo *et al.* (2014) verificaram a eficiência das faixas de fibra semelhante a parênquima para a segregação de espécies de *Tachigali* (Leguminosae); Pace *et al.* (2014) identificaram diversas sinapomorfias anatômicas na madeira que suportam gêneros da família Bignoniaceae. Especificamente, o uso de atributos da anatomia da madeira para fins filogenéticos tem permitido a obtenção de resultados consistentes, que comprovam a eficiência desses caracteres quando comparados com filogenias baseadas em dados moleculares (Olson 2002, Lens *et al.* 2007, 2012).

Em Ericales, muitos estudos validam a significância dos caracteres anatômicos da madeira para a sistemática do grupo, uma vez que fornecem informações que estão em concordância com as hipóteses filogenéticas apresentadas para o grupo (em Ericales - Lens *et al.* 2007, em Primuloideae – Lens *et al.* 2005a, em Balsaminoideae – Lens *et al.* 2005b). Lens *et al.* (2007) reúnem dados sobre a anatomia da madeira de 52 espécies de Ericales, e os

combinam com os dados moleculares da Ordem gerados por Schönenberger *et al.* (2005), a fim de demonstrar o valor sistemático de caracteres da madeira, destacando a importância de se conduzir estudos nessa área, e concluem que os caracteres da madeira foram informativos quando combinados aos moleculares. No trabalho supracitado utilizou-se apenas uma espécie de cada gênero para representá-lo.

Além disso, alguns autores têm utilizado aspectos da anatomia foliar para a segregação de espécies e táxons, como por exemplo: Diane *et al.* (2003), em espécies de Heliotropiaceae; Matias *et al.* (2007), em espécies de *Echinodorus* Rich. (Alismataceae); e Oliveira *et al.* (2011), em *Campomanesia* Ruiz & Pav. (Myrtaceae). A ornamentação epicuticular, por exemplo, é uma das características epidérmicas mais informativas à taxonomia em folhas (p.ex: Solereder 1908; Metcalfe & Chalk 1979, Barthlott *et al.* 1998; Moraes *et al.* 2011), e tem sido utilizada na identificação de espécies, por exemplo, em *Trifolium* L. – Fabaceae (Zoric *et al.* 2009) e em *Posoqueria* Aubl. – Rubiaceae (Arruda *et al.* 2010).

Em Primulaceae, Freitas (2003) utilizou caracteres anatômicos das folhas como recurso adicional à taxonomia de espécies de *Myrsine* do sul e sudeste do Brasil, observando diferenças significativas, principalmente, em relação à ornamentação da cutícula na epiderme foliar. Luna *et al.* (2013) e Carrijo (2011) identificaram caracteres da anatomia foliar diagnósticos para a segregação de quatro espécies de *Stylogyne* A.DC., gênero neotropical de Primulaceae (Myrsinoideae), dentre os quais destacam-se a forma do sistema vascular no pecíolo, a coloração e forma das cavidades secretoras, e a ornamentação epicuticular.

A anatomia vegetal em Primulaceae é utilizada para elucidar diferentes questões, a saber: a) taxonômicas, por exemplo, para esclarecer a distinção entre espécies de um mesmo gênero (Solereder 1908, Metcalfe & Chalk 1950, Otegui 1998, Luna *et al.* 2013); b) filogenéticas (Lens *et al.* 2007); c) de origem e desenvolvimento de estruturas secretoras (Luna *et al.* 2014); e d) de origem e desenvolvimeto de peças florais (Caris & Smets 2004). Na Tabela 1 são apresentados alguns dos principais trabalhos aplicando dados anatômicos vegetativos e reprodutivos Primulaceae para resolver algumas dessas questões.

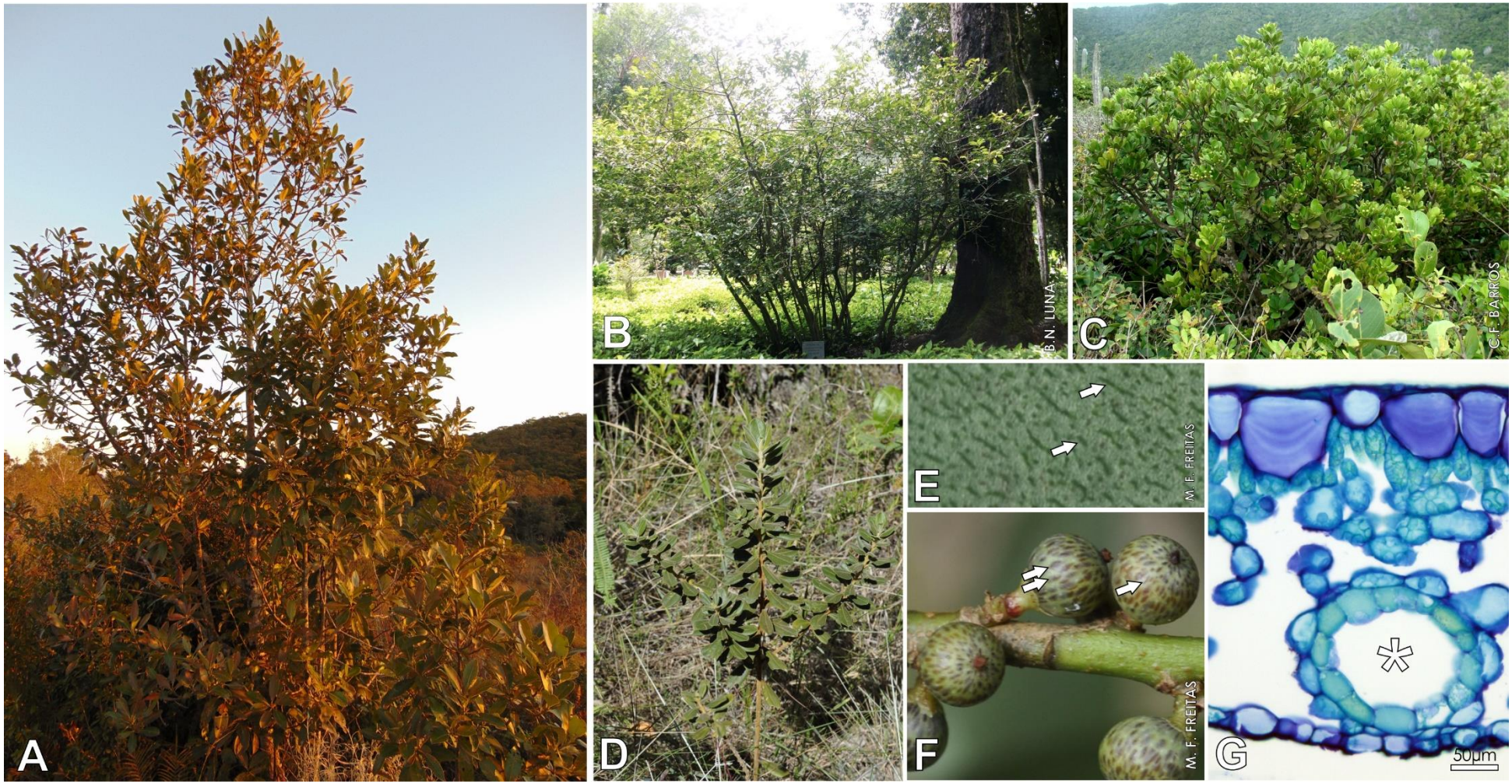

Figura 3 – Aspectos gerais de Myrsinoideae (A, B, D-G) e Theophrastoideae (C). A- Hábito arbóreo de *Myrsine gardneriana*. B – Hábito arbustivo de *Ardisia humilis*. C – Hábito arbustivo de *Jacquinia armillaris*. D– Hábito sub-arbustivo de *M. glazioviana*. E – Estruturas secretoras vistas à olho nu na face abaxial das folhas de *M. lineata*. F – Estruturas secretoras nos frutos (seta branca). G – Secção transversal do terço médio da folha de *M. umbellata* evidenciando a cavidade secretora no mesofilo (*).

Tabela 1 - Estudos anatômicos em Primulaceae: gêneros e número de espécies analisados, abordagem do trabalho e autores.

Gêneros(s) analisado(s) e número de spp.	**Abordagem**	**Autor**
	Utilização de dados anatômicos para propor a filogenia da ordem	Anderberg & Stahl (1995)
Aegiceras (1)	Glândulas de sal em *Aegiceras corniculatum* (L.) Blanco	Cardale *et al.* (1971)
Samolus	Ontogenia floral associada à delimitação do posicionamento do gênero	Caris & Smets (2004)
Maesa (3)	Ontogenia floral associada à delimitação do posicionamento do gênero	Caris *et al.* (2000)
Primula (4)	Micromorfologia dos tricomas glandulares	Colombo *et al.* (2014)
Ardisia (3) *Aegiceras* (1) *Embelia* (3) *Maesa* (2) *Myrsine* (2)	Células perfuradas do raio	Dayal *et al.* (1984)
Primula (1)	Micromorfologia dos tricomas glandulares	Fico *et al.* (2007)
27 gêneros	Anatomia comparada da madeira do clado "primulóide"	Lens *et al.* (2005a)
	Anatomia da madeira aplicada à filogenia	Lens *et al.* (2007)
Lysimachia (1)	Ontogenia das cavidades secretoras	Lersten (1986)
Stylogyne (4)	Anatomia foliar aplicada à taxonomia	Luna *et al*. (2013)
Myrsine (2)	Ontogenia das estruturas secretoras foliares	Luna *et al.* (2014)
Maesa *Aegiceras* *Embelia*	Ontogenia floral e implicações taxonômicas e filogenéticas	Ma & Saunders (2003)
Primula (1)	Adaptação anatômica da raiz	Micco & Aronne (2012)
Androsace (1) *Cortusa* (1) *Hottonia* (1) *Primula* (1) *Soldanella* (1) *Anagallis* (2) *Cyclamen* (1) *Glaux* (1) *Lysimachia* (2) *Trientalis* (1) *Samolus* (1)	Micromorfologia das sementes e estrutura do endosperma | dados aplicados à sistemática	Morozowska *et al.* (2011)
Myrsine (2)	Células perfuradas de raio	Otegui (1994)
Myrsine (1)	Análise das estruturas associadas à produção de hidroxibenzoquinonas	Otegui *et al.* (1997)
Myrsine (1)	Caracterização anatômica da semente	Otegui *et al.* (1998)
Myrsine (1)	Morfologia floral	Otegui *et al.* (1999)
Myrsine (1)	Anatomia da madeira	Pinheiro & do Carmo (1993)
Clavija *Jacquinia*	Anatomia foliar associada à taxonomia	Ståhl (1989)
Maesa (2)	Anatomia da folha, flor e fruto aplicada à sistemática	Utteridge (1998)
Primula (1)	Micromorfologia das folhas e análise das estruturas associadas à produção de flavonóides	Vitalini *et al.* (2011)
Stimpsonia (1) *Ardisiandra* (1)	Ontogenia floral aplicada à sistemática	Wanntorp *et al.* (2012)

HIPÓTESES

- A anatomia foliar e da madeira fornecem dados informativos à taxonomia e filogenia de Primulaceae;
- A etapa inicial de desenvolvimento das cavidades secretoras e dos tricomas glandulares é igual em todas as espécies;
- A ontogenia, tendo o contexto filogenético como base, permite a inferência dos mecanismos que geraram a variedade das estruturas secretoras;
- As subfamílias e os gêneros de Primulaceae formam grupos monofiléticos;
- O trajeto evolutivo das estruturas secretoras em Primulaceae deve ser percorrido de forma similar em todos os clados que contêm estruturas morfologicamente semelhantes;

OBJETIVOS

Geral

- Identificar os caracteres anatômicos úteis à taxonomia e filogenia de espécies lenhosas Neotropicais de Primulaceae e prover uma hipótese filogenética para as espécies lenhosas Neotropicais de Primulaceae, baseada nos dados anatômicos da foha e da madeira;

Específicos

- Descrever a anatomia da madeira e da folha de espécies lenhosas Neotropicais de Primulaceae;
- Identificar os caracteres diagnósticos anatômicos nas folhas e madeira;
- Caracterizar e identificar as estruturas secretoras foliares e descrever o processo de desenvolvimento das cavidades/ductos secretores e dos tricomas glandulares;
- Comparar a ontogênese das estruturas secretoras com as descritas para espécies de outros gêneros da família Primulaceae e caracterizar o processo de formação dos tricomas glandulares em diversos estágios de desenvolvimento foliar;
- Caracterizar histoquimicamente a composição química da secreção produzida pelas cavidades secretoras;
- Reconstruir a evolução dos estados de caráter da anatomia da folha e da madeira;

ÁREAS DE ESTUDO E MATERIAL BOTÂNICO

Os táxons foram selecionados de modo a abranger a maior variação morfológica possível dentre os gêneros Neotropicais de Primulaceae, contemplando os táxons lenhosos da família. As espécies coletadas foram selecionadas com base nos locais de ocorrência e disponibilidade de coleta. Desta maneira, foram selecionados seis gêneros: *Ardisia*, *Clavija*, *Cybianthus*, *Jacquinia*, *Myrsine* e *Stylogyne*, abrangendo as subfamílias Myrsinoideae e Theophrastoideae. As coletas de folha e lenho foram realizadas entre março de 2013 a junho de 2016 (Anexo I). Além disso, foram utilizadas amostras coletadas pela Dra. Maria de Fátima Freitas. As espécies coletadas estão sumarizadas na Tabela 2 e o mapa com os locais de coleta é apresentado na Figura 4. As subfamílias Primuloideae e Maesoideae não ocorrem no Neotrópico, desta maneira, não foram amostradas para o desenvolvimento deste trabalho.

As amostras foram processadas no Laboratório de Botânica Estrutural (LBE) do Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Os testemunhos foram depositados no Herbário Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (RB), e as amostras do lenho e as lâminas permanentes foram depositadas na Xiloteca do Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (RBw) e as lâminas de folhas e ontogenia serão depositadas no laminário do LBE.

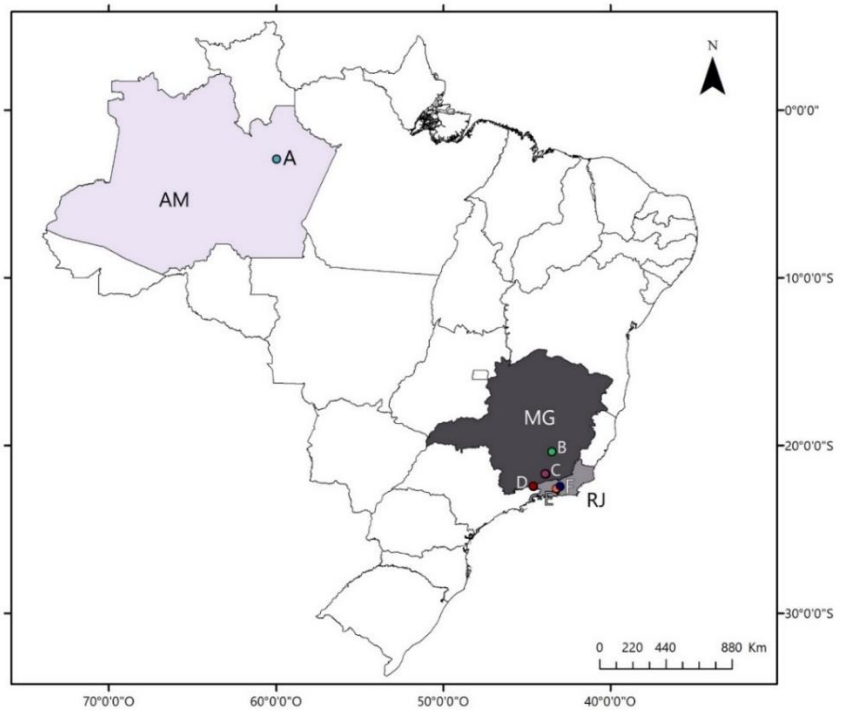

Figura 4 – Mapa com alguns dos locais de coletas realizadas entre 2013-2016. A – Reserva Florestal Adolfo Ducke (AM); B – Parque Nacional do Caparaó e APA Andorinhas (MG); C – Parque Estadual do Ibitipoca (MG); D – Parque Nacional do Itatiaia (RJ); E – Bosque da Barra e Restinga Grumari (RJ); F - Parque Estadual Costa do Sol (RJ).

Na Figura 5 são apresentadas as etapas metodológicas desenvolvidas. A metodologia detalhada é apresentada separadamente em cada capítulo.

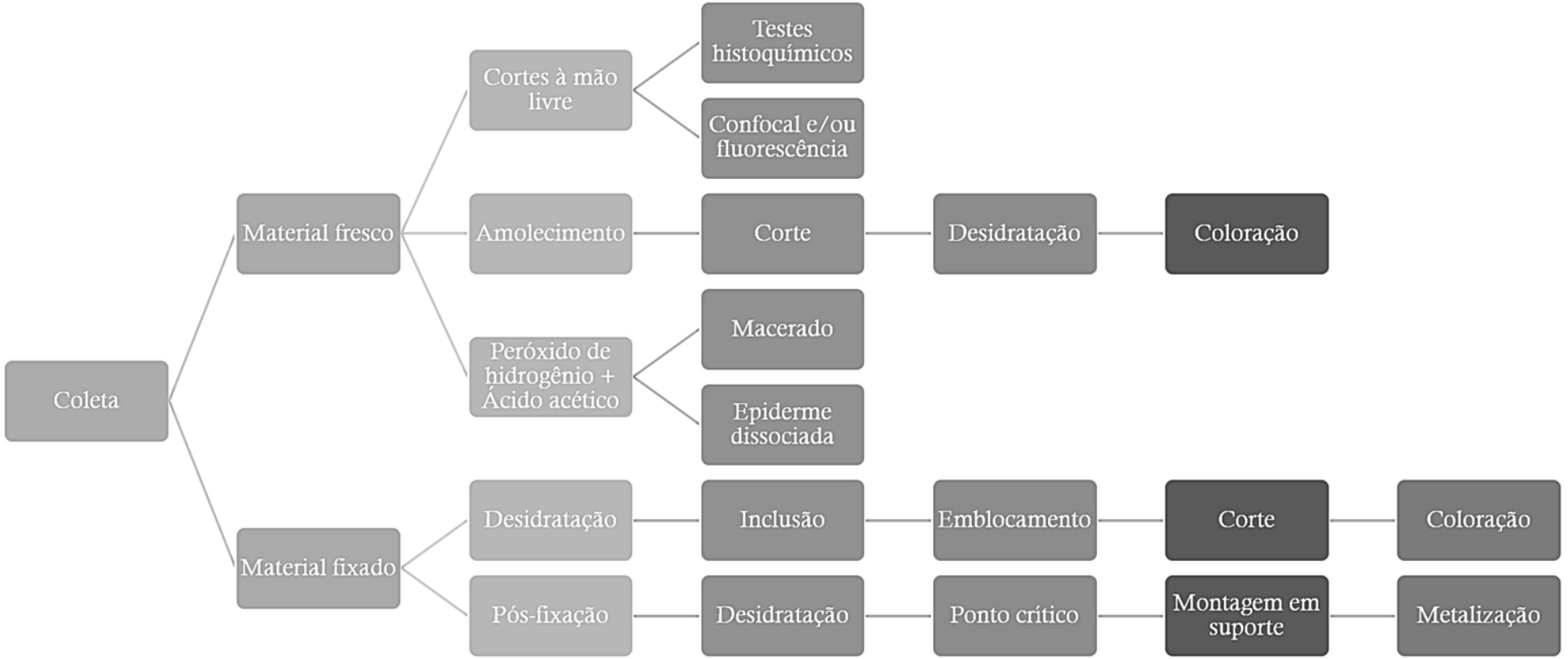

Figura 5 – Esquema dos procedimentos anatômicos desenvolvidos.

Tabela 2 – Espécies de Primulaceae selecionadas e locais de coleta. PE – Parque Estadual; PM – Parque Municipal; PN – Parque Nacional.

Espécies	Local de coleta	Habitat	Hábito	Voucher	RBw	Slide collection
Myrsinoideae						
***Ardisia guianensis* (Aubl.) Mez**	Teresópolis - RJ	Mata Atlântica | Floresta ombrófila	Arbusto	RB 462085	-	-
***Ardisia humilis* Vahl**	Arboreto JBRJ – RJ	Mata Atlântica | Floresta ombrófila	Arbusto	RBv 2014	10303	2722
***Ardisia solanacea* Roxb.**	Arboreto JBRJ – RJ	Mata Atlântica | Floresta ombrófila	Arbusto	RBv 2094	10304	2723
		Mata Atlântica | Floresta ombrófila	Arbuto	RBv 2199	-	-
		Mata Atlântica | Floresta ombrófila	Arbusto	RBv 2091	-	-
***Cybianthus brasiliensis* (Mez) G. Agostini**	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Floresta ombrófila	Arbusto/Arvoreta	RB 605169	10305	2800
***Cybianthus densiflorus* Miq.**	Reserva Ducke – AM	Floresta Amazônica	Sub arbusto	INPA 191486	10312	2809
***Cybianthus glaber* A. DC.**	Parque Nacional do Itatiaia – RJ	Mata Atlântica | Floresta ombrófila	Arbusto monopodial	RB 636647	10173	2772
		Mata Atlântica | Floresta ombrófila	Arbusto monopodial	RB 636645	-	-
***Cybianthus guyanensis* (A. DC.) Miq.**	Reserva Ducke – AM	Floresta Amazônica	Arbusto	INPA 178624	10306	2808
	Reserva Ducke – AM	Floresta Amazônica	Arbusto	INPA 178964	10307	2776
	Reserva Ducke – AM	Floresta Amazônica	Arvoreta	INPA 190110	10308	2777
	Reserva Ducke – AM	Floresta Amazônica	Arvoreta	INPA 178628	10309	2810
	Reserva Ducke – AM	Floresta Amazônica	Arbusto	INPA 208941	10310	2779
	Reserva Ducke – AM	Floresta Amazônica	Arbusto	-	10311	2826
***Cybianthus nemoralis* (Mez) G. Agostini**	Ilhéus- BA	Mata Atlântica | Mata higrófila	Arbusto	CEPEC 93447	10316	2832
***Cybianthus verticillatus* (Vell.) G. Agostini**	Parque Nacional do Itatiaia – RJ	Mata Atlântica | Floresta ombrófila	Arbusto monopodial	RB 586284	-	-
***Cybianthus venezuelanus* Mez**	Reserva Ducke – AM	Floresta Amazônica	Sub-arbusto	INPA 190119	10315	2775
***Myrsine balansae* Mez (Otegui)**	Mauá da Serra - PR	-	Árvore	UEC 115510	-	-
	Mauá da Serra - PR	-	Árvore	UEC 155521	-	-
***Myrsine congesta* Sw. (Pipoly)**	Mina do Sapo - MG	-	Sub-arbusto	BHCB 153534	-	-

***Myrsine coriacea* (Sw.) R. Br. Ex Roem. & Schult.**	Estrada para Quatis-Fumaça - RJ	Mata Atlântica | Floresta secundária	Árvore	RB 583202	-	-
	Parque Nacional da Serra dos Órgãos - RJ	Mata Atlântica | Floresta ombrófila	Árvore	RB 582964	-	-
	Arboreto JBRJ	Mata Atlântica	Árvore	RB 380806	-	-
	Estrada para Paraty – RJ	Mata Atlântica | Floresta secundária	Árvore	RB 583204	10321	2726
	Paraty - RJ	Mata Atlântica | Floresta secundária	Árvore	-	10322	2727
	Parque Nacional do Caparaó - MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 609489	10323	2801
	Bosque da Barra – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10324	2818
***Myrsine emarginella* Miq.**	Serra de Itacolomi - MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 380828	-	-
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Árvore	RB 605187	10325	2803
***Myrsine gardneriana* A. DC.**	Fazenda Conquista - PR	-	Árvore	RB 380621	-	-
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Arbusto	-	10326	2796
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605197	10327	2825
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605202	10331	2797
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Árvore	RB 609476	10328	2814
	Parque Nacional do Caparaó - MG	Mata Atlântica | Floresta ombrófila	Arbusto	RB 609490	10329	2815
	Parque Nacional do Caparaó - MG	Mata Atlântica | Floresta ombrófila	Arbusto	RB 609497	10330	2813
***Myrsine glazioviana* Warm.**	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Sub-arbusto	RB 695201	-	-
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Sub-arbusto	RB 605184	10332	2782
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Sub-arbusto	RB 605189	10333	2794
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Campo rupestre	Sub-arbusto	RB 605198	10334	2795
***Myrsine guianensis* (Aubl.) Kuntze**	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	-	10335	-
	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	RB 583201	10336	2771

***Myrsine guianensis* (Aubl.) Kuntze**	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Pontal do Atalaia	Árvore	RB 583200	10337	2729
	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Pontal do Atalaia	Arvoreta	-	10338	2728
	Restinga Grumari – RJ	Mata Atlântica | Restinga	Arvoreta	-	10339	2731
	Restinga Grumari – RJ	Mata Atlântica | Restinga	Arvoreta	-	10340	2732
	Restinga Grumari – RJ	Mata Atlântica | Restinga	Arvoreta	-	10341	2828
	Restinga Grumari – RJ	Mata Atlântica | Restinga	Arvoreta	RB 584693	10342	-
***Myrsine hermogenesii* (Jung-Mend. & Bernacci) M.F. Freitas & Kin.-Gouv.**	Parque Nacional da Tijuca- RJ	Mata Atlântica | Floresta ombrófila	Arvoreta	RB 152822	-	-
***Myrsine lancifolia* Mart.**	Serra de Itacolomi - MG	-	Arbusto	UEC 140880	-	-
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Floresta ombrófila	Arvoreta	-	10343	2802
	APA Andorinhas – MG	-	Arbusto	RB 606214	10344	2812
	APA Andorinhas – MG	-	Arbusto	RB 606213	-	-
***Myrsine lineata* (Mez) Imkhan.**	Parque Nacional da Bocaina - SP	Mata Atlântica | Floresta ombrófila	Arvoreta/ Árvore	UEC 113228	-	-
	Parque Nacional do Itatiaia – RJ	Mata Atlântica | Floresta ombrófila	Arvoreta/ Árvore	-	10176	2773
	Parque Nacional do Itatiaia – RJ	Mata Atlântica | Floresta ombrófila	Arvoreta/ Árvore	-	10346	2827
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Floresta ombrófila	Arvoreta/ Árvore	RB 605196	10347	2798
***Myrsine parvifolia* A. DC.**	Praia Seca – RJ	Mata Atlântica | Restinga	Arbusto	-	10348	2829
	Praia Seca – RJ	Mata Atlântica | Restinga	Arbusto	-	10349	2830
	Praia Seca – RJ	Mata Atlântica | Restinga	Arbusto	RB 636649	10350	2831
	Ilha do Cardoso - SP	Mata Atlântica| Restinga	Arbusto	UEC 140877	-	-
***Myrsine parvula* (Mez) Otegui**	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 605183	-	-
	Parque Estadual do Ibitipoca - MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 605178	10352	2805
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 605180	10351	2816
***Myrsine rubra* M.F. Freitas & Kin.-Gouv.**	Bosque da Barra – RJ	Mata Atlântica | Floresta ombrófila	Árvore	RB 444753	10358	2817

***Myrsine rubra* M.F. Freitas & Kin.-Gouv.**	Bosque da Barra – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10359	2819
	Bosque da Barra – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10360	2824
	Bosque da Barra – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10361	2820
	Bosque da Barra – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10362	2823
	Bosque da Barra – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10363	2821
***Myrsine squarrosa* (Mez) M.F. Freitas & Kin. - Gouv.**	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605168	-	-
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605176	-	-
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605172	-	-
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605186	10353	2836
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605200	10354	2837
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Campo rupestre	Arbusto	RB 605179	10355	2804
***Myrsine umbellata* Mart.**	Parque Nacional do Itatiaia – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10174	2838
	Parque Nacional do Itatiaia – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10175	-
	Parque Nacional do Itatiaia – RJ	Mata Atlântica | Floresta ombrófila	Árvore	-	10356	2806
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 605170	10357	2799
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 605182	-	-
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 605177	-	-
	Parque Estadual do Ibitipoca – MG	Mata Atlântica | Floresta ombrófila	Árvore	RB 605181	-	-
***Myrsine venosa* A.DC.**	Ilha do Cardoso - SP	Mata Atlântica	Arvoreta	UEC 140881	-	-
	Ilha do Cardoso - SP	Mata Atlântica	Arbusto	UEC 140879	-	-
	Parque Nacional da Tijuca- RJ	Mata Atlântica | Floresta ombrófila	Árvore	RB 583205	-	-
***Myrsine villosissima* Mart.**	APA Andorinhas – MG	Mata Atlântica	Sub-arbusto	RB 606215	10364	2811

***Stylogyne atra* Pipoly**	Reserva Ducke – AM	Floresta amazônica	Arbusto	INPA 190118	10366	2781
	Reserva Ducke – AM	Floresta amazônica	Arbusto	INPA 178964	10365	2780
***Stylogyne depauperata* Mez**	Parque Nacional da Tijuca- RJ	Mata Atlântica | Floresta ombrófila	Arbusto	RB 444752	-	-
***Stylogyne pauciflora* Mez**	Prainha – RJ	Mata Atlântica	Arbusto	-	10367	2774
	Teresópolis - RJ	Mata Atlântica | Floresta ombrófila	Arbusto	RB 462100	-	-
***Stylogyne sordida* Mez**	Parque Estadual da Pedra Branca - RJ	Mata Atlântica | Floresta ombrófila	Arbusto	RB 404211	-	-
***Stylogyne warmingii* Mez**	Fazenda Fortaleza - MG	-	Arbusto	RB 468128	-	-
Theophrastoideae						
***Clavija nutans* (Vell.) B.Ståhl**	Ribeirão Preto – SP	Mata Atlântica	Arbusto	RB 502407	-	2835
***Clavija spinosa* Vell. (Mez)**	Itaipuaçu, Niterói – RJ	Mata Atlântica	Arbusto	RB 535194	-	2833
***Clavija weberbaueri* Mez**	Alto Solimões – AM	Floresta Amazônica	Arbusto	RB 117379	-	2834
***Jacquinia armillaris* Jacq.**	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	RB 583208	10172	2724
	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	RB 584966	10317	2767
	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	-	10318	2725
	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	-	10319	2768
	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	-	10320	2769
	Parque Estadual Costa do Sol – RJ	Mata Atlântica | Restinga	Arbusto	-		2770

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CAPÍTULO 1

LEAF ANATOMY OF FIVE NEOTROPICAL GENERA OF PRIMULACEAE

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Abstract

Premise of research. The present study tackles the general question of whether the assessment of leaf anatomical characters has potential utility in characterizing clades and taxa at various levels of the taxonomic hierarchy of the Primulaceae.

Methodology. Fully expanded field-collected leaves of 33 species from five genera were sampled. The material was subjected to anatomical procedures by light microscopy, SEM, confocal microscopy and epifluorescence microscopy. PCAs were carried out to test the validity of leaf anatomical features as a method of separating the species and genera. In addition, to understand character evolution, some leaf anatomical characters were plotted on a DNA phylogeny.

Pivotal results. The basic leaf anatomical structure was shared by all species: dorsiventral mesophyll, single-layered epidermis and mesophyll cells containing druses. In contrast, other attributes, such as trichome types, stomata, and cuticular ornamentation display diversity, which is helpful in defining groups within the Neotropical Primulaceae. In addition, several apomorphies for genera and subfamilies could be identified. The subfamily Myrsinoideae can be characterized by its secretory cavities and ducts. The only representative of the Theophrastoideae we studied stood out by its extraxylary fiber bundles. *Cybianthus* can be segregated from other genera by its paracytic stomata, *Myrsine* by the presence of an additional bundle above the vascular system in the midrib, *Stylogyne* and *Ardisia* by the weakly dorsiventral mesophyll and *Jacquinia* by the presence of bundles of extraxylary fibers in the mesophyll and marginal sclerenchyma.

Conclusions. Based on multivariate analysis, it was possible to validate the efficacy of leaf anatomical characters in species and genus segregation and, based on these results it is further possible to use such data as the basis for future taxonomic delimitation.

Keywords: cuticular sculpturing, secretory cavities, trichome types, principal components analysis, character optimization.

Introduction

Primulaceae (order Ericales) comprises 2,500 species circumscribed in 58 genera (APG IV 2016). This pantropical family includes herbs, shrubs and trees and is characterized by haplostemonous flowers with sympetalous corolla, stamens opposite the petals, free central placentation, bitegmic tenuinucellate ovules and nuclear endosperm (Källersjö et al. 2000). In the Brazilian Flora, the family is represented by 140 species in 11 genera (BFG 2015; Freitas & Carrijo 2015).

In previous circumscriptions, Primulaceae was treated as a sister group of Maesaceae, Myrsinaceae and Theophrastaceae (Källersjö et al. 2000), but following phylogenetic analysis based on morphological and molecular data (Anderberg & Ståhl 1995; Anderberg et al. 1998, 2000, 2002; 2007; Caris & Smets 2004; Oh et al. 2008), those closely related families were subordinated as subfamilies within Primulaceae. In this sense, Primulaceae now comprises the subfamilies Maesoideae, Myrsinoideae, Primuloideae and Theophrastoideae (APG III 2009; APG IV 2016). The monophyly of Primulaceae is well established; however, the relationship among genera, especially those from the Neotropics, requires further elucidation (Stevens 2001 onwards).

Anatomical data are highly informative to researchers pursuing studies in taxonomy, phylogeny and ecology (Baas et al. 1982, 2000; Metcalfe & Chalk 1979, Olson 2005). For example, Lens et al. (2007) assessed wood anatomical data from 52 species from Ericales and combined them with molecular data from Schönenberger et al. (2005) in order to prove their systematic value. In Primulaceae, the larger morphological revisions of Neotropical genera, such as *Ardisia* Sw. subgenus *Auriculardisia* (Ricketson & Pipoly 2003), *Clavija* Ruiz & Pav. (Ståhl 1989, 1995), *Cybianthus* subgenus *Grammadenia* Mart. (Pipoly 1987), *Jacquinia* L. (Ståhl 1989, 1992), *Myrsine* L. (Freitas 2003; Freitas & Kinoshita 2015) and *Stylogyne* A.DC. (Carrijo 2011; Carrijo et al. 2012) briefly assessed leaf anatomical characters and pointed out their significance for the systematics and taxonomy of each

genus. Metcalfe & Chalk (1950) comprehensively summarized all earlier leaf anatomical literature.

The present study tackles the general question of whether the assessment of leaf anatomical characters has potential utility in characterizing clades and taxa at various levels of the taxonomic hierarchy. To accomplish this, we inventoried leaf anatomical diversity in 33 species and 5 genera of Primulaceae, mainly representing the subfamily Myrsinoideae, and one species of the subfamily Theophrastoideae. We also plotted leaf anatomical features on a molecular phylogeny, in order to understand trait evolution.

Material and methods

Material examined

Seventy-five specimens representing 33 species from 5 genera and two Neotropical subfamilies of Primulaceae (Myrsinoideae: *Ardisia* Sw. 3 studied species/450 species in total, *Cybianthus* Mart. 7/161, *Myrsine* L. 17/155 and *Stylogyne* A.DC. 5/60; and Theophrastoideae: *Jacquinia* L. 1/35) were collected at different sites in Brazil: Parque Estadual da Costa do Sol (22°48′0″ S and 41°55′51″ W), Parque Nacional do Itatiaia (22°25′33″ S and 44°37′15″ W), Parque Estadual do Ibitipoca (21°41′48″ S and 43°53′50″ W), Reserva Ducke (59°58′ 38″ S and 2°55′46″ W) and in the arboretum of Jardim Botânico do Rio de Janeiro (22°58′0″ S and 43°13′28″ W). When available, at least three specimens of each species were analyzed and for each three leaves from the third or fourth node were sampled. All analyzed specimens and voucher information are listed in Appendix 1. For further information on *Stylogyne* leaf anatomy, see Luna et al. (2013). Classification of trichomes followed Theobald et al. (1979).

Light microscopy (LM)

After collection, mature leaves were fixed for 48 h in a solution of 2.5 % glutaraldehyde and 4.0 % formaldehyde buffered with 0.05 $mol^{-1}L$ sodium cacodylate buffer, pH 7.2, at room temperature (Barros & Miguens 1998). The samples were dehydrated in a graded ethanol series and embedded in methacrylate resin (Historesin, Leica, Nussloch, Heidelberg, Germany). Thin cross sections of 4-6 μm were made in a Leica RM2245 semi-automated rotary microtome, stained with toluidine blue 0.05 % in 0.1 $mol^{-1}L$ phosphate buffer (Feder & O'Brien 1968), and sealed with Entellan (Merck). Cuticular macerations were performed using Franklin's solution (Franklin 1945), stained with 1% safranin, and mounted in glycerin 50% (Johansen 1940).

Histochemical tests were performed on freehand sections of mature fresh leaves from three individuals each of *Ardisia humilis* Vahl, *A. solanacea* Roxb., *Cybianthus brasiliensis* (Mez) G. Agostini, *Myrsine guianensis* (Aubl.) Kuntze, *M. glazioviana* Warm., *M. umbellata* Mart. and *Stylogyne pauciflora* Mez. Sudan III (Johansen 1940) was used for lipids, Nile blue (Cain, 1947) for neutral and acid lipids, and NADI reagent (David & Carde 1964) for essential oil and resins. Slides were examined and documented with an *Olympus* BX 50 light microscope equipped with an *Olympus* DP73 digital camera.

Scanning electron microscopy (SEM)

For SEM analysis, mature leaves were fixed as described for light microscopy and subsequently rinsed three times with the same buffer, dehydrated in a graded ethanol series (Barros & Miguens 1998), critical-point-dried using CO_2 in a Leica EM CPD030 apparatus, covered with 20 nm of gold in an Emitech K550X apparatus, and observed under a Zeiss EVO 040 scanning electron microscope.

Epifuorescence and Confocal microscopy

For epifluorescent and confocal microscopy, fragments of cuticular macerations were used. Auramin O 0.05% (Excitation (Ex) – 450-480 nm/ Emission (Em) – 515 nm) was used to stain the cuticular membrane (Considine & Knox 1979), and Calcofluor (Ex - 360-370 nm/ Em – 420 nm) was used for staining cellulose (Herth & Schnepf 1980). Epifluorescence images were taken with an *Olympus* DP73 camera attached to an *Olympus* BX50 epifluorescence microscope and in a Zeiss Axio Imager 2. The confocal images were taken using the LAS AF LITE (ver. 2.6.0) software (Leica Microsystems) in a Leica *TCS SPE* laser scanning confocal microscope.

Statistical analysis

Principal components analysis (PCA) were carried out to test the validity of leaf anatomical features as a method of separating the species and genera (Manly 1994). PCA analyses were performed with Statistica v. 7.0 (for Windows). Twenty-one characters were selected for the analysis.

Phylogenetic analysis and character evolution

Previous phylogenetic analysis did not combine the selected genera analyzed in this study. We therefore inferred phylogenetic relationships from *mat*K sequences of 8 Primulaceae species, representing the four subfamilies, using Genbank accessions (Appendix 2). As outgroups, we selected *Manilkara zapota* (L.) P. Royen and *Pouteria torta* (Mart.) Radlk. both species from Sapotaceae, sister group of Primulaceae (Schönenberger et al. 2005).

A heuristic search was performed with 5000 repetitions and 100 trees per replication, using TBR swapping, based on the character optimization method ACCTRAN (accelerated transformation optimization; Farris 1970, Swofford & Maddison 1987), with unordered characters of equal weight and the retention of multiple most parsimonious trees

(MAXTREE), using maximum parsimony in PAUP* version 4.0b10 for Windows (Swofford 2002). Each node support value was evaluated by bootstrap analysis, performed with random addition sequence of taxa, using TBR for 1000 replicates (Felsenstein 1985). To trace their evolution, eight leaf anatomical characters were plotted using parsimony optimization on the strict consensus cladogram obtained in the phylogenetic analysis, using the software Mesquite (Maddison and Maddison 2016) with parsimony optimization. The characters and character states plotted were: mucilage cells in the epidermis (absent: 0; present: 1), secretory cavities/ducts (absent: 0; present: 1), marginal sclerenchyma (absent: 0; present: 1), mesophyll arrangement (dorsiventral: 0; weakly dorsiventral: 1), mesophyll cells with invaginations (0: absent; 1: present), bundles of extraxylary fibres (absent: 0; present 1), hypodermis (absent: 0; present: 1). Additional anatomical data on *Primula* (Primuloideae)*, Maesa* (Maesoideae)*, Manilkara* and *Pouteria* (Sapotaceae) were taken from the literature (Metcalfe & Chalk 1950, Utteridge 1998, Monteiro et al. 2007)

Results

Table 1 presents a summary of petiole, midrib and leaf blade characters.

Petiole and midrib (Figure 1)

The outline of the petiole and midrib can be flat-convex (Fig. 1A), biconvex (Fig. 1B), or concave-convex/grooved (Fig. 1C). Epidermal cells can be square to procumbent with a flat surface (Fig. 1D), or they can be convex (Fig. 1E).

In the cortex of both petiole and midrib, the number of cell layers in the angular collenchyma varies from 3 to 5 (Fig. 1D). In all species, with the exception of *Jacquina armillaris*, secretory cavities are observed (Fig. 1A-C). Brachysclereids may be present in the cortex (Fig. 1F). In the cortex of both petiole and midrib, some cells may contain druse crystals (Fig. 1G) or prismatic crystals (Fig. 1H).

The vascular system in the petiole can be arc-shaped, with or without incurved margins (Fig. 1A and 1B), or it can be V-shaped with incurved margins (Fig. 1C). *Jacquinia armillaris* has three separate bundles in the petiole (Fig. 1I).

In the midrib, the vascular system can be arc-shaped, with or without incurved margins (Fig. 1J). It can also possess an additional bundle above the vascular system with an opposed arrangement (Fig. 1K), and/or it can be V-shaped, with or without incurved margins, with an almost closed vascular system (Fig. 1L).

The vascular bundle in the midrib may be surrounded by a fiber sheath, as seen in almost all species (Fig. 1K-L). On the other hand, the sheath may be composed of parenchyma cells, as observed in *Cybianthus verticillatus*, in which xylem tissue is well developed (Fig. 1J). The number of layers in this sheath usually varies from 2 to 4 in all Myrsinoideae analyzed in the present study, while in *Jacquinia* (Theophrastoideae), the sheath is very asymmetric with an abaxial fiber cap of 10 layers of fibers (Fig. 1M).

Secretory cavities also occur in the pith of petiole and midrib (Fig. 1K); these cavities contain a mix of substances testing positive for proteins (Fig. 1N), resin (Fig. O), essential oils (Fig. 1P) and lipids (Fig. 1Q).

Leaf blade

Epidermal cells (Figure 2)

All analyzed species have a single-layered epidermis composed of square or procumbent cells (Fig. 2A). In both adaxial and abaxial surface, mucilaginous idioblasts, which are characterized by their size and a high concentration of polyssacharides in the cytoplasm, can be seen (Fig. 2B). The cuticle is rather thin in most samples of *Ardisia*, *Cybianthus* and *Stylogyne*, (~2-3µm) but thicker in *Myrsine* (~7-9 µm) and in *Jacquinia* (~10µm).

The anticlinal walls of the epidermal cells vary from straight (Fig. 2C) to slightly sinuous (Fig. 2D) or highly sinuous (Fig. 2E). The patterns vary between species or between the

adaxial and abaxial epidermis of the same species (Table 1). The surface of the outer periclinal walls of both epidermal surfaces varies from smooth (Fig. 2F) to striated (Fig. 2G). Striae can occur on all epidermal cells or can be restricted to the stomatal areas, as observed in *A. humilis*, *C. verticillatus* and *S. sordida* (Fig. 2H).

Stomata are restricted to the lower leaf surface in most species, except *A. solanacea* and *S. atra*, which have amphistomatic leaves (Fig. 2I). In these species, stomata are morphologically similar on both surfaces; the difference is that the lower surface has a higher stomatal frequency (~50/mm²). Stomata are located at the same level as other epidermal cells (Fig. 2A), and they can be anomocytic (Fig. 2J), anisocytic to helicocytic (Fig. 2K), or paracytic (Fig. 2L). In some species, paracytic and anomocytic stomata occur together (Table 1).

Trichomes (Figure 3)

All studied species have globular multicellular trichomes (**Type 0**) (Fig. 3A-D). These trichomes can be found on both sides of the leaf surface at the same level as other epidermal cells (Fig. 3B), or depressed below the leaf surface, as observed in *Jacquinia armillaris* (Fig. 3C-D).

Most diversity of trichomes was observed among the *Cybianthus* species, which presented a variety of types. **Type 1** - Peltate scales, as observed in *C. brasiliensis* (Fig. 3E-F) and *C. guyanensis* (Fig. 3G-H). The multicellular head, when mature, can form a contiguous structure (Fig. 3F). **Type 2** - Peltate, in which the head cell presents an irregular growth, resulting an asymmetric shape such as that found in *C. densiflorus* (Fig. 3I) and *C. nemoralis*. **Type 3** - Peltate, rounded, with a large multicellular head (>30 cells) and three basal cells, as found in *C. densiflorus* (Fig. 3J) and *C. venezuelanus* (Fig. 3K). **Type 4 -** Stalked stellate trichome, which is present in *C. venezuelanus* (Fig. 3L) and in *Ardisia humilis. Myrsine coriacea* has branched glandular trichomes (**Type 5,** Fig. 3M). Simple multicellular non-glandular

trichomes (**Type 6,** Fig. 3N-O) occur in *Cybianthus verticillatus*, *Myrsine congesta*, *M. glazioviana* and *M. vilosissima*. *Jacquinia armillaris* presents branched non-glandular trichomes found only on the petiole (**Type 7,** Fig. 3P); and *M. vilosissima* exhibits glandular capitate trichomes (**Type 8,** Fig. 3Q).

Mesophyll (Figure 4)

The general aspects of the leaf blade are similar among species from different genera. The dorsiventral mesophyll is a common feature shared by almost all genera analyzed (Fig 4A and 4C). However, *Ardisia* and *Stylogyne* have weakly dorsiventral mesophyll without a distinct spongy layer (Fig. 4B). In both cases, adaxial palisade-like cells show wall invaginations. Palisade parenchyma is composed of 1 or 2 layers, and the spongy parenchyma presents layers of 4 to 10 cells. Crystalliferous cells with druses are very frequent (Fig. 4B).

Jacquinia armillaris has a hypodermis composed of 2 or 3 layers. Continuous bundles of extraxylary sclerenchyma fibers are found in the subepidermal portion (Fig. 4D-F). Vascular bundles are collateral and surrounded by a sclerenchymatic sheath (Fig. 4B).

Hydathodes were observed in the leaf margin of *Myrsine* species, with a well-defined epithem, composed of modified parenchyma cells and stomata (Fig. 4 G-H).

With the exception of *Jacquinia armillaris*, nearly all species had secretory cavities randomly distributed through the mesophyll (Fig. 4A and 4G). The cavities are intercellular, surrounded by an epithelium. Histochemical analysis revealed the presence of mixed substances in the secretion. Figure 4I shows the control without any staining. Lipids (Fig. 4J-K), resins (Fig. 4L) and essential oils (Fig. 4M) were all detected.

Comparison between genera (Figure 5)

Principal component analysis of the leaf anatomical characters reveals that the sum of the first three PC factors accounted for 72 % of the total variance. The loadings of each character used in the principal components analysis are shown in Table 2. The characters with major scores that contributed to the formation of the groups observed in the PCA were the separated bundles in the petiole, presence of an additional bundle above the vascular system with an opposed arrangement, paracytic stomata, type 7 trichome, secretory cavities, hypodermis, bundles of extraxylary fibres, marginal sclerenchyma, and cells with wall invaginations in the mesophyll.

The first factor accounts for 36% of the total variance, with separated bundles in the midrib, type 7 trichomes, extraxylary fibers, hypodermis and marginal sclerenchyma having the highest negative correlation (-0.96) and secretory cavities having the highest positive correlation (0.96). The second factor accounts for 19% of total variance, with additional bundle in the midrib having the highest negative correlation (-0.70). The third factor corresponds to 17% of total variance with cells with wall invaginations in the mesophyll having the highest positive correlation (0.70) and paracytic stomata having the highest negative correlation (-0.70).

Phylogenetic analysis

From 560 base pair characters in the *mat*K sequences, 428 were constant, 41 were parsimony-uninformative and 91 were parsimony-informative characters. Parsimony analysis resulted in a single tree with 166 steps, the consistency index was 0.90, the retention index was 0.91 and the homoplasy index was 0.09. All consensus and bootstrap trees displayed the same well-resolved topology. Sequence variation in *mat*K strongly supports the monophyly of Primulaceae (100% bootstrap value) and resolves the four subfamilies (Fig. 6A).

Character evolution (Fig. 6B-D and 7A-D)

Mucilage cells in the epidermis were found only in Myrsinoideae species (Fig. 6B). Stomatal type shows some homoplasy within Primulaceae: anisocytic stomata support the *Ardisia, Myrsine* and *Stylogyne* clade; *Cybianthus* has anomocytic and paracytic stomata, like the outgroup Sapotaceae, and *Jacquinia* and other Theophrastoideae have anomocytic stomata (Fig. 6C). Secretory cavities are an apomorphy for Myrsinoideae and have evolved independently in Maesoideae (Fig. 6D). The poorly developed mesophyll (Fig. 7A) and the presence of mesophyll cells with invaginations (Fig. 7D) is a feature shared by *Ardisia* and *Stylogyne* (Fig. 7A) and presented in a few *Cybianthus* species (Table 1). Hypodermis and extraxylary fibres appeared as an apomorphy for *Jacquinia* (Theophrastoideae, Fig. 7B). Marginal sclerenchyma apparently evolved independently in *Jacquinia* (Theophrastoideae) and *Pouteria* (Sapotaceae) (Fig. 7C).

Discussion

Our observations are in agreement with the literature on Primulaceae (Solereder 1908, Metcalfe & Chalk 1950, Ståhl 1992, Otegui & Maldonado 1998, Gostin et al. 2011 and Luna et al. 2013), but greatly expand our knowledge due to a wider and/or very different taxon sampling. As far as we know, the presence of hydathodes in *Myrsine* and the observation of an additional bundle above the vascular system in the midrib or the petiole are reported here for the first time.

Table 1 shows some features to be more common in one genus than in the other genera, but very few features by themselves are diagnostic at the genus level. However, when subjected to PCA *Cybianthus* and *Myrsine* formed their own clusters, while *Ardisia* and *Stylogyne*, formed a single cluster, and it was not possible to segregate these two genera. This condition highlights the difficulty of segregating such closely related genera based solely on morphological characters (Anderberg & Ståhl 1995, Bernacci & Jung-Mendaçolli

2000), and in the present study, species from these genera shared the presence of cells with invaginations in the mesophyll layer adjacent to the adaxial epidermis and the weakly dorsiventral mesophyll.

The only representative of the Theophrastoideae subfamily does not fit into any of the clusters, which is in accordance with the phylogenetic analysis where Theophrastoideae emerged as a sister group of Primuloideae and Myrsinoideae. This segregation was affected by the presence of the following characters: extraxylary foliar sclerenchyma, marginal sclerenchyma, type 7 trichomes, anomocytic stomata, and separated vascular bundles in the petiole, as observed in *Jacquinia armillaris* (Theophrastoideae), but absent in the analyzed Myrsinoideae species.

Metcalfe & Chalk (1950) reported the presence of branched hairs in *J. armillaris*, and in the present work, we observed this type of trichome only on the petiole. *J. armillaris* also shows depressed glandular trichomes, as reported for many Theophrastoideae species (Metcalfe & Chalk 1950: 864; Ståhl 1991).

All Myrsinoideae species analyzed here display secretory cavities in the mesophyll, midrib and petiole, and the presence of several chemical substances was detected, such as lipids, resin and essential oils, indicating that the secretion is composed of a mix of substances, as reported by Luna et al. (2014) in *Myrsine coriacea* and *M. venosa*.

Generally, among leaf anatomical features in angiosperms, the epidermis provides the most informative characters for taxonomy (Solereder 1908; Stace 1965; Metcalfe & Chalk 1979; Barthlott et al. 1998), and it has been used in the identification of many species (Sampaio et al. 2014; Zhang et al. 2014). Our observations showed several differences among Myrsinoideae genera that could be detected by the type of stomata, variety of indumentum, sinuosity of anticlinal walls ornamentation of periclinal walls and presence of mucilage idioblasts in the epidermis.

Variation in the indumentum is also useful for taxonomic and phylogenetic studies, and in Primulaceae, Otegui & Maldonado (1998), studying *Myrsine*, and Fico et al. (2007), studying *Primula* L, used the anatomical aspects of trichomes to segregate species in each genus. Große (1908) observed that non-glandular hairs were very rare among Myrsinoideae (=Myrsinaceae). In fact, most common trichomes are peltate with a glandular head, as observed in all species herein studied. However, *Myrsine congesta*, *M. glazioviana* and *M. vilosissima* present numerous multiseriate non-glandular trichomes distributed on both the adaxial and abaxial surfaces. These species are xeric shrubs and their dense indumentum of non-glandular hairs is possibly related with the protection against high temperatures, water loss and excessive light (Ehlringer 1984, Wagner et al. 2004). Non-glandular trichomes were also found in *Cybianthus verticillatus* but they were much less abundant and were found mainly above the midrib.

Many authors have reported the presence of peltate trichomes in Primulaceae (Metcalfe & Chalk 1950; Otegui & Maldonado 1998; Fico et al. 2007; Luna et al. 2013; Luna et al. 2014), and we found these structures in all analyzed species. Peltate trichomes display several morphological variations, depending on the species, such as flattened scales, peltate with irregular head, peltate with large multicellular head, as well as stellate and branched trichomes.

Cybianthus is divided into ten subgenera, according to Agostini (1980), and species studied by us belong to subgenus *Conomorpha* (*C. brasiliensis* and *C. guyanensis*), *Cybianthus* (*C. verticillatus*, *C. venezuelanus*) and *Weilgetia* (*C. densiflorus* and *C. nemoralis*). A prominent feature characterizing the subgenus *Conomorpha* is the presence of type 1 peltate scales trichomes, as reported by Pipoly (1996, 1998) in *C. cuatrecasasii* Pipoly, *C. idroboi* Pipoly and *C. montanus* (Lundell) G. Agostini.

Adaptions of anatomical characters to environmental conditions

Leaf anatomical characters may also reflect adaptation to environmental conditions (Rôças et al. 1997). It is noteworthy that species of *Ardisia, Stylogyne* and some *Cybianthus* are shrubs or subshrubs that occur under similar shaded conditions (BFG 2015) and the presence of a poorly developed mesophyll may be related to the habitat in which they occur. *Jacquinia armillaris* is found in coastal sandy environments from Brazil (BFG 2015), called *restingas*, which are characterized by extreme abiotic factors such as high salinity, high light intensity and regular flooding (Lacerda et al. 1993) and several leaf anatomical characters may be related to this extreme condition, such as the thick cuticle above both abaxial and adaxial epidermis, the development of several layers in the palisade mesophyll and the position of the peltate trichomes in sunken areas of the epidermis. The indumentum of xeric *Myrsine* species may also be adaptive (see above).

Phylogenetic significance of leaf anatomical characters

Tracing characters on the *mat*K phylogeny made it possible to notice that homoplasy is relatively limited in the leaf anatomy of the Primulaceae. Both Myrsinoideae and Theophrastoideae species are well supported by leaf anatomy. Although extraxylary fibres were only observed in the one Theophrastoideae species, Ståhl (1987, 1989, 1991) also observed their presence in other Theophrastoideae, rendering them a synapomorphy for the whole subfamily (fig. 7B).

A diagnostic feature of all Myrsinoideae that also distinguishes them from the other Primulaceae subfamilies is the presence of secretory cavities distributed in all plant organs (Judd et al. 2008) and we observed that they are a synapomorphy for Myrsinoideae, but which apparently also has evolved independently in Maesoideae. Mucilaginous epidermal idioblasts are restricted to Myrsinoideae species and appear as an apomorphy for the subfamily, but seem to have been lost in some species.

Within Myrsinoideae, the closely related genera *Ardisia* and *Stylogyne* emerged as sister groups and the presence of cells with invaginations in the mesophyll and the weakly developed mesophyll support this relationship. The presence of an additional bundle with an opposed arrangement in the vascular system of *Myrsine* species was one of the features characterizing the genus. Metcalfe and Chalk (1950) consider the petiole as an important taxonomic attribute as it is little influenced by environmental factors and according to character optimization, this feature may be considered an autapomorphy to *Myrsine*.

Additional anatomical data on Maesoideae and Primuloideae are needed to trace leaf anatomical characters on the phylogeny.

Conclusions

Our results support the current classification of Primulaceae (Anderberg et al. 1998, 2000, 2002, 2007), as we observed the segregation of the four subfamilies in the phylogenetic analysis, and give a general overview on how the woody Neotropical genera are related. In addition, based on multivariate analysis, our work validated the efficacy of leaf anatomical characters in species and genus segregation.

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Legends

Figure 1 – Petiole and midrib. A-R Light microscopy. O-R – Histochemical tests. A, Flat convex petiole of *Cybianthus densiflorus*. B, Biconvex petiole of *Myrsine umbellata*. C, Concave-convex petiole of *C. brasiliensis.* D, Epidermal cells with a flat surface (arrow). E, Epidermal cells with the lumen strongly convex to the outer surface (»). F, Brachysclereids in the cortex of the petiole of *C. verticillatus* (bold arrow). G, Druses in *M. balansae* (◄). H, Prismatic crystals in *A. humilis* (♦). I, Vascular system in the petiole of *Jacquinia armillaris*, evidencing the separated bundles. J, Midrib of *C. verticillatus*, evidencing the arc-shaped vascular system. Note the absence of the fiber sheath. K, Vascular system in the midrib of *M. lineata*, evidencing the additional bundle with an opposed arrangement (rectangle). L, midrib of *Stylogyne atra* with a V-shaped vascular system with strongly incurved margin. M, Arc-shaped vascular system of *J. armillaris* outlined by several fiber layers. The secretion was stained within the secretory tracts of the mesophyll, midrib and petiole with N, Comassie blue for protein detection; O, Cupric acetate for resin; P, NADI reagent for essential oils and Q, Sudan III for lipids.*, secretory cavities; xyl, xylem; phl, phloem; sh, sheath.

Figure 2 – General aspects of leaf epidermis of Primulaceae. A-D; I, K and L – Light microscopy. F-H – Scanning electron microscopy. E and I – Confocal microscopy. A, *C. verticillatus* in epifluorescence with Auramin and Calcofluor. Arrowhead showing the stomata in the abaxial epidermis. B, Transversal section of *Myrsine umbellata*, evidencing idioblasts in the epidermis with a mucilaginous content (arrow). C, Frontal view of *Myrsine parvifolia* epidermis with straight anticlinal walls. D, *M. lancifolia* epidermis in frontal view, evidencing the undulate anticlinal walls. E, *Cybianthus guyanensis* epidermis with sinuous

anticlinal walls. F, Smooth outer periclinal wall in *Stylogyne atra*. G, Striated periclinal walls in *Cybianthus venezuelanus*. H, partially striated epidermis of *Ardisia humilis*, with striae arranged in the subsidiary cells. I, Stomata in the adaxial epidermis of *A. solanacea*. J, Anomocytic stomata in *C. guyanensis*. K, Anisocytic stomata in *S. atra*. L, Paracytic stomata in *Cybianthus venezuelanus*.

Figure 3 – Trichome diversity in Neotropical Primulaceae. A, C, E, G, L and Q – Scanning electron microscopy. B, D, F, H, J, M-P – Light microscopy. I – Epifluorescence microscopy. K – Confocal microscopy. A, Peltate trichome in *Myrsine guianensis*. B, Transverse section of the peltate trichome in *M. lineata*. C and D Peltate trichome sunken in the epidermis in *Jacquinia armillaris*. E and F, Type 1 trichomes in *C. brasiliensis*. G and H, Type 1 trichomes in *C. guyanensis.* I, Type 2 trichomes in *C. densiflorus*. J, Transverse section of type 3 trichome in *C. densiflorus*. K, Frontal view of type 3 trichome in *C. venezuelanus*. L, Type 4 trichome in *C. venezuelanus.* M, Type 5 trichome in *M. coriacea.* Type 6 trichomes in *Cybianthus verticillatus* (N) and *M. congesta* (O). P, Type 7 trichome in *J. armillaris*. Q, Type 8 trichome in *M. villosissima*.

Figure 4 – General aspects of Primulaceae mesophyll. A-M – Light microscopy. I-M – Histochemical tests. A, Dorsiventral mesophyll of *Myrsine gardneriana*. Secretory cavities (*). B, Weakly dorsiventral mesophyll of *Stylogyne*; note cells with invaginations (rectangle). C, Dorsiventral mesophyll of *Jacquinia armillaris*. D, Detail of the mesophyll of *J. armillaris* showing the hypodermis and the bundles of extraxylary fibers. E, Frontal view of *J. armillaris* leaf, showing the continuous bundles of extraxylary fibers that occur throughout the subepidermal portion of the mesophyll (arrows). Arrowheads indicate the glandular trichomes distributed along the epidermis. F, Leaf margin of *J. armillaris*,

showing the marginal fibers. G and H, hydathodes in *M. lineata*. ◙, epithem; arrowhead, modified stomata. I, Transversal section of the mesophyll without any treatment of *M. guianensis*, showing translucent content in the secretory cavity. J and K, Secretory cavities in *M. glazioviana* stained with Sudan III and Nile blue, respectively, for lipid detection. L – Secretory cavity in *M. umbellata* stained with cupric acetate for resin detection. M – Secretory cavity in *M. umbellata* stained with NADI reagent for essential oil detection. *, secretory cavities; pp, palisade parenchyma; lp, lacunose parenchyma; ◄, druses.

Figure 5 - 3D plot of principal component analysis (PCA). Factor 1 - 36%, factor 2 - 19%, factor 3 - 17%. ◙ - *Ardisia*; ■ - *Cybianthus*; ● – *Jacquinia*; ♦ - *Myrsine*; ▲- *Stylogyne*; 1 - *Ardisia guianensis*, 2 – *A. humilis*, 3 – *A. solanacea*, 4 - *Cybianthus brasiliensis,* 5 – *C. densiflorus,* 6 – *C. glaber,* 7 – *C. guyanensis*, 8 - *C. nemoralis,* 9 – *C. venezuelanus*, 10 – *C. verticillatus*, 11 - *Jacquinia armillaris*, 12 - *Myrsine balansae*, 13 – *M. congesta*, 14 – *M. coriacea,* 15 – *M. emarginella*, 16 – *M. gardneriana*, 17 – *M. glazioviana*, 18 – *M. guianensis*, 19 – *M. hermogenesii*, 20 – *M. lancifolia*, 21 – *M. lineata*, 22 – *M. parvifolia*, 23 – *M. parvula*, 24 – *M. rubra*, 25 – *M. squarrosa*, 26 – *M. umbellata*, 27 – *M. venosa*, 28 – *M. villosissima*, 29 – *Stylogyne atra*, 30 – *S. depauperata*, 31 – *S. pauciflora*, 32 – *S. sordida*, 33 – *S. warmingii*.

Figure 6 – Phylogeny and character evolution of Primulaceae. A – Strict consensus tree based on *mat*K sequences with bootstrap values above the nodes. In bold the analyzed genera. B-D – Leaf anatomical characters optimized on the strict consensus cladogram. Character names and states are indicated in the boxes.

Figure 7 – A-D – Leaf anatomical characters optimized on the strict consensus cladogram. Character names and states are indicated in the boxes.

Table 1 – Summary of Neotropical Primulaceae leaf anatomical features

Species	Petiole				Midrib			Stomata		Epidermis						Mesophyll			
	Outline	Vascular system	Sclerenchymatic sheath	Brachysclereids	Outline	Vascular bundle	Sclerenchymatic sheath	Type	Position	Trichomes (types)	Anticlinal wall/adaxial	Anticlinal wall/abaxial	Abaxial surface	Adaxial surface	Mucilaginous idioblasts	Type	Secretory cavities	Druses	Prismatic crystals
MYRSINOIDEAE																			
Ardisia																			
Ardisia guianensis	F	AIM	-	-	B	AIM	+	Ani	H	0	St	St	Str	Str	+	D	+	+	-
A. humilis	F	AIM	+	+	B	VSM	+	Ani	H	0;4	St	St	Str	Ps	+	D	+	+	+
A. solanacea	F	AIM	-	-	F	AIM	+	Ani	A	0	Ss	Ss	Str	Ps	-	WD	+	+	-
Cybianthus																			
Cybianthus brasiliensis	Cc	VIM	-	-	Cc	VIM	+	Par;Ano	H	1	Si	Si	Str	Sm	+	D	+	-	+
C. densiflorus	F	AIM	-	-	F	AIM	+	Par	H	0;2;3	Si	Si	Str	Str	-	D	+	-	-
C. glaber	F	VIM	-	+	B	VIM	+	Par	H	0	Si	Si	Str	Str	-	WD	+	+	-
C. guyanensis	F	VIM	-	+	F	VIM	+	Par;Ano	H	1	Si	Si	Str	Str	+	D	+	-	-
C. nemoralis	C	AIM	-	-	F	AIM	+	Par	H	2	Si	Si	Str	Str	-	D	+	+	-
C. venezuelanus	F	Arc	-	+	B	ACM	+	Par	H	3;4	Si	Si	Str	Str	-	D	+	-	-
C. verticillatus	F	Arc	-	+	F; B	Arc	+	Par	H	6	Si	Si	Str	Str	-	WD	+	+	-
Myrsine																			
Myrsine balansae	F	AIM	-	-	B	AIM*	+	Ani	H	0	Ss	Ss	Str	Sm	+	D	+	+	-
M. congesta	F	ACM	-	-	F	Arc*	+	Ani	H	0;6	Si	Si	Sm	Sm	+	D	+	+	-
M. coriacea	F	ACM	-	-	B	Arc*	+	Ani	H	0;5	St	Ss	Str	Str	+	D	+	+	-

M. emarginella	F	AIM*	+	+	B	AIM*	+	Ani	H	0	St	Ss	Sm	Sm	-	D	+	+	-
M. gardneriana	F	ACM*	+	+	F	AIM*	+	Ani	H	0	St	St	Sm	Sm	+	D	+	+	-
M. guianensis	F	AIM	-	-	F	AIM*	+	Ani	H	0	Ss	Si	Sm	Ps	+	D	+	+	-
M. glazioviana	C	ACM	+	-	Cc	Arc	+	Ani;Ano	H	0;6	St	St	Sm	Sm	+	D	+	+	-
M. hermogenesii	F	AIM	+	-	Cc; F	Arc	+	Ani	H	0	Si	Si	Str	Sm	+	D	+	+	-
M. lancifolia	F	AIM	+	-	B	AIM*	+	Ani	H	0	Ss	Ss	Str	Sm	+	D	+	+	-
M. lineata	F	ACM	-	-	F	AIM*	-	Ani	H	0	Si	Si	Str	Str	-	D	+	+	-
M. parvifolia	F	AIM	-	-	F	AIM*	+	Ani	H	0	St	St	Sm	Sm	+	D	+	+	-
M. parvula	F	AIM	+	-	F	AIM*	+	Ani	H	0	Si	Si	Str	Str	+	D	+	+	-
M. rubra	F	ACM*	+	-	F	AIM*	+	Ani	H	0	St	Ss	Str	Str	+	D	+	+	-
M. squarrosa	F	ACM	+	-	B	ACM*	+	Ani	H	0	Ss	Ss	Sm	Sm	-	D	+	+	-
M. umbellata	C	ACM	+	-	B	Arc*	+	Ani	H	0	St	Ss	Str	Str	+	D	+	+	-
M. venosa	C	ACM	-	-	F	Arc	+	Ani	H	0	Si	Si	Str	Str	+	D	+	+	-
M. villosissima	Cc	ACM	+	-	F	Arc	+	Ani	H	0;6;8	Si	Si	Sm	Sm	-	D	+	+	-
Stylogyne																			
Stylogyne depauperata	F	AIM	+	+	F	Arc*	+	Ani	H	0	St	St	Str	Str	-	D	+	+	-
S. pauciflora	F	VIM	-	-	F	VCM*	+	Ani	H	0	Ss	Ss	Str	Str	-	D	+	+	-
S. sordida	F	Arc	-	+	B	VCM	+	Ani	H	0	St	St	Ps	Sm	-	D	+	+	-
S. atra	F	VIM	+	+	F	VIM*	+	Ani	A	0	Ss	Ss	Sm	Sm	+	WD	+	-	-
S. warmingii	F	VIM	+	-	B	ACM	+	Ani	H	0	St	St	Str	Str	-	WD	+	+	-
THEOPHRASTOIDEAE																			
Jacquinia																			
Jacquinia armillaris	F	S	+	-	B	Arc	+	Ano	H	0;7	St	St	Sm	Sm	-	D	-	+	-

F, flat-convex; C, circular; Cc, concave-convex; AIM, arc with incurved margin; ACM, arc with closed margin; VIM, "V" shape with incurved margin; S, separated bundles; * - Presence of accessory bundle; -, absent; +, present; B, biconvex; A, Amphistomatic; H, hypostomatic; Ani, anisocytic; Ano, anomocytic; Par, paracytic; Ps, partially striated; Si, sinuous; Sm, smooth; Ss, slightly sinuous, St, straight; Str, striated; D, dorsiventral; WD, weakly dorsiventral. Trichome types 0-8 see figure 3 and text.

Table 2 – Loadings of the three factors with the greatest influence on principal components analyses.

Characters	**Factor 1**	**Factor 2**	**Factor 3**
"V" shape in the petiole	0.167314	0.571208	0.313972
Separate bundles in midrib	**-0.965135**	0.046330	0.212336
Additional bundle in midrib	0.250291	**-0.703024**	0.244525
"V" shape in the midrib	0.211535	0.683243	0.432203
Anisocytic stomata	0.395144	-0.586999	0.582823
Anomocytic stomata	-0.555550	0.278965	-0.276576
Paracytic stomata	-0.009529	0.595915	**-0.700000**
Trichome | Type 0	0.030279	-0.484649	0.690951
Trichome | Type 7	**-0.965135**	0.046330	0.212336
Adaxial surface | anticlinal wall slightly sinuous	0.219664	-0.298539	0.436745
Abaxial surface | anticlinal wall slightly sinuous	0.234834	-0.396838	0.398132
Abaxial surface | ondulate periclinal wall	-0.581641	-0.223368	0.045400
Adaxial surface | ondulate periclinal wall	-0.769557	-0.054882	0.123008
Dorsiventral mesophyll	-0.285316	-0.659030	-0.609818
Weakly dorsiventral mesophyll	0.285316	0.659030	0.609818
Secretory cavities	**0.965135**	-0.046330	-0.212336
Bundles of extraxylary fibers	**-0.965135**	0.046330	0.212336
Hypodermis	**-0.965135**	0.046330	0.212336
Brachysclereids	0.169052	0.583560	-0.027716
Marginal sclerenchyma	**-0.965135**	0.046330	0.212336
Cells with invaginations in mesophyll	0.275416	0.447795	**0.730661**
Cumulative % of eigenvalues	35.67	54.82	72.17

Appendix 1

Data on collected species, voucher and growth form. Symbols for growth form: S = shrub, Ss = sub-shrub, T = tree.

Myrsinoideae

Ardisia guianensis (Aubl.) Mez: RB 462085 (S); *Ardisia humilis* Vahl: RBv 2014 (S); *Ardisia solanacea* Roxb.: RBv 2094, RBv 2199, RBv 2091 (S); *Cybianthus brasiliensis* (Mez) G.Agostini: RB 605169 (S); *Cybianthus densiflorus* Miq.: INPA 191486 (Ss); *Cybianthus glaber* A.DC.: RB 636647, RB 636645 (S); *Cybianthus guyanensis* (A.DC.) Miq.: INPA 178624, INPA 178964, INPA 190110, INPA 178628, INPA 208941 (T); *Cybianthus nemoralis* (Mez) G.Agostini: CEPEC 93447 (S); *Cybianthus verticillatus* (Vell.) G.Agostini: RB 586284 (S); *Cybianthus venezuelanus* Mez: INPA 190119 (Ss); *Myrsine balansae* Mez (Otegui): UEC 115510, UEC 155521 (T); *Myrsine congesta* Sw. (Pipoly): BHCB 153534 (S); *Myrsine coriacea* (Sw.) R.Br. ex Roem. & Schult.: RB 380806, RB 582964, RB 583204, RB 583202 (T); *Myrsine emarginella* Miq.: RB 605187, RB 380828 (T); *Myrsine gardneriana* A.DC.: RB 380621, RB 605188, RB 605197, RB 605166, RB 605202 (T); *Myrsine glazioviana* Warm.: RB 605189, RB 605198, RB 605201, RB 605185 (S); *Myrsine guianensis* (Aubl.) Kuntze: RB 583201, RB 584963, RB 583200 (T); *Myrsine hermogenesii* (Jung-Mend. & Bernacci) M.F.Freitas & Kin.-Gouv.: RB 152822 (T); *Myrsine lancifolia* Mart.: UEC 140880, RB 606214, RB 606213 (T); *Myrsine lineata* (Mez) Imkhan.: UEC 113228, RB 605196, RB 605174 (T); *Myrsine parvifolia* A.DC.: UEC 140877, UEC 140878, RB 636649 (S); *Myrsine parvula* (Mez) Otegui: RB 605180, RB 605178, RB 605183 (T); *Myrsine rubra* M.F.Freitas & Kin.-Gouv.: RB 444753 (T); *Myrsine squarrosa* (Mez) M.F.Freitas & Kin.-Gouv.: RB 605168, RB 605186, RB 605176, RB 605172 (S); *Myrsine umbellata* Mart.: RB 605182, RB 605177, RB 605181, RB 605170 (T); *Myrsine venosa* A.DC.: UEC 140881, UEC 140879, RB 583205 (T); *Myrsine villosissima* Mart.: RB 380829, RB 606215 (S); *Stylogyne*

depauperata Mez: RB 444752 (S); *Stylogyne pauciflora* Mez: RB 462100 (S); *Stylogyne atra* Pipoly: INPA 190118, INPA 178964, INPA 209091 (S); *Stylogyne sordida* Mez: RB 404211 (S); *Stylogyne warmingii* Mez: RB 468128 (S).

Theophrastoideae

Jacquinia armillaris Jacq.*:* RB 369866, RB 583208. RB 584966 (S).

Appendix 2

Data on Genbank accessions for each species, subfamily and outgroup.

Maesoideae - *Maesa lanceolata* Forssk.: JF270859.1. **Myrsinoideae -** *Ardisia humilis* Vahl JF416272.1; *Ardisia solanacea* Roxb: JF416278.1; *Cybianthus peruvianus (*A.DC.) Miq.: KF981353.1; *Myrsine coriacea* (Sw.) R. Br. ex Roem. & Schult.: JQ588471.1, JQ588473.1; *Stylogyne turbacensis* (Kunth) Mez: AY839952.1. **Primuloideae** - *Primula stricta* Hornem.: KC475526.1. **Theophrastoideae -** *Jacquinia arborea* Vahl: KJ012648.1; *Jacquinia keyensis* Mez: KJ772869.1. **Sapotaceae -** *Manilkara zapota* (L.) P. Royen: GU135011.1; *Pouteria torta* (Mart.) Radlk.: JQ626418.1.

Figure 1 – Petiole and midrib. A-R Light microscopy. O-R – Histochemical tests. A, Flat convex petiole of *Cybianthus densiflorus*. B, Biconvex petiole of *Myrsine umbellata*. C, Concave-convex petiole of *C. brasiliensis.* D, Epidermal cells with a flat surface (arrow). E, Epidermal cells with the lumen strongly convex to the outer surface (»). F, Brachysclereids in the cortex of the petiole of *C. verticilatus* (bold arrow). G, Druses in *M. balansae* (◄). H, Prismatic crystals in *A. humilis* (♦). I, Vascular system in the petiole of *Jacquinia armillaris*, evidencing the separated bundles. J, Midrib of *C. verticillatus*, evidencing the arc-shaped vascular system. Note the absence of the fiber sheath. K, Vascular system in the midrib of *M. lineata*, evidencing the additional bundle with an opposed arrangement (rectangle). L, midrib of *Stylogyne atra* with a V-shaped vascular system with strongly incurved margin. M, Arc-shaped vascular system of *J. armillaris* outlined by several fiber layers. The secretion was stained within the secretory tracts of the mesophyll, midrib and petiole with N, Comassie blue for protein detection; O, Cupric acetate for resin; P, NADI reagent for essential oils and Q, Sudan III for lipids.*, secretory cavities; xyl, xylem; phl, phloem; sh, sheath.

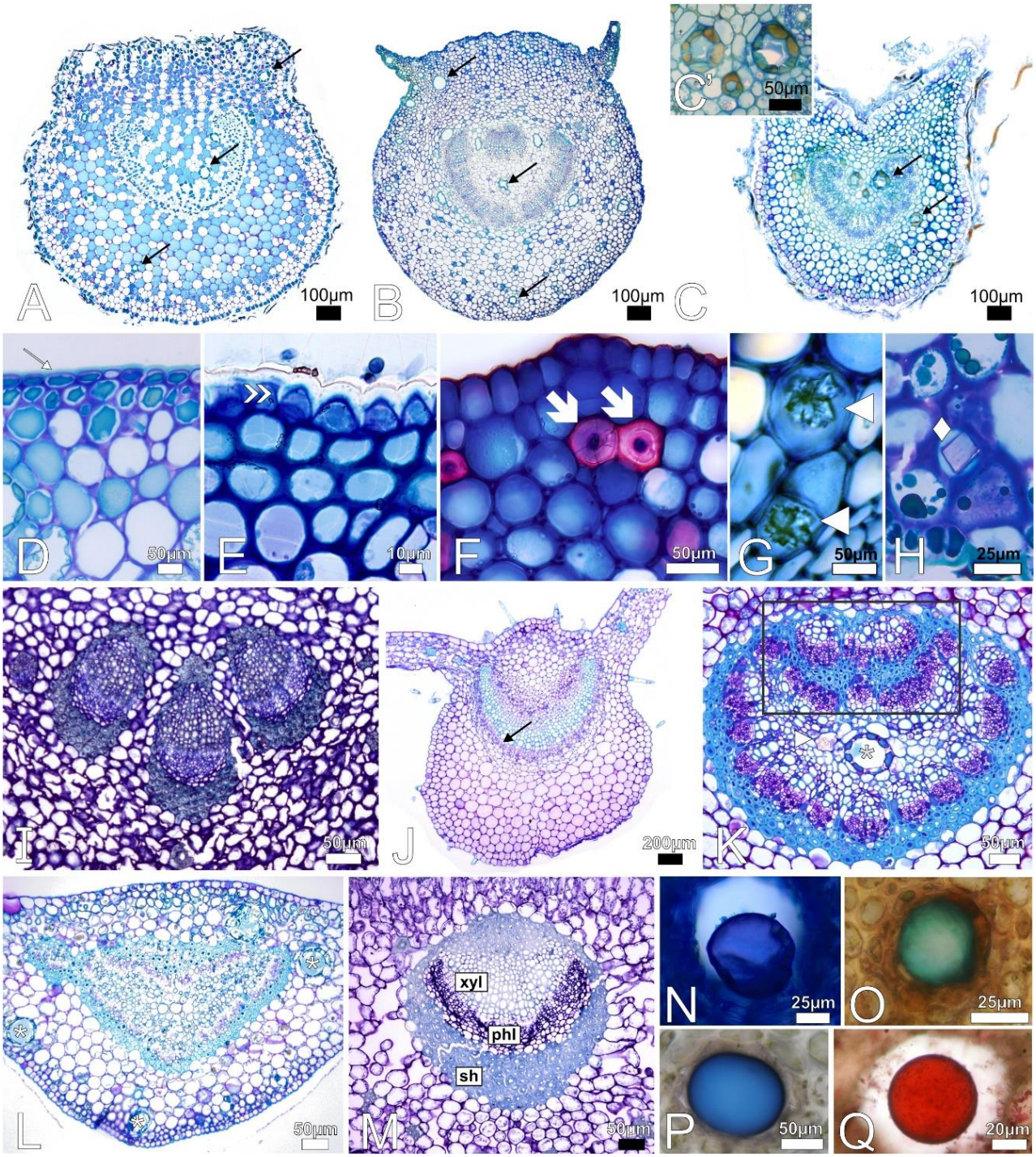
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Figure 2 – General aspects of leaf epidermis of Primulaceae. A-D; I, K and L – Light microscopy. F-H – Scanning electron microscopy. E and I – Confocal microscopy. A, *C. verticilatus* in epifluorescence with Auramin and Calcofluor. Arrowhead showing the stomata in the abaxial epidermis. B, Transversal section of *Myrsine umbellata*, evidencing idioblasts in the epidermis with a mucilaginous content (arrow). C, Frontal view of *Myrsine parvifolia* epidermis with straight anticlinal walls. D, *M. lancifolia* epidermis in frontal view, evidencing the undulate anticlinal walls. E, *Cybianthus guyanensis* epidermis with sinuous anticlinal walls. F, Smooth outer periclinal wall in *Stylogyne atra*. G, Striated periclinal walls in *Cybianthus venezuelanus*. H, partially striated epidermis of *Ardisia humilis*, with striae arranged in the subsidiary cells. I, Stomata in the adaxial epidermis of *A. solanacea*. J, Anomocytic stomata in *C. guyanensis*. K, Anisocytic stomata in *S. atra*. L, Paracytic stomata in *Cybianthus venezuelanus*.

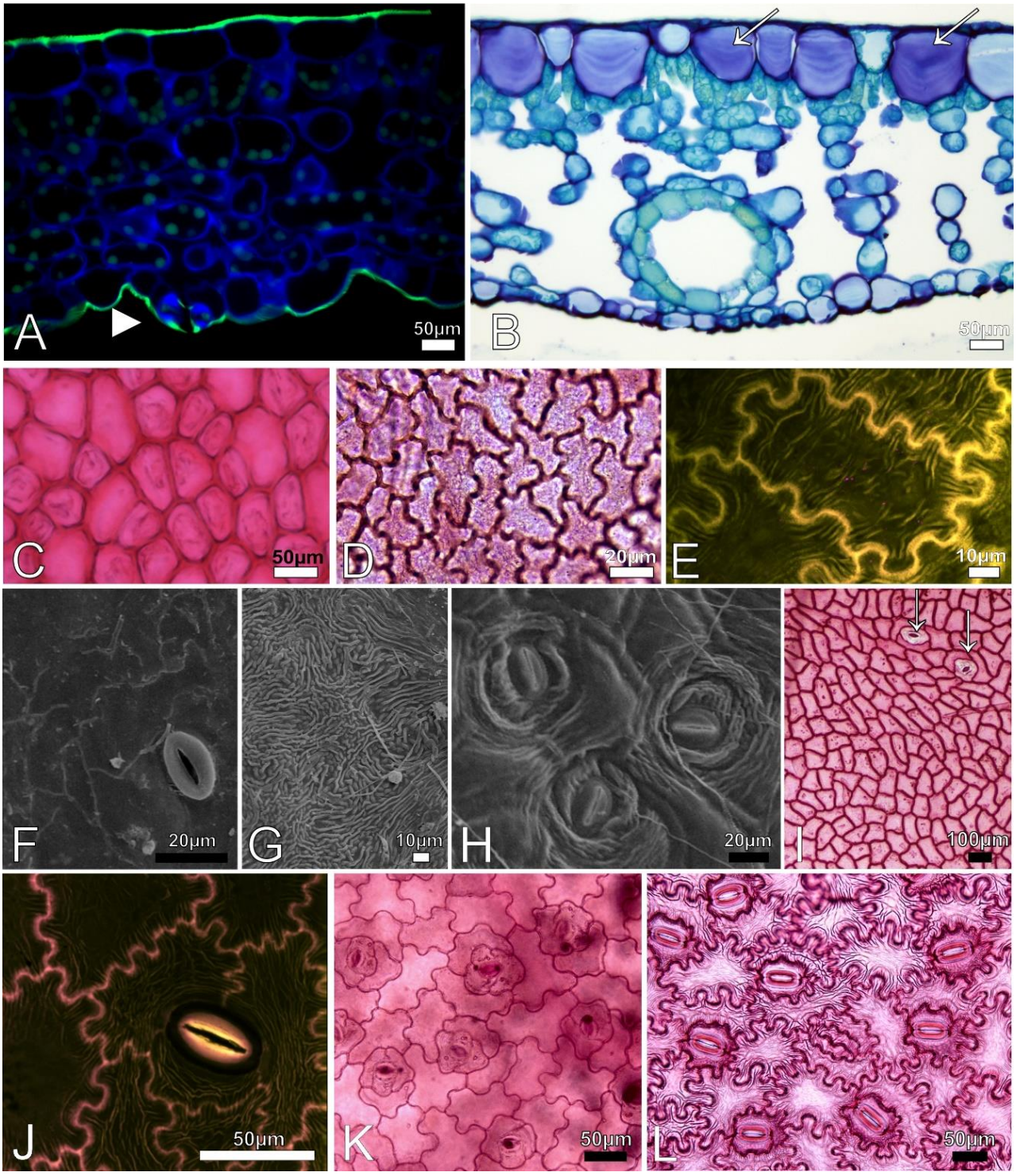
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Figure 3 – Trichome diversity in Neotropical Primulaceae. A, C, E, G, L and Q – Scanning electron microscopy. B, D, F, H, J, M-P – Light microscopy. I – Epifluorescence microscopy. K – Confocal microscopy. A, Peltate trichome in *Myrsine guianensis*. B, Transverse section of the peltate trichome in *M. lineata*. C and D Peltate trichome sunken in the epidermis in *Jacquinia armillaris*. E and F, Type 1 trichomes in *C. brasiliensis*. G and H, Type 1 trichomes in *C. guyanensis.* I, Type 2 trichomes in *C. densiflorus*. J, Transverse section of type 3 trichome in *C. densiflorus*. K, Frontal view of type 3 trichome in *C. venezuelanus*. L, Type 4 trichome in *C. venezuelanus.* M, Type 5 trichome in *M. coriacea.* Type 6 trichomes in *Cybianthus verticillatus* (N) and *M. congesta* (O). P, Type 7 trichome in *J. armillaris*. Q, Type 8 trichome in *M. villosissima*.

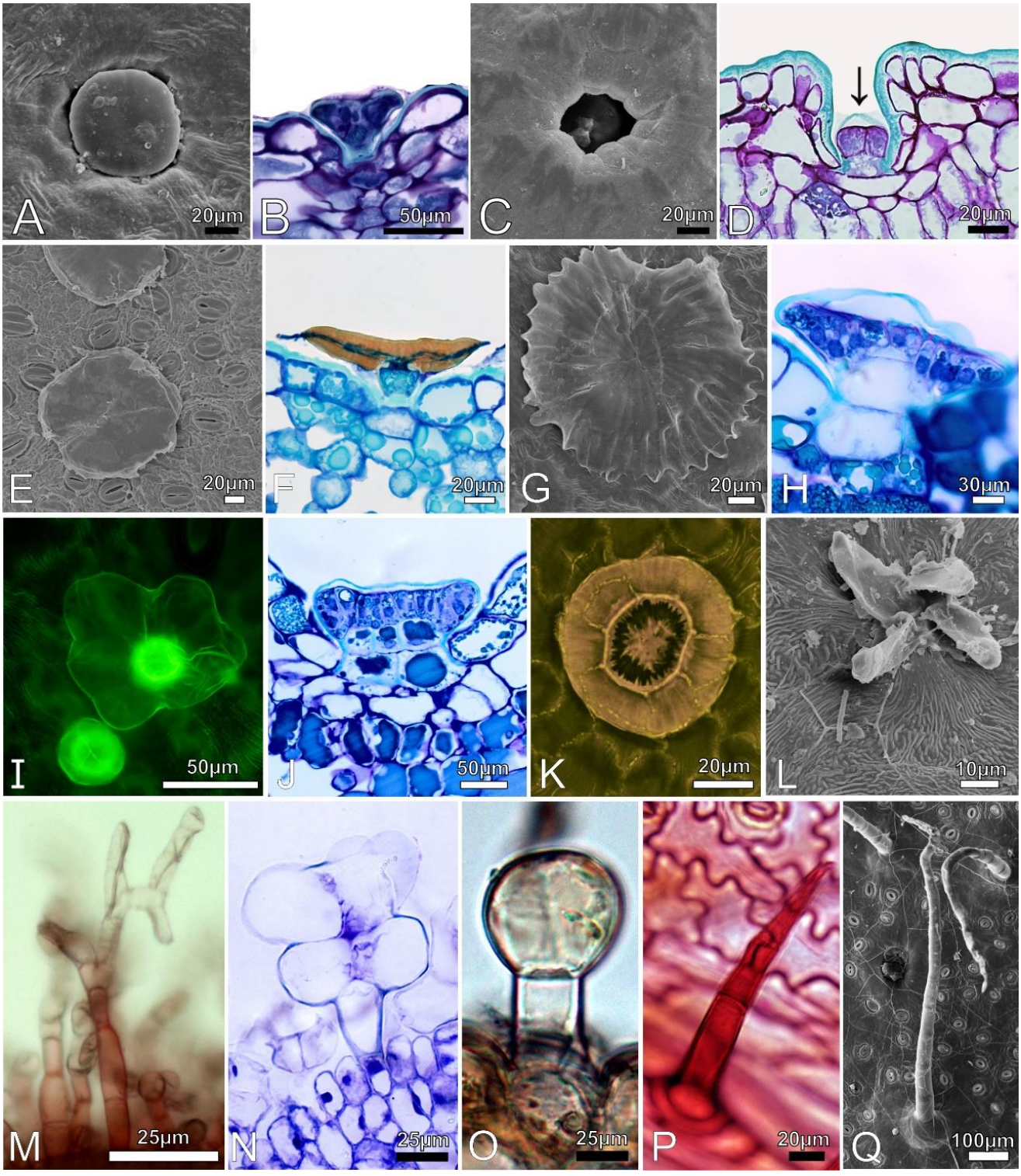
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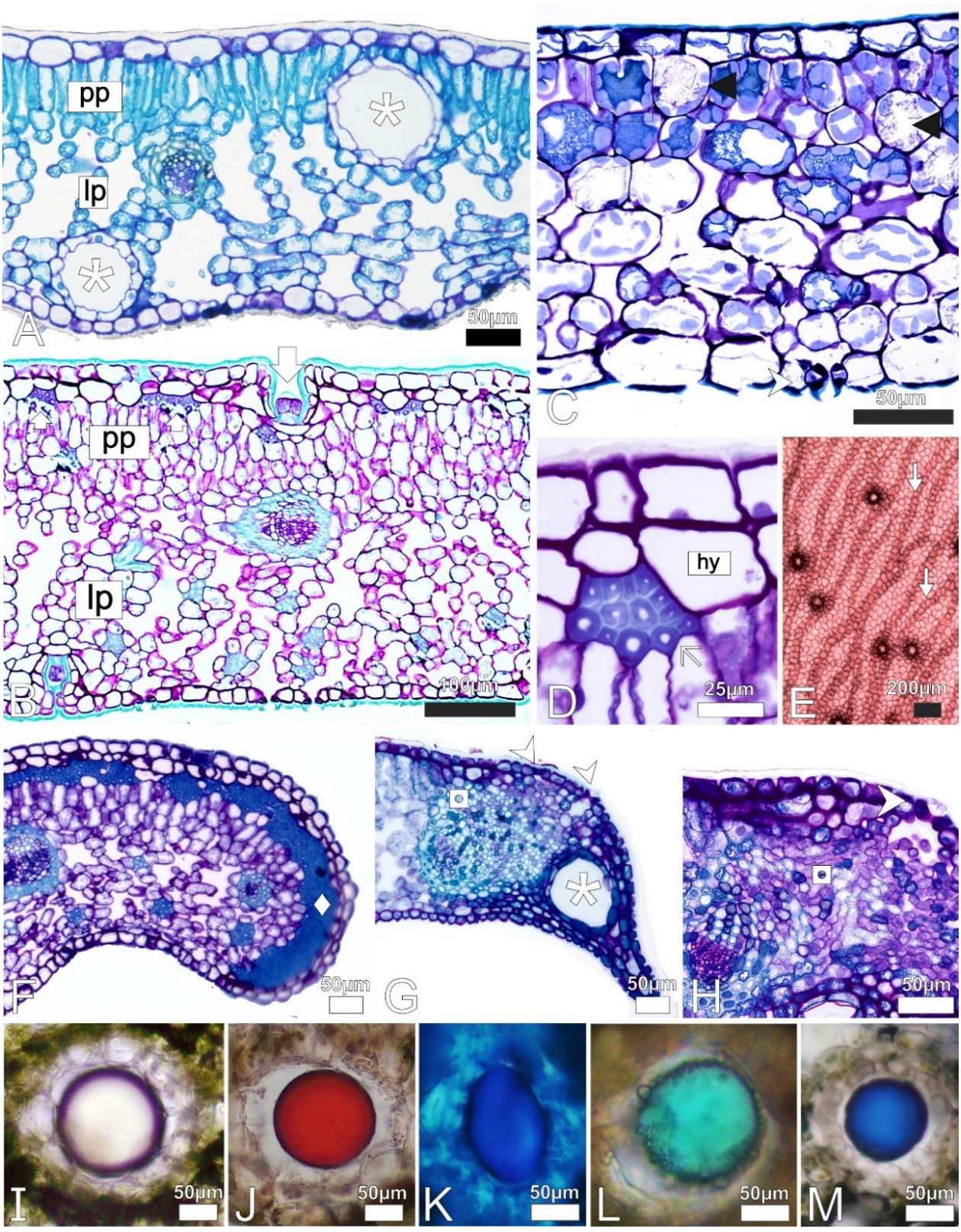
Figure 4 – General aspects of Primulaceae mesophyll. A-M – Light microscopy. I-M – Histochemical tests. A, Dorsiventral mesophyll of *Myrsine gardneriana*. Secretory cavities (*). B, Weakly dorsiventral mesophyll of *Stylogyne*; note cells with invaginations (rectangle). C, Dorsiventral mesophyll of *Jacquinia armillaris*. D, Detail of the mesophyll of *J. armillaris* showing the hypodermis and the bundles of extraxylary fibers. E, Frontal view of *J. armillaris* leaf, showing the continuous bundles of extraxylary fibers that occur throughout the subepidermal portion of the mesophyll (arrows). Arrowheads indicate the glandular trichomes distributed along the epidermis. F, Leaf margin of *J. armillaris*, showing the marginal fibers. G and H, hydathodes in *M. lineata*. ◙, epithem; arrowhead, modified stomata. I, Transversal section of the mesophyll without any treatment of *M. guianensis*, showing translucent content in the secretory cavity. J and K, Secretory cavities in *M. glazioviana* stained with Sudan III and Nile blue, respectively, for lipid detection. L – Secretory cavity in *M. umbellata* stained with cupric acetate for resin detection. M – Secretory cavity in *M. umbellata* stained with NADI reagent for essential oil detection. *, secretory cavities; pp, palisade parenchyma; lp, lacunose parenchyma; ◄, druses.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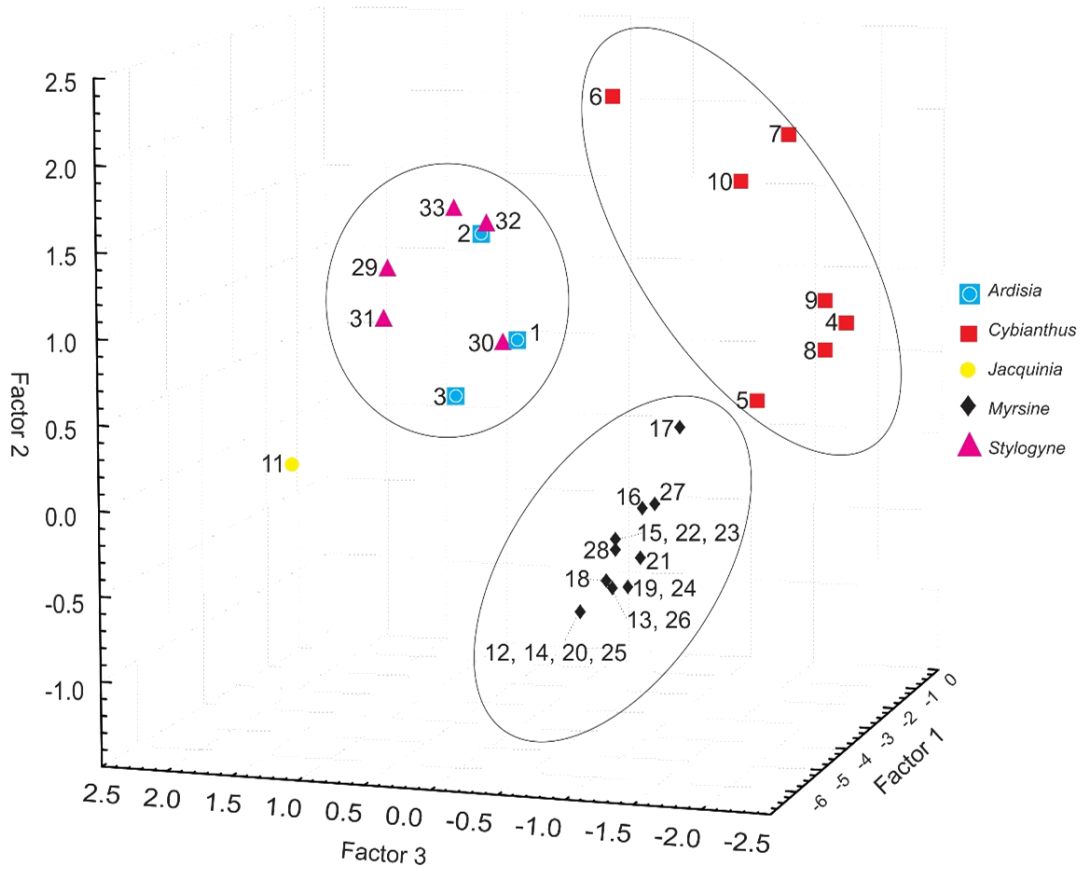

Figure 5 - 3D plot of principal component analysis (PCA). Factor 1 - 36%, factor 2 - 19%, factor 3 - 17%. ◙ - *Ardisia*; ■ - *Cybianthus*; ● – *Jacquinia*; ♦ - *Myrsine*; ▲- *Stylogyne*; 1 - *Ardisia guianensis*, 2 – *A. humilis*, 3 – *A. solanacea*, 4 - *Cybianthus brasiliensis,* 5 – *C. densiflorus,* 6 – *C. glaber,* 7 – *C. guyanensis*, 8 - *C. nemoralis,* 9 – *C. venezuelanus*, 10 – *C. verticilatus*, 11 - *Jacquinia armillaris*, 12 - *Myrsine balansae*, 13 – *M. congesta*, 14 – *M. coriacea,* 15 – *M. emarginella*, 16 – *M. gardneriana*, 17 – *M. glazioviana*, 18 – *M. guianensis*, 19 – *M. hermogenesii*, 20 – *M. lancifolia*, 21 – *M. lineata*, 22 – *M. parvifolia*, 23 – *M. parvula*, 24 – *M. rubra*, 25 – *M. squarrosa*, 26 – *M. umbellata*, 27 – *M. venosa*, 28 – *M. villosissima*, 29 – *Stylogyne atra*, 30 – *S. depauperata*, 31 – *S. pauciflora*, 32 – *S. sordida*, 33 – *S. warmingii*.

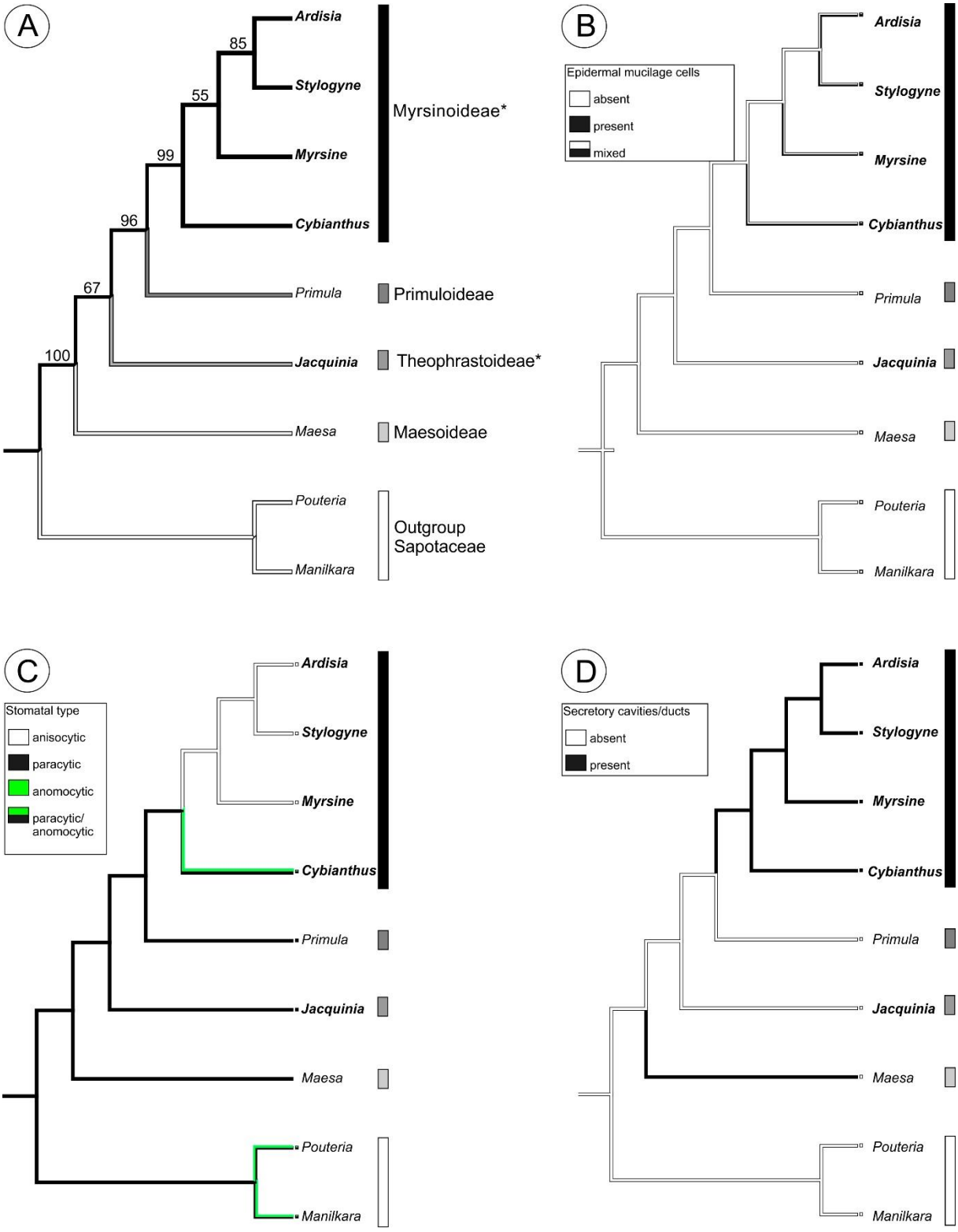

Figure 6 – Phylogeny and character evolution of Primulaceae. A – Strict consensus tree based on *mat*K sequences with bootstrap values above the nodes. In bold the analyzed genera. B-D – Leaf anatomical characters optimized on the strict consensus cladogram. Character names and states are indicated in the boxes.

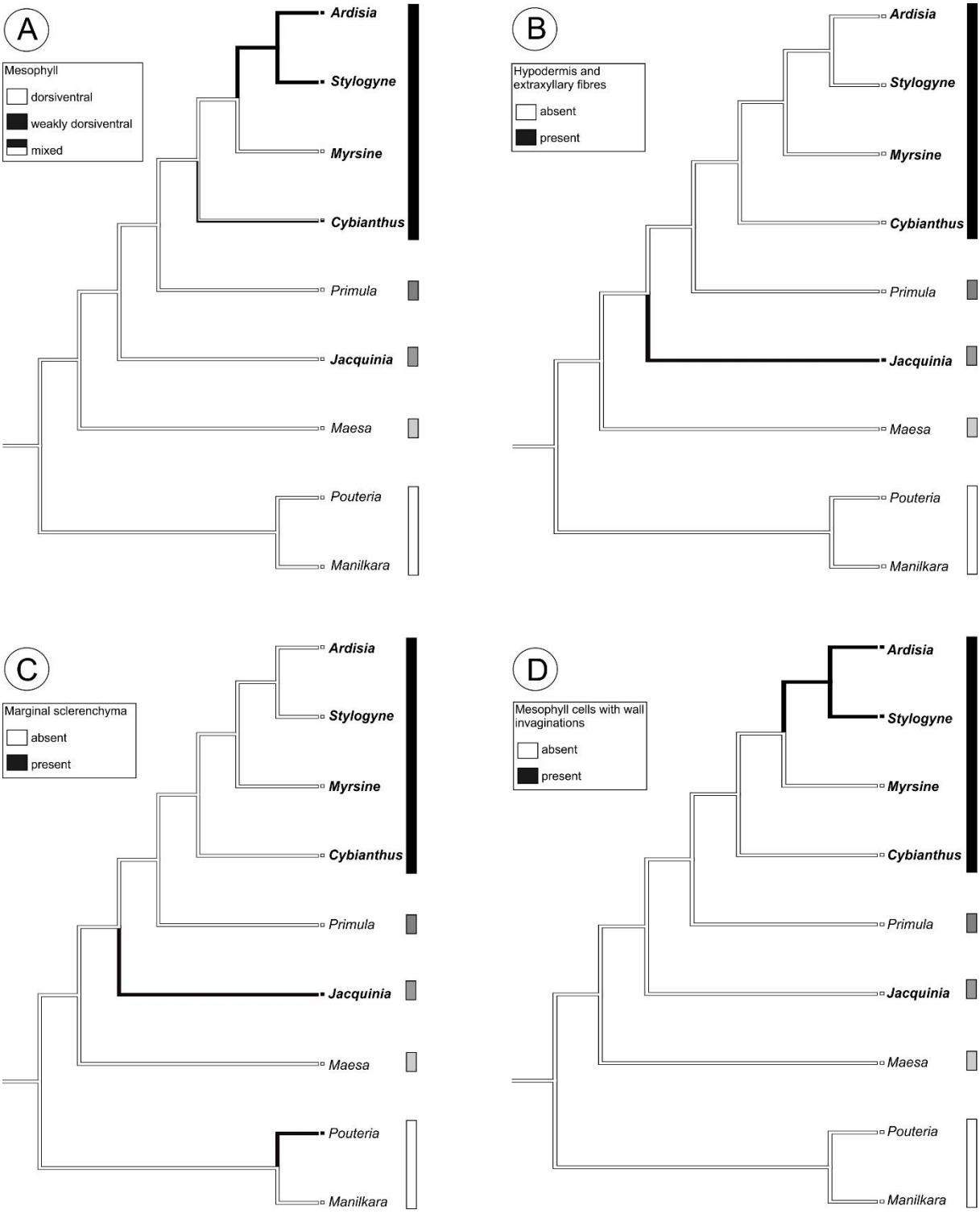

Figure 7 – A-D – Leaf anatomical characters optimized on the strict consensus cladogram. Character names and states are indicated in the boxes

Capítulo 2

WOOD ANATOMY OF SIX NEOTROPICAL GENERA OF PRIMULACEAE.

Este capítulo foi escrito de acordo com as normas do periódico *Botanical Journal of the Linnean Society*

Title: Wood anatomy of six Neotropical genera of Primulaceae.

Abstract

The present work aims to describe the wood anatomy of six genera (27 species) of Neotropical Primulaceae species in order to: 1) identify diagnostic characters at species, genera and subfamily level, 2) to trace wood anatomical characters on a molecular phylogeny and 3) to find anatomical features with potential for future combined morphological and molecular phylogenetic analysis. Wood anatomical analysis were performed according to standard procedures in light microscopy and scanning electron microscopy. The wood of the analyzed Neotropical Primulaceae are characterized by the presence of diffuse porosity, simple perforation plates, septate fibres and scanty paratracheal axial parenchyma, which bears a resemble to previously studied species. Besides that, data analysis demonstrated that the wood anatomy of Theophrastoideae species present rays > 10 cells in width and short vessel elements, while Myrsinoideae present breakdown areas in rays, and the vessel elements are longer. *Ardisia* and *Stylogyne* present scalariform intervessel pits, *Myrsine* exhibit breakdown areas in rays, two *Cybianthus* species that belong to the same subgenera present particularities, such as the presence of scalariform perforation plate in *C. nemoralis*, and the absence of rays in *C. densiflorus*; and *Jacquinia* lacks septate fibres. In summary, wood anatomical characters were able to segregate genera and subfamilies.

Keywords: Primuloid clade – Myrsinoideae – Raylessness – Amazonia – character optimization.

Introduction

During the advent and sophistication of phylogenetic analysis, members of Primulaceae passed through substantial changes until the rise of the recent circumscription of the family (APG III 2009 and APG IV 2016). After several classifications − as the replacement of genera (Anderberg et al. 1995; 1998), the establishment of the old genera *Maesa* to the family Maesaceae (Anderberg et al. 2000), followed by several generic realignments (Källersjo et al. 2000)–APG III (2009) reclassified the Primuloid clade that was previously formed by Maesaceae, Myrsinaceae, Theophrastaceae and Primulaceae and subordinated those families as subfamilies within Primulaceae *sensu lato* (order Ericales) (Stevens *onwards* 2001).

In this current classification, woody Primulaceae species are grouped in the pantropical Myrsinoideae, the Neotropical Theophrastoideae and in the paleotropical Maesoideae. Primuloideae, the remaining subfamily, circumscribes the herbaceous species and is restricted to the temperate regions of the Northern Hemisphere (Stevens 2001 *onwards*). The family comprise 2590 species grouped in 58 genera (APG III). Those subfamilies share a set of morphological characters as the haplostemonous flowers with sympetalous corolla, stamens opposite the petals, free central placentation, bitegmic tenuinucellate ovules and nuclear endosperm (Källersjö et al. 2000).

Anatomical approaches provide relevant information to taxonomic, phylogenetic and ecological studies (Baas et al. 2000, Olson 2005). Specifically, the use of wood anatomical data for phylogenetic purposes has allowed obtaining consistent results, which proves the efficacy of these characters when compared with phylogenies based on molecular data (e.g.: Olson 2002, Lens et al. 2007; 2012).

In Ericales, many studies validate the significance of wood anatomy for the group systematic, as they provide information that are in agreement with the molecular phylogenies of the family (in Ericales - Lens et al. 2007, in Primuloideae – Lens et al.

2005a, in Balsaminoideae – Lens et al. 2005b). Lens *et al.* (2007) gathered data on the wood anatomy of 52 Ericales species and combined it with the molecular data from Schönenberger et al. (2005) in order to demonstrate the systematic value of these characters, highlighting the importance of conducting studies in this area.

Lens et al. (2005a) provided a detailed study on the wood anatomy of the Primuloid clade (APG II classification = Primulaceae), analyzing 78 species grouped in 27 genera. Besides this, only few studies were conducted detailing the aspects of the wood anatomy of Primulaceae (Metcalfe and Chalk 1950, Dayal et al. 1984, Otegui 1994) and to date, none of the previous studies circumscribed woody species from Brazil.

In this work, we present the wood anatomy of Neotropical Myrsinoideae and Theophrastoideae with the description of 6 genera (27 species), discussing the wood anatomical aspects in the light of systematic and phylogeny.

Material and methods

Sampled material

Sampling were performed exclusively from material collected in protected areas from Brazil. Table 1 provides collected species, sites of collection and voucher information. In total, 73 samples of 27 species, varying from sub-shrubs to trees, were collected by a non-destructive method with increment borer, at breast height for trees and at the base for shurbs and sub-shrubs. The number of collected samples per species varied according to the availability of the individuals.

Light microscopy

Wood sections were cut in three planes, and their thickness ranged from 10-25 μm. All samples were bleached, stained with safranin and astra blue (Bukatsch 1972, modified), dehydrated (Johansen 1940; Sass 1958) and mounted in Entellan®. Macerations were

prepared with Franklin solution (Franklin 1945). Dissociated cell elements were stained with aqueous safranin 1% and mounted on semi-permanent slides with glycerin 50% (Strasburger 1924).

The images for descriptions and measurements were obtained with an Olympus BH2 light microscope with a digital image processing system (Cell Sens) fitted with a video camera (Olympus SC30). Images were obtained with an Olympus BX50 attached with an Olympus DP73 digital camera. Descriptions, measurements and wood anatomical terminology followed the "IAWA List of Microscopic Features for Hardwood Identification" (IAWA Committee 1989). Crystals were observed under the polarized light in a Zeiss Axio Imager 2.

Scanning electron microscopy

For scanning electron microscopy, sections were air-dried and after the complete dehydration, fragments were mounted in stubs and were covered with 20 nm of gold in an Emitech K550X apparatus, and observed under a Zeiss EVO 040 scanning electron microscope from Laboratório de Botânica Estrutural from Instituto de Pesquisas Jardim Botânico do Rio de Janeiro.

Data analysis

Principal components analysis (PCA) was used to order species, qualitative and quantitative wood anatomy features, showing the factors with more variance (Ludwig & Reynolds 1988). PCA analyses were performed with Statistica v. 7.0 (for Windows) using thirty-two wood anatomical characters.

To test the efficacy of wood anatomical characters in segregating the subfamilies subordinated to Primulaceae, it was performed a PCA merging results from this research with those obtained by Lens et al. (2005a).

Character evolution

To trace evolution of wood anatomical characters from the Neotropical Primulaceae, there were plotted six characters using parsimony optimization on the strict consensus cladogram obtained in the phylogenetic analysis based on *mat*K sequences (Luna et al. 2017 – chapter 1), using the software Mesquite (Maddison and Maddison 2016) with parsimony optimization. The characters and character states plotted were: breakdown areas in rays (absent: 0; present: 1), type of perforation plates (simple: 0; scalariform: 1), rays (present: 0; absent: 1), ray width (1-3 cells: 0; 3-6 cells: 1; > 10 cells: 2), vessel element length (0: <350μm; 1: >350μm) and axial parenchyma (0: absent; 1: present). Additional anatomical data on *Maesa, Manilkara* and *Pouteria* were assessed from the literature (Metcalfe & Chalk 1950, Lens et al. 2005a and Wheeler 2011). Primuloideae species are not woody, then the characters were not optimized for this subfamily.

Results

Species are presented according to the APG IV (2016) classification. A survey of the wood anatomy results is presented in Table 2.

Myrsinoideae

Ardisia (Figure 1; Table 2)

Growth rings absent or scarcely distinct marked by flattened latewood fibres (Fig. 1A). Wood diffuse-porous. Vessels solitary, in radial multiples of 2-4 in *A. humilis* and 2-8 in *A. solanacea* (Fig. 1A) or in clusters (rare). Vessel outline circular to oval (Fig. 1B). Vessel elements with simple perforation plates (Fig. 1B). Intervessel pits alternate (Fig. 1C) and minute. Vessel-ray pits similar to intervessel pits in shape and size or tending to form scalariform pits (coalescent pit apertures – Fig. 1D and 1E). Septate (Fig. 1F), thin- to thick-walled, with simple to minutely bordered pits. Axial parenchyma scanty

paratracheal; apotracheal difuse (2-4 cells). Rays of 2-10 cells in width, >1 mm high, 1-4 rays per mm (Fig. 1H), heterocellular, integrated by square and upright cells mixed throughout the ray; presence of aggregated rays, sheath cells present (Fig. 1G). Prismatic crystals present in upright and square ray cells (Fig. 1I).

Cybianthus **(Figure 2; Table 2)**

Growth rings indistinct (Fig. 2A). Wood diffuse-porous (Fig. 2A and 2B). Vessels solitary or in radial multiples of 2-10, circular to oval outline. Vessel elements with simple perforation plates (Fig. 2C) and with exclusively scalariform plates with 2-7 bars in *C. nemoralis* (Fig. 2D). Intervessel pits alternate and minute (Fig. 2D and 2F); presence of coalescent pit aperture. Vessel-ray pits similar to intervessel pits in shape and size. Septate and non-septate fibres, thin- to thick-walled, with simple to minutely bordered pits. *C. guyanensis* present portions with very thick-walled fibres (Fig. 2G). Axial parenchyma absent or scanty paratracheal; apotracheal diffuse (2-5 cells). Rays entirely absent in *Cybianthus densiflorus* (Fig. 2I). Rays ≥1 mm high, heterocellular, integrated by upright and square cells mixed throughout the ray (Fig. 2H), 2-10 cells in width, 1-5 per mm, presence of sheath cells. Prismatic crystals present in ray cells. Pith including secretory cavities in *C. brasiliensis* and in *C. guyanensis* (Fig. 2J).

Myrsine **(Figure 3; Table 2)**

Growth rings distinct, marked by thick-walled and radially flattened latewood fibres (Fig. 3A). Wood diffuse-porous (Fig. 3A and 3B). Vessels solitary, in radial multiples of 2-8, circular to oval outline (Fig. 3B). Vessel elements with simple perforation plates (Fig. 3C). Intervessel pits alternate and minute (Fig. 3D). Vessel-ray pits similar to intervessel pits in shape and size. Septate and non-septate fibres, thin- to thick-walled, with simple to minutely bordered pits. Axial parenchyma paratracheal or scanty paratracheal. Rays of 2-

22 cell in width, >1 mm, 1-5 per mm, heterocellular integrated by square, procumbent and upright cells mixed throughout the ray, presence of sheath cells, presence of aggregated rays. Rays with two distinct sizes in *M. glazioviana* (2-5 cells/10-15 cells) and in *M. villosissima* (2-4/22). Breakdown areas in rays in *M. emarginella*, *M. gardneriana*, *M. guianensis*, *M lancifolia*, *M. parvula*, *M. rubra*, *M. squarrosa* and *M. umbellata* (Fig. 3E-G). Pith including secretory cavities in all species (Fig. 3I). Prismatic crystals present in square, procumbent and upright cells (Fig. 3J).

Stylogyne **(Figure 4; Table 2)**

Growth rings distinct, marked by thick-walled and radially flattened latewood fibres (Fig. 4A). Wood diffuse-porous (Fig. 4A-B). Vessels solitary, in radial multiples of 2-8 elements, circular to oval outline (Fig. 4A-B). Vessel elements with simple perforation plates (Fig. 4C). Intervessel pits alternate and minute (Fig. 4D) or scalariform (Fig. 4E). Vessel-ray pits similar to intervessel pits in shape and size or tending to form scalariform pits (coalescent pit apertures). Septate and non-septate (rare) fibres, thin- to thick-walled, with simple to minutely bordered pits. Axial parenchyma absent or scanty paratracheal. Rays of 2-5 cell in width (Fig. 4F), >1 mm high, 2-4 per mm, heterocellular integrated by square and upright cells mixed throughout the ray (Fig. 4G), presence of sheath cells (Fig. 4F), presence of aggregated rays. Prismatic crystals present in square and upright ray cells (Fig. 4G).

Theophrastoideae

Clavija **(Figure 5; Table 2)**

Growth rings indistinct. Wood diffuse-porous (Fig. 5B). Vessels solitary, in radial multiples of 2-6 elements, or in clusters of 3-5 cells (Fig. 5B). Vessel outline circular to oval. Vessel elements with simple perforation plates. Intervessel pits alternate and minute.

Vessel-ray pits similar to intervessel pits in shape and size. Septate and non-septate fibres, thin- to thick-walled, with simple to minutely bordered pits. Axial parenchyma scanty or absent. Rays of 3-8 cells in width, >1mm high (Fig. 5E), integrated by procumbent and square cells mixed throughout the ray (Fig. 5G), presence of sheath cells.

Jacquinia **(Figure 5; Table 2)**

Growth rings indistinct. Wood diffuse-porous (Fig. 5A). Vessels solitary, in radial multiples of 2-8 elements, or in clusters of 3-5 cells (Fig. 5A). Vessel outline circular to oval. Vessel elements with simple perforation plates (Fig. 5C). Intervessel pits alternate (Fig. 5D) and minute. Vessel-ray pits similar to intervessel pits in shape and size. Non-septate fibres, thin- to thick-walled, with simple to minutely bordered pits. Axial parenchyma scanty or absent. Rays of two distinct sizes: >10 cells in width and 5-7 cells in width, >1mm high (Fig. 5F), integrated by procumbent and square cells mixed throughout the ray (Fig. 5H), presence of sheath cells, presence of aggregated rays. Prismatic crystals present in the procumbent ray cells.

Comparisons between genera and subfamilies **(Figures 6 and 7)**

Wood characters contributed to genus segregation as demonstrated by the analysis (Fig. 6). Principal component analysis of the wood anatomical characters reveals that the sum of the first three PC factors accounted for 49 % of the total variance. The loadings of each character used in the principal components analysis are shown in Table 3. The characters with major scores contributed to the formation of two groups observed in PCA, one formed by Myrsinoideae genera (*Ardisia*, *Cybianthus*, *Myrsine* and *Stylogyne*), other by Theophrastoideae (*Clavija* and *Jacquinia*) (Fig. 6).

The first factor accounts for 23% of the total variance, with the presence of indistinct growth rings, vessel element length <350 μm, the absence of axial parenchyma and rays

composed by square and procumbent cells having the highest positive correlation (0.84) and the presence of distinct growth rings with the highest negative correlation (-0.83). The second factor accounts for 14% of total variance, with the ray composition of upright and square cells (0.84) and the growth rings slightly distinct (0.80) having the highest positive correlation. The third factor corresponds to 12% of total variance with the presence of vessel elements length ranging between 350-500 μm (0.84) with the highest positive correlation.

Results from principal components analysis merging current data with those obtained by Lens et al. (2005) revealed the presence of four distinct groups, one formed by species from Maesoideae, other by Theophrastoideae species, the third one by the rayless Myrsinoideae species and the fourth by the remaining Myrsinoideae species with rays (Fig. 7). The character with highest values (> |0.7|) in the first factor were the vessel length > 350 μm (-0.77), > 4 rays/mm (0.73) and rays integrated by square, procumbent and upright ray cells (0.86). In the second factor the characters with highest correlation were the vessel tangential diameter ranging from 20-50 μm (-0.74) and > 50 μm (0.72); and in the third factor the characters having the highest correlation were the presence of unisseriate rays (0.72) and rays of two distinct sizes (0.69).

Character optimization (Figs. 8 and 9)

Breakdown areas in rays were found only in *Myrsine* species (Fig. 8A). In general, simple perforation plates are a synapomorphy for Primulaceae, however, in some *Cybianthus* (Myrsinoideae) and *Maesa* (Maesoideae) scalariform perforation plates had evolved independently (Fig. 8B). The absence of rays is a gain for *Cybianthus densiflorus* and was not observed in any other of the Neotropical species (Fig. 8C). Rays with more than 10 cells in width (Fig. 8D), vessel elements shorter than 350 μm (Fig. 9A) and the absence of axial parenchyma (Fig. 9B) is a synapomorphy for *Jacquinia* (Theophrastoideae).

Discussion

Wood anatomy of Primulaceae, in general, are characterized by the presence of diffuse porosity, simple perforation plates, libriform septate fibres, scanty paratracheal parenchyma, and multiseriate rays (Metcalfe & Chalk 1950; Dayal et al. 1984; Pipoly III 1987; Carlquist 1992; Lens et al. 2005a). These features were also observed in both Myrsinoideae and Theophrastoideae species analyzed in the present work, with a few exceptions.

However, despite those above-mentioned similarities, the compared subfamilies differ in some aspects. Theophrastoideae present shorter vessel elements (196-308 μm), rays with more than 10 cells in width, axial parenchyma absent or extremely rare and rays composed by procumbent and square cells, while Myrsinoideae present higher vessel elements (319-1242 μm), *breakdown areas in rays*. Maesoideae differs from the other woody subfamilies due to the presence of unisseriate rays in the xylem (Lens et al. 2005a).

Besides that, useful characters to distinguish genera and species were the presence or absence of rays, and the cellular composition of rays. Within Theophrastoideae, *Clavija* can be distinguished from *Jacquinia* by the presence of septate fibres. In Myrsinoideae, although their wood structure is considered rather uniform despite their broader habitat occurrence (Lens et al. 2005a), some characters when combined, were able to distinguish genera. *Ardisia*, *Cybianthus* and *Stylogyne* can be segregated from *Myrsine* by the presence of alternate to scalariform intervessel and vessel-ray pits and rays composed by square and upright cells; and *Mysine* differ from the other genera by the presence of *breakdown areas in rays* and multicellular rays composed by procumbent, square and upright cells.

It is known, however, that wood characters may be influenced by environmental factors reflecting ecological adaptations (Carlquist 2001). As stated by Carlquist (2012), the presence of shorter vessels is usually related to dry environments; in this sense, it is possible to assume that in *Jacquinia armillaris* (Theophrastoideae) the presence of shorter vessels

may also be related to the environmental condition from where this species occur, the sandy coastal regions from Brazil (BFG 2015).

The scarcity or absence of axial parenchyma and the presence of septate fibres, both features observed in all Primulaceae species, are strictly related as discussed by Carlquist (2015a), who observed that in species that lacks axial parenchyma, in general, occur septate fibres which acts in the vertical flow in the xylem. Similarly, upright ray cells that occur in all Myrsinoideae species, also contributes to the vertical flow in the wood.

Myrsinoideae present a special type of secretory structure in rays, which are described as breakdown areas in rays in the CSIRO family key for hardwood identification (Ilic 1987) and is the terminology used by Lens et al. (2005a). Such structures were also described in *Embelia ribes* Burm. f., *E. tsjeriamcottam* A.DC., *Myrsine capitellata* Wall. and in *M. semiserrata* Wall. by Dayal et al. (1984) as perforated ray cells. Otegui (1994) observed the occurrence of real perforated ray cells in two *Rapanea* (=*Myrsine*).

Otegui et al. (1998a) described the breakdown areas in rays as a cluster of idioblasts in *M. laetevirens* (Mez) Arechav., the same authors stated that until more detailed aspects about the development of these structures are known, it is not possible to make inferences about their possible homology. In the present study, we classify *breakdown areas in rays* as a type of secretory structure in the ray, as they possess a content. These structures were only observed among *Myrsine* species, and appear as a disjunction between the cell wall of two or more adjacent ray cells. Lens et al. (2005a) also found in *Aegiceras* Gaertn., *Badula* Juss., *Discocalyx* Mez, *Embelia* Burm. f. and *Parathesis* (A. DC.) Hook. f. We did not observe such structures in *Clavija* nor in *Jacquinia* and there are no records about the presence of this type of secretory structure in Primuloideae, Theophrastoideae or Maesoideae.

Lens et al. (2005), in a combined analysis using wood anatomy and molecular data in the Primuloid clade (=Primulaceae) concluded that these structures represent a synapomorphy of Myrsinoideae (=Myrsinaceae), as they were only found in genera

circumscribed in this subfamily. There are no records about the specific function of these structures. Lens et al. (2007) mentioned that these structures constitutes a cavity, however they are undoubtly different from the secretory cavities found in the leaves (Luna et al. 2013), flowers (Otegui & Cocucci 1999), fruits, and in the pith of the wood. Althought, it was not possible to trace the exactly ontogeny of this structure it is possible to presume that once they are found in rays, they are derived from the ray initials from the vascular cambium.

According to Agostini (1980), the genus *Cybianthus* is divided into ten subgenera and the analyzed species studied in this work are grouped into three of the total subegenera: *Conomorpha* (*C. brasiliensis* and *C. guyanensis*), *Cybianthus* (*C. glaber* and *C. venezuelanus*) and *Weilgetia* (*C. densiflorus* and *C. nemoralis*). According to PCA, none of the characters were able to support species according to their subgenera, however some features were specifically found in *Weilgetia* species as the presence of vessel elements with scalariform perforation plate in *C. nemoralis* and the absence of rays in *C. densiflorus*. It is believed that scalariform perforation is a primitive characteristic in relation to the simple perforation plates (Bailey 1944, Lens et al. 2016) which may indicate that this subgenus circumscribes species that retains ancestral characters.

We reported here, for the first time, the absence of rays in the Neotropical *Cybianthus densiflorus*, a species from the Amazonia Forest. According to Carlquist (2015b), raylessness is a manifestation of secondary woodiness and reflects juvenilistic ontogeny, which means the lack of horizontal subdivisions in ray initials as opposed to the commonness of such divisions in truly woody species. In Primulaceae, such phenomena were previously observed in two species from Myrsinoideae subfamily: *Lysimachia kalalauensis* Skottsb. (Carlquist 1974) and *Coris monspeliensis L.* (Lens et al. 2005a). The significance of the absence of rays appears to be a gain in mechanical strength for a narrow stem in which radial conduction of photosynthates is minimal (Carlquist 2013).

The presence of secondary woodiness is believed to be an additional evidence for the herbaceous origin of a taxon (Carlquist 1992). Lens et al. (2005a), based on the results of wood anatomy of Primulaceae species, discuss that seems to be unlikely that the possible ancestral of the Primuloid clade (=Primulaceae) is herbaceous as suggested by Anderberg et al. (2001). However, this new evidence in *Cybianthus* indicates the need of further investigations in this area especially in the subgenus *Weilgetia*.

Tracing the wood anatomical characters over the stric consensus tree was possible to assess wood anatomical synapomorphies for *Jacquinia*: the absence of axial parenchyma, vessel elements shorter than 350 μm and rays with more than 10 cells in width. For Myrsinoideae the synapomorphies that support the subfamily is the presence of vessel elements longer than 350 μm and the presence of rays with 3-6 cells in width, which has also evolved independently in the subfamily Maesoideae.

In summary, based on wood anatomy it was possible to segregate the Neotropical Primulaceae subfamilies from each other and from the remaining woody subfamily, Maesoideae. Moreover, when combining characters, it was possible to segregate genera. According to the results obtained in the present studies, the more suitable characters for this type of approach are the cellular ray composition, the presence of secretory structures in ray and variation of vessel length, frequency and diameter.

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Legends

Table 1 – Material of Primulaceae used for wood anatomical analyzes with voucher information, collection site and growth form.

Table 2 – Survey of wood anatomical data of Primulaceae.

Table 3 – Loadings of the first three factors of the principal components analysis with wood anatomical characters of Neotropical Primulaceae.

Table 4 - Loadings of the first three factors of the principal components analysis with wood anatomical characters of Maesoideae, Myrsinoideae and Theophrastoideae.

Figure 1 – Wood anatomy of *Ardisia*. A – D – *A. solanacea*. E – H – *A. humilis*. A – Growth ring boundaries. Wood diffuse porous. B – Vessel element with simple perforation plate. C – Intervessel pits alternate with distinct borders. D – Inner view of a vessel-ray pitting, under SEM, evidencing the tendency to form scalariform pits (►). E – Outer view of a vessel-ray pitting, under LM, evidencing pits with distinct borders. F – Septate fibres (arrow). G – Multiseriate rays with. H – Prismatic crystals in the upright and square ray cells.

Figure 2 – Wood anatomy of *Cybianthus*. A – *C. verticilatus*. B – *C. venezuelanus*. C – G – *C. guyanensis.* H – *C. densiflorus.* I – *C. brasiliensis.* A – Indistinct growth ring boundaries. B – Wood diffuse-porous. C – Simple perforation plates. D – Intervessel pits alternate with distinct borders, outer view. E – Inner view of intervessel pits. F – Very thick-walled fibres. G – Square and upright ray cells. H – Absence of rays. I – Secretory cavities in the pith.

Figure 3 – Wood anatomy of *Myrsine*. A-C and E-F – Light microscopy; D and G – Scanning electron microscopy; I – Epifluorescence and polarized microscopy. A - Distinct

growth boudaries in *M. lineata*. B – Wood diffuse-porous in *M. emarginella*. C – Vessel elements with simpe perforation plates in *M. glazioviana*. D – Alternate intervascular pits in *M. glazioviana*. E – Multicellular rays *M. lancifolia*. Detail of the ray showing breakdown areas in rays *M. guianensis* (F) and *M. squarrosa* (G). H – Fresh section of *M. umbellata*, showing the breakdown area in ray (arrow head), without any type of dying. I – Radial section of *M. umbellata* stained with Sudan III, showing the secretion within the breakdown area in ray. J – Starch grains in the cells of the rays, stained with Lugol. K – Pith, showing secretory cavities in *M. lineata*. L – Prismatic crystals in the ray of *M. gardneriana*.

Figure 4 – Wood anatomy of *Stylogyne*. A – *S. pauciflora*. B-G – *S. rodriguesiana.* A – Growth ring boundaries. C – Simple perforation plate. D – Intervessel pit alternate. E – Scalariform pit. F – Multiseriate ray. G – Heterocellular rays.

Figure 5 – Wood anatomy of *Jacquinia*. A – Wood diffuse-porous. B – Simple perforation plates. C – Intervessel pits alternate. D – Multiseriate rays. E – Procumbent ray cells.

Figure 6 – 3D plot of three factor from principal components analysis. Factor 1 - 27.97%, Factor 2 – 19.91%, Factor 3 - 11.89%.1 – *Ardisia humilis*, 2 – *A. solanacea*, 3 –*Cybianthus brasiliensis*, 4 – *C. densiflorus*, 5 – *C. glaber*, 6 – *C. guyanensis*, 7 – *C. nemoralis*, 8 – *C. venezuelanus*, 9 – *Jacquinia armillaris*, 10 – *Clavija nutans*, 11 – *C. spinosa*, 12 – *C. weberbaueri*, 13 – *Myrsine coriacea*, 14 – *M. emarginella*, 15 – *M. gardneriana*, 16 – *M. glazioviana*, 17 – *M. guianensis*, 18 – *M. lancifolia*, 19 – *M. lineata*, 20 – *M. parvifolia*, 21 – *M. parvula*, 22 – *M. rubra*, 23 – *M. squarrosa*, 24 – *M. umbellata*, 25 – *M. villosissima*, 26 – *Stylogyne atra,* 27 – *S. pauciflora*.

Figure 7 – 3D plot of three factor from principal components analysis merging data from current work and those from Lens et al. (2005a).

Figure 8 – Wood anatomical characters optimized on the strict consensus cladogram from Luna et al. (2017 – chapter 1). Characters and their states are indicated in the boxes.

Figure 9 – Wood anatomical characters optimized on the strict consensus cladogram from Luna et al. (2017 – chapter 1). Characters and their states are indicated in the boxes.

Table 1 - Material of Primulaceae used for wood anatomical analyzes with voucher information, collection site and growth form.

Species	Collection site	Growth form	RBw	Slide collection
	MYRSINOIDEAE			
Ardisia humilis Vahl	Arboreto JBRJ – RJ	Shrub	10303	2722
Ardisia solanacea Roxb.	Arboreto JBRJ – RJ	Shrub	10304	2723
Cybianthus brasiliensis (Mez) G. Agostini	Parque Estadual do Ibitipoca - MG	Small tree / shrub	10305	2800
Cybianthus densiflorus Miq.	Reserva Ducke – AM	Subshrub	10312	2809
	Reserva Ducke – AM	Subshrub	10314	2807
	Reserva Ducke – AM	Subshrub	10313	2778
Cybianthus glaber A. DC.	Parque Nacional do Itatiaia – RJ	Shrub	10173	2772
Cybianthus guyanensis (A. DC.) Miq.	Reserva Ducke – AM	Small tree / shrub	10306	2808
	Reserva Ducke – AM	Small tree / shrub	10307	2776
	Reserva Ducke – AM	Small tree / shrub	10308	2777
	Reserva Ducke – AM	Small tree / shrub	10309	2810
	Reserva Ducke – AM	Small tree / shrub	10310	2779
	Reserva Ducke – AM	Small tree / shrub	10311	2826
Cybianthus nemoralis (Mez) G. Agostini	Ilhéus- BA	Shrub	10316	2832
Cybianthus venezuelanus Mez	Reserva Ducke – AM	Shrub	10315	2775
Myrsine coriacea (Sw.) R. Br. Ex Roem. & Schult.	Estrada para Paraty – RJ	Tree	10321	2726
	Paraty - RJ	Tree	10322	2727
	Parque Nacional do Caparaó - MG	Tree	10323	2801
	Bosque da Barra – RJ	Tree	10324	2818
Myrsine emarginella Miq.	Parque Estadual do Ibitipoca - MG	Tree	10325	2803
Myrsine gardneriana A. DC.	Parque Estadual do Ibitipoca - MG	Small tree	10326	2796
	Parque Estadual do Ibitipoca - MG	Small tree	10327	2825
	Parque Estadual do Ibitipoca - MG	Small tree	10331	2797
	Parque Estadual do Ibitipoca - MG	Small tree	10328	2814
	Parque Nacional do Caparaó - MG	Small tree	10329	2815
	Parque Nacional do Caparaó - MG	Small tree	10330	2813
Myrsine glazioviana Warm.	Parque Estadual do Ibitipoca - MG	Shrub	10332	2782
	Parque Estadual do Ibitipoca - MG	Shrub	10333	2794
	Parque Estadual do Ibitipoca - MG	Shrub	10334	2795
Myrsine guianensis (Aubl.) Kuntze	Parque Estadual Costa do Sol – RJ	Shrub	10335	-
	Parque Estadual Costa do Sol – RJ	Shrub	10336	2771
	Parque Estadual Costa do Sol – RJ	Shrub	10337	2729
	Parque Estadual Costa do Sol – RJ	Shrub	10338	2728
	Restinga Grumari – RJ	Small tree	10339	2731
	Restinga Grumari – RJ	Small tree	10340	2732

	Restinga Grumari – RJ	Small tree	10341	2828
	Restinga Grumari – RJ	Small tree	10340	-
Myrsine lancifolia Mart.	Parque Estadual do Ibitipoca - MG	Tree	10343	2802
	APA Andorinhas – MG	Tree	10344	2812
Myrsine lineata (Mez) Imkhan.	Parque Nacional do Itatiaia – RJ	Tree	10176	2773
	Parque Nacional do Itatiaia – RJ	Tree	10346	2827
	Parque Estadual do Ibitipoca - MG	Tree	10347	2798
Myrsine parvifolia A. DC.	Praia Seca – RJ	Shrub	10348	2829
	Praia Seca – RJ	Shrub	10349	2830
	Praia Seca – RJ	Shrub	10350	2831
Myrsine parvula (Mez) Otegui	Parque Estadual do Ibitipoca - MG	Tree	10352	2805
	Parque Estadual do Ibitipoca – MG	Tree	10351	2816
Myrsine rubra M.F. Freitas & Kin.-Gouv.	Bosque da Barra – RJ	Tree	10358	2817
	Bosque da Barra – RJ	Tree	10359	2819
	Bosque da Barra – RJ	Tree	10360	2824
	Bosque da Barra – RJ	Tree	10361	2820
	Bosque da Barra – RJ	Tree	10362	2823
	Bosque da Barra – RJ	Tree	10363	2821
Myrsine squarrosa (Mez) M.F. Freitas & Kin. - Gouv.	Parque Estadual do Ibitipoca – MG	Shrub	10353	2836
	Parque Estadual do Ibitipoca – MG	Shrub	10354	2837
	Parque Estadual do Ibitipoca – MG	Shrub	10355	2804
Myrsine umbellata Mart.	Parque Nacional do Itatiaia – RJ	Tree	10174	2838
	Parque Nacional do Itatiaia – RJ	Tree	10175	-
	Parque Nacional do Itatiaia – RJ	Tree	10356	2806
	Parque Estadual do Ibitipoca – MG	Tree	10357	2799
Myrsine villosissima Mart.	APA Andorinhas – MG	Shrub	10364	2811
Stylogyne atra Pipoly	Reserva Ducke – AM	Shrub	10366	2781
	Reserva Ducke – AM	Shrub	10365	2780
Stylogyne pauciflora Mez	Prainha – RJ	Shrub	10367	2774
	THEOPHRASTOIDEAE			
Clavija nutans (Vell.) B.Ståhl	Ribeirão Preto – SP	Shrub	-	2835
Clavija spinosa Vell. (Mez)	Itaipuaçu, Niterói – RJ	Shrub	-	2833
Clavija weberbaueri Mez	Alto Solimões – AM	Shrub	-	2834
Jacquinia armillaris Jacq.	Parque Estadual Costa do Sol – RJ	Shrub	10172	2724
	Parque Estadual Costa do Sol – RJ	Shrub	10317	2767
	Parque Estadual Costa do Sol – RJ	Shrub	10318	2725
	Parque Estadual Costa do Sol – RJ	Shrub	10319	2768
	Parque Estadual Costa do Sol – RJ	Shrub	10320	2769
	Parque Estadual Costa do Sol – RJ	Shrub	-	2770

Table 2 – Survey of Primulaceae wood anatomical characters. Values are minimum-mean-maxima.

Species	VF	VL	TD	A	VWT	IP	CP	FL	FWT	SF	RF	BAR
Myrsinoideae												
Ardisia humilis	36-52-61	446-584-772	26-54-83	1226-2454-3889	4-6-8	2-3-4	+	691-945-1085	7-9-11	+	1-2-4	-
A. solanacea	40-56-72	591-1242-1823	33-45-56	905-1623-2406	4-5-6	2-3-4	+	1409-2128-2578	6-9-12	+	2-3-4	-
Cybianthus brasiliensis	98-104-111	278-367-504	34-48-69	601-1540-2405	3-6-8	2-3-4	-	442-554-660	6-8-10	-	2-3-4	-
C. densiflorus	140-174-204	235-340-412	12-17-21	244-384-601	3-4-5	0.8-1-2	-	320-390-495	3-4-6	+	-	-
C. guyanensis	34-89-173	283-610-1101	16-32-53	352-992-2298	2-3-5	1-2-3	+	440-966-1436	4-8-16	+	1-2-5	-
C. nemoralis	50-65-95	285-347-443	25-31-39	449-795-1353	2-4-5	1-2-3	+	358-493-631	4-6-7	+	2-3-4	-
C. venezuelanus	160-209-258	328-431-535	21-30-46	398-792-1282	3-4-5	1-2-3	-	504-634-798	5-6-8	+	1-2-3	-
C. verticilatus	130-167-228	344-548-643	19-24-31	279-622-1045	1-3-4	1-2-3	+	491-755-1120	5-8-12	-	2-3-4	-
Myrsine coriacea	25-31-44	205-504-944	45-70-105	1324-3718-6371	3-4-6	1-2-3	+	442-817-1239	5-9-16	+	1-2-3	+
M emarginella	75-93-108	344-464-573	30-42-54	729-1876-2728	3-5-8	2-3-4	-	488-672-948	5-7-9	+	2-3-5	+
M. gardneriana	48-106-166	239-384-587	16-35-50	497-1345-3955	2-4-7	1-2-4	+	376-580-894	4-7-12	+	1-2-4	+
M. glazioviana	133-202-272	172-319-458	15-30-44	318-870-1794	2-3-6	2-3-4	+	343-529-1067	4-6-9	+	1-3-5	-
M. guianensis	25-48-93	313-550-749.3	31-54-88	808-2699-5495	2-5-10	1-2-4	+	586-813-1090	7-10-14	+	1-2-5	+
M. lancifolia	74-116-145	300-499-691	25-40-69	777-1540-3106	1-3-5	2-3-4	-	469-703-874	9-15-21	+	1-2-3	+
M. lineata	85-120-160	300-499-720	20-30-40	359-1050-1794	1-3-4	1-3-4	+	486-781-1124	5-8-14	+	2-3-4	-
M. parvifolia	67-120-158	220-405-557	20-33-50	600-1195-2192	2.5-4-6	0.8-1.5-3	+	438-613-894	5-8-11	+	1-2-3	+
M. parvula	61-89-134	204-438-651	26-42-63	716-1763-3897	2-3-5	0.7-1-3	-	373-620-922	6-8-12	+	2-3-4	+
M. rubra	59-87-140	320-474-661	26-50-82	906-2331-4180	2-3-5	1-2-3	-	433-724-1023	4-7-10	+	1-2-4	+
M. squarrosa	80-115-202	278-389-536	20-34-48	601-1363-2863	2-3-6	2-3-5	+	345-558-800	4-7-11	+	2-3-4	+
M. umbellata	30-54-71	292-462-715	22-46-65	840-2169-3360	1-2-3	1-2-3	+	523-765-1167	4-7-10	+	2-3	+
M. villosissima	136-152-176	327-464-704	29-38-47	1353-1670-2516	2-3-5	2-2.5-3	-	419-572-821	4-7-9	+	1-2-2	-
Stylogyne atra	41-68-102	257-395-529	15-24-35	359-777-1353	2-3-5	1-1.5-2	+	428-606-916	4-7-10	+	2-3-4	-
S. pauciflora	67-80-95	222-385-530	20-29-37	446-836-1410	3-4-5	1-2-3	+	533-687-829	4-5-8	+	2-2-4	-
Theophrastoideae												
Clavija nutans	146-194-253	205-308-444	17-20-26	402-655-974	1.4-2.5-4	1-2-3	-	662-1037-1749	4-5-7	+	4	-
C. spinose	111-122-133	119-196-320	15-20-26	285-465-703	1.5-2.5-3	1-2-3	-	211-330-466	4-5-7	+	2-3-4	-
C. weberbaueri	95-98-99	186-307-403	30-40-55	233-384-497	4-6-8	0.7-1-1.4	-	341-423-512	4-6-7	+	4-6	-
Jacquinia armillaris	53-90-135	123-198-363	11-24-50	657-1982-2516	2-3-6	1-2-3	-	253-385-607	4-8-14	-	1-2-3	-

Notes: VF = Vessel frequency (/mm^2); VL = Vessel length (µm); TD = Tangential diameter (µm); A = Area; VWT = Vessel wall thickening (µm); IP = intervessel pit diameter (µm); VR = vessel-ray pit diameter (µm); CP = coalescent pits; FL = Fibre length (µm); FWT = Fibre wall thickening; SF = Septate fibres; RF = Ray frequency (/mm'); BAR = Breakdown areas in rays.

Table 3 – Loadings of the first three factors of the principal components analysis with wood anatomical characters of Neotropical Primulaceae.

Characters	**Factor 1 23%**	**Factor 2 14%**	**Factor 3 12%**
Growth rings distinct	**-0.831314**	-0.381714	0.061407
Growth rings slightly distinct	0.245664	**0.804310**	0.118333
Growth rings indistinct	**0.842548**	-0.422390	-0.221957
Vessel frequency: 30-50 vessels/mm²	0.405397	-0.569917	0.295375
Vessel frequency: 50-100 vessels/mm²	-0.454198	0.093299	0.230484
Vessel frequency: 100-200 vessels/mm²	0.254491	0.247299	-0.349894
Vessel frequency: > 200 vasos/mm²	-0.127073	0.163887	-0.230341
Vessel length < 350 µm	**0.824241**	-0.096752	0.019762
Vessel length 350-500 µm	-0.750183	-0.150991	-0.416625
Vessel length 500-650 µm	-0.000580	-0.048982	**0.848946**
Vessel length > 650µm	-0.043692	0.169162	0.164752
Vessel tangential diameter 20-50 µm	0.047805	0.189786	-0.714149
Vessel tangential diameter >50 µm	-0.088301	-0.310502	0.780028
Scalariform perforation plate	0.216499	0.352280	0.152409
Simple perforation plate	-0.216499	-0.352280	-0.152409
Septate fibres	-0.380405	-0.094004	0.029695
Fibre lenght 300-500 µm	0.783149	-0.177543	-0.105661
Fibre lenght 500-700 µm	-0.405831	0.336583	-0.598957
Fibre lenght 700-900 µm	-0.310398	-0.362481	0.364085
Fibre lenght 900-1100 µm	0.215498	0.072309	0.459777
Fibre lenght >1100 µm	-0.043692	0.169162	0.164752
Axial parenchyma rare or absent	**0.842548**	-0.422390	-0.221957
Rays with > 10 cells width	0.497258	-0.302244	-0.113233
Raios de dois tamanhos distintos	-0.232668	-0.097973	-0.171943
< 4 raios/mm	-0.153263	-0.492229	0.125447
Rays with > 1 mm height	-0.153263	-0.492229	0.125447
Upright and square ray cells	0.108929	**0.843702**	0.349252
Square and procumbent ray cells	**0.831667**	-0.429694	-0.233478
Upright, square and procumbent ray cells	-0.704313	-0.515102	-0.179734
Breakdown areas in rays	-0.559582	-0.377840	-0.201917

Table 4 - Loadings of the first three factors of the principal components analysis with wood anatomical characters of Maesoideae, Myrsinoideae and Theophrastoideae.

Characters	Factor 1	Factor 2	Factor 3
Growth rings distinct	0.502536	-0.669196	-0.096647
Growth rings slightly distinct	0.239044	0.238738	0.546520
Growth rings indistinct	-0.517750	0.672985	0.081501
Vessel frequency: <30 vessels/mm²	0.148982	0.490024	-0.194127
Vessel length < 350 µm	**-0.778410**	-0.204793	0.092841
Vessel length 350-500 µm	0.404123	-0.459036	-0.096408
Vessel length 500-650 µm	0.375029	0.425604	0.204449
Vessel length > 650µm	0.038862	0.352838	-0.310165
Vessel tangential diameter 20-50 µm	-0.098517	**-0.749280**	0.175070
Vessel tangential diameter >50 µm	0.128392	**0.729895**	-0.191786
Sclariform perforation plates	0.112218	0.301426	0.301056
Simple perforation plates	0.041182	0.005702	-0.155934
Scalarifom pits	0.263498	0.519090	0.141004
Septate fibres	0.576136	0.176813	-0.175379
Fibre lenght 300-500 µm	-0.688704	-0.128054	0.081430
Fibre lenght 900-1100 µm	0.198934	0.383822	0.062253
Fibre lenght >1100 µm	0.101129	0.513940	-0.025264
Unisseriate rays	0.223442	0.296609	**0.721600**
Rays with > 10 cells width	-0.664761	0.169842	-0.381448
Rays of two distinct sizes	0.301991	0.166978	**0.696857**
< 4 rays/mm	**0.736117**	-0.064777	0.224951
Rays > 1 mm height	0.323717	0.282535	-0.480962
Rays integrated by square, procumbent and upright cells	**0.863197**	0.265699	-0.018912
Breakdown areas in rays	0.380644	-0.101689	-0.223695
Rayless wood	-0.331497	-0.288991	0.371633
Prysmatic crystals	0.262835	-0.151980	-0.349464

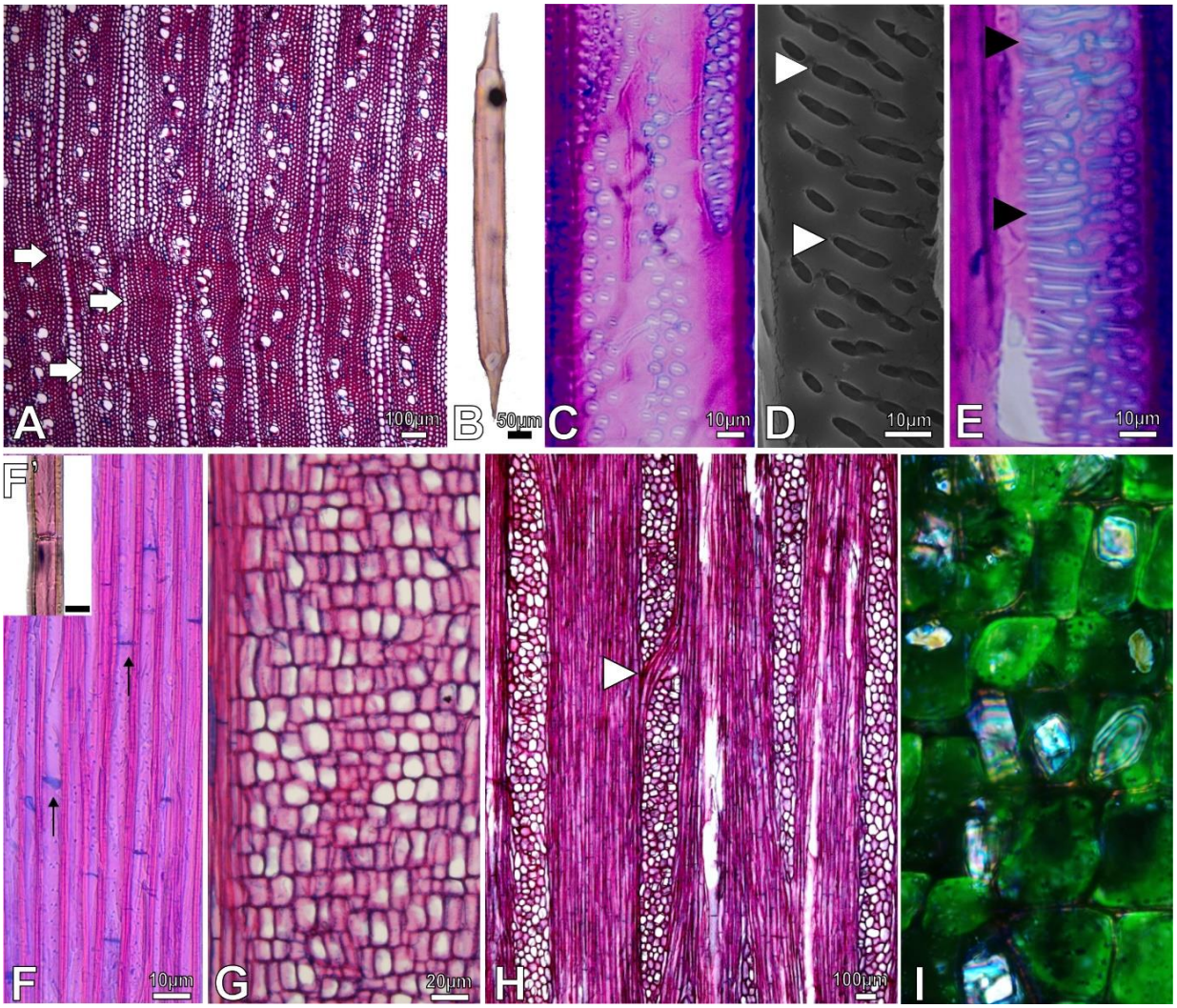
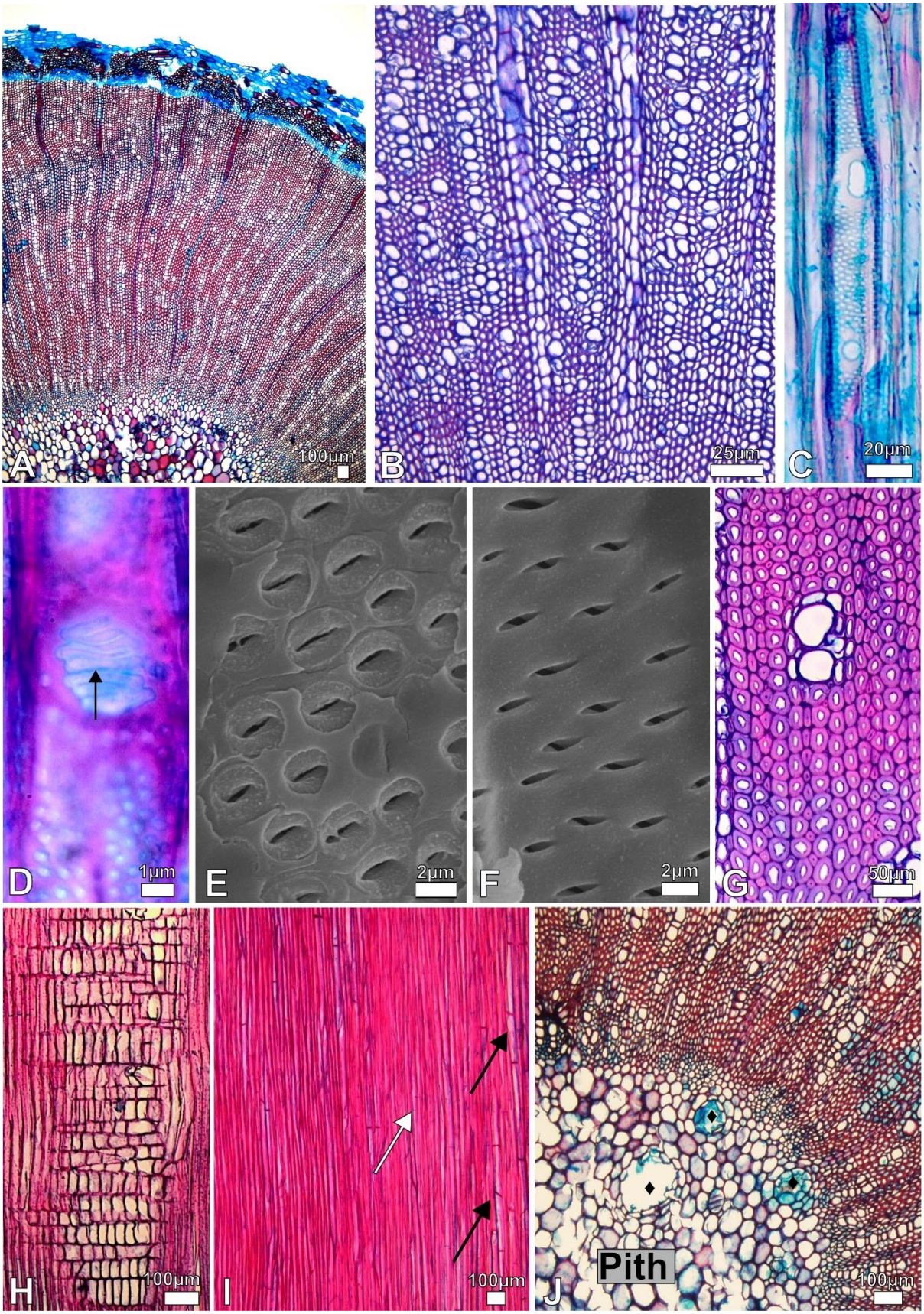


Figure 1 – Wood anatomy of *Ardisia*. A-C and E-G – Light microscopy. D – Scanning electron microscopy. H – Epifluorescence and polarized microscopy. A – D – *A. solanacea*. E – H – *A. humilis*. A – Growth ring boundaries (arrows). Wood diffuse porous. B – Vessel element with simple perforation plate. C – Intervessel pits alternate with distinct borders. D – Inner view of a vessel-ray pitting, under SEM, evidencing the tendency to form scalariform pits (►). E – Outer view of a vessel-ray pitting, under LM, evidencing pits with distinct borders. F – Septate fibres (arrow), F' presents the septa in detail. G – Radial longitudinal of multiseriate rays. H – Tangential longitudinal section of multiseriate rays. Arrow head indicate aggregated rays. I – Prismatic crystals in the upright and square ray cells.

Figure 2 – Wood anatomy of *Cybianthus*. A-D and G-J – Light microscopy; E and F – Scanning electron microscopy. A – *C. verticilatus*. B – *C. venezuelanus*. C – G – *C. guyanensis.* H – *C. densiflorus.* I – *C. brasiliensis.* A – Indistinct growth ring boundaries. B – Wood diffuse-porous. C – Simple perforation plates. D – Intervessel pits alternate with distinct borders, outer view. E – Inner view of intervessel pits. F – Very thick-walled fibres. G – Square and upright ray cells. H – Absence of rays. I – Secretory cavities in the pith.


A
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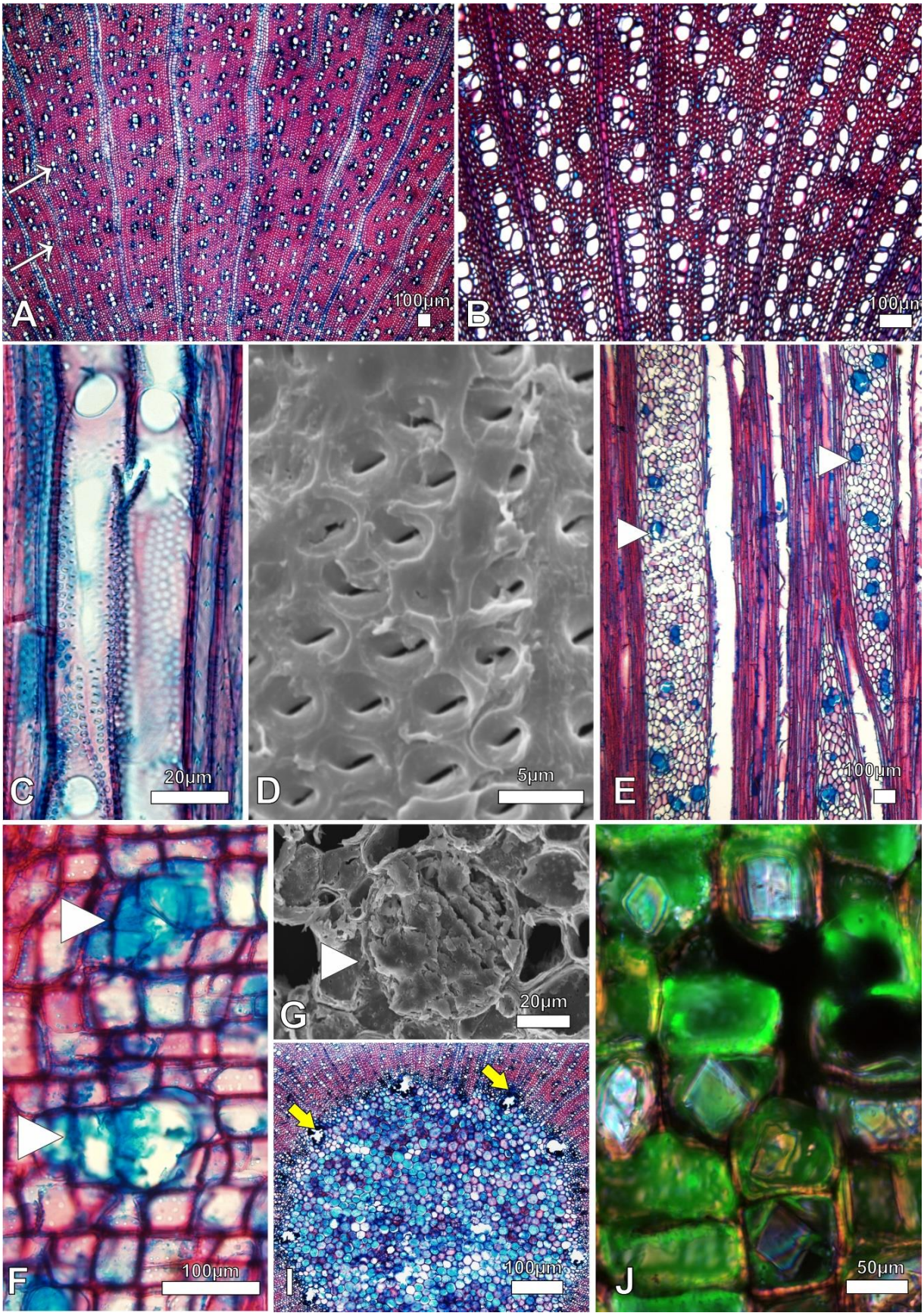
B
25µm
C
20µm
D
1µm
E
2µm
F
2µm
G
50µm
H
100µm
I
100µm
J
Pith
100µm

Figure 3 – Wood anatomy of *Myrsine*. A-C and E-F – Light microscopy; D and G – Scanning electron microscopy; I – Epifluorescence and polarized microscopy. A - Distinct growth boudaries in *M. lineata*. B – Wood diffuse-porous in *M. emarginella*. C – Vessel elements with simpe perforation plates in *M. glazioviana*. D – Alternate intervascular pits in *M. glazioviana*. E – Multicellular rays *M. lancifolia*. Detail of the ray showing breakdown areas in rays *M. guianensis* (F) and *M. squarrosa* (G). H – Fresh section of *M. umbellata*, showing the breakdown area in ray (arrow head), without any type of dying. I – Radial section of *M. umbellata* stained with Sudan III, showing the secretion within the breakdown area in ray. J – Starch grains in the cells of the rays, stained with Lugol. K – Pith, showing secretory cavities in *M. lineata* (yellow arrows). L – Prismatic crystals in the ray of *M. gardneriana*.


A
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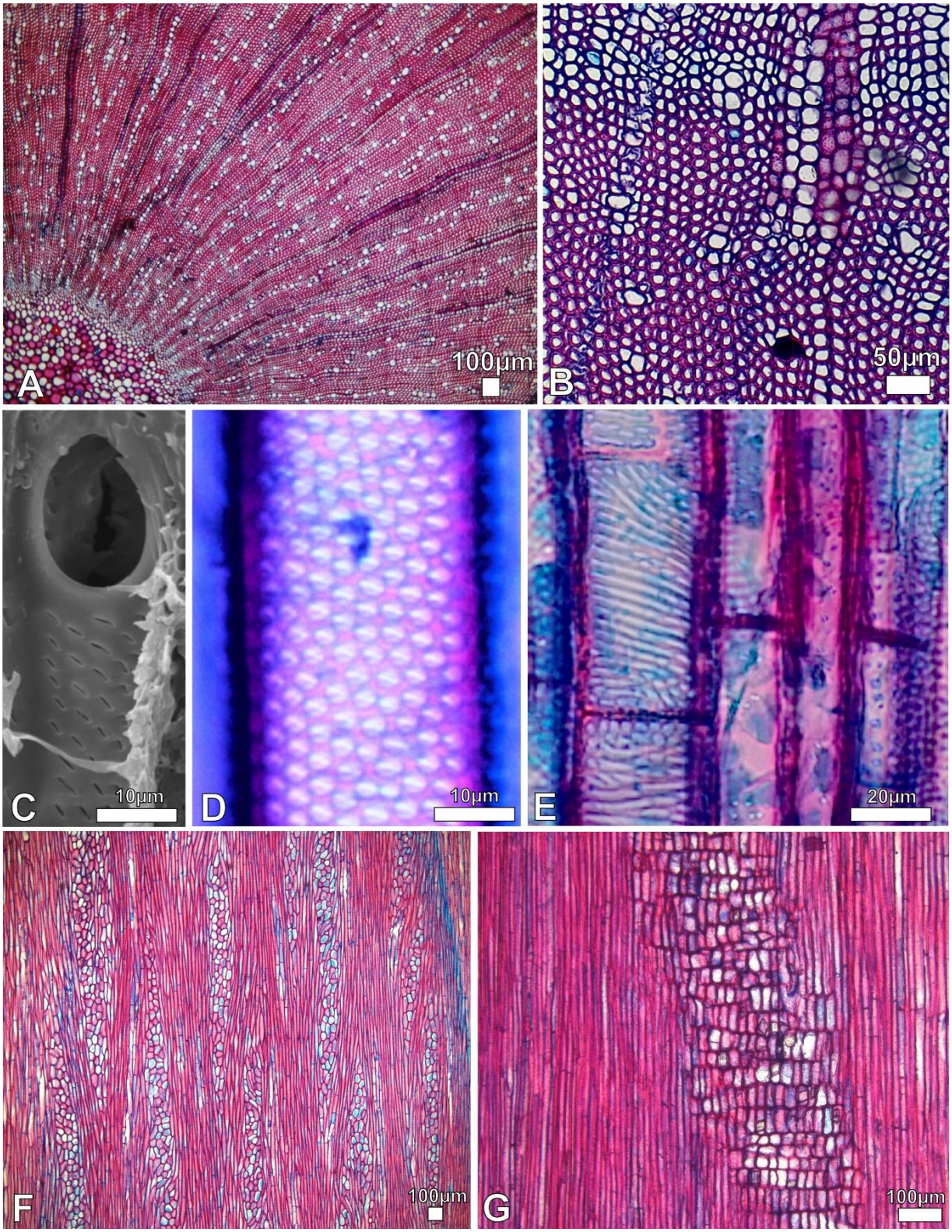

Figure 4 – Wood anatomy of *Stylogyne*. A, B and D-G – Light microscopy; C – Scanning electron microscopy. A – *S. pauciflora*. B-G – *S. rodriguesiana.* A – Growth ring boundaries. C – Vessel element with simple perforation plate. D – Intervessel pit alternate. E – Scalariform pits. F – Multiseriate ray. G – Heterocellular rays.

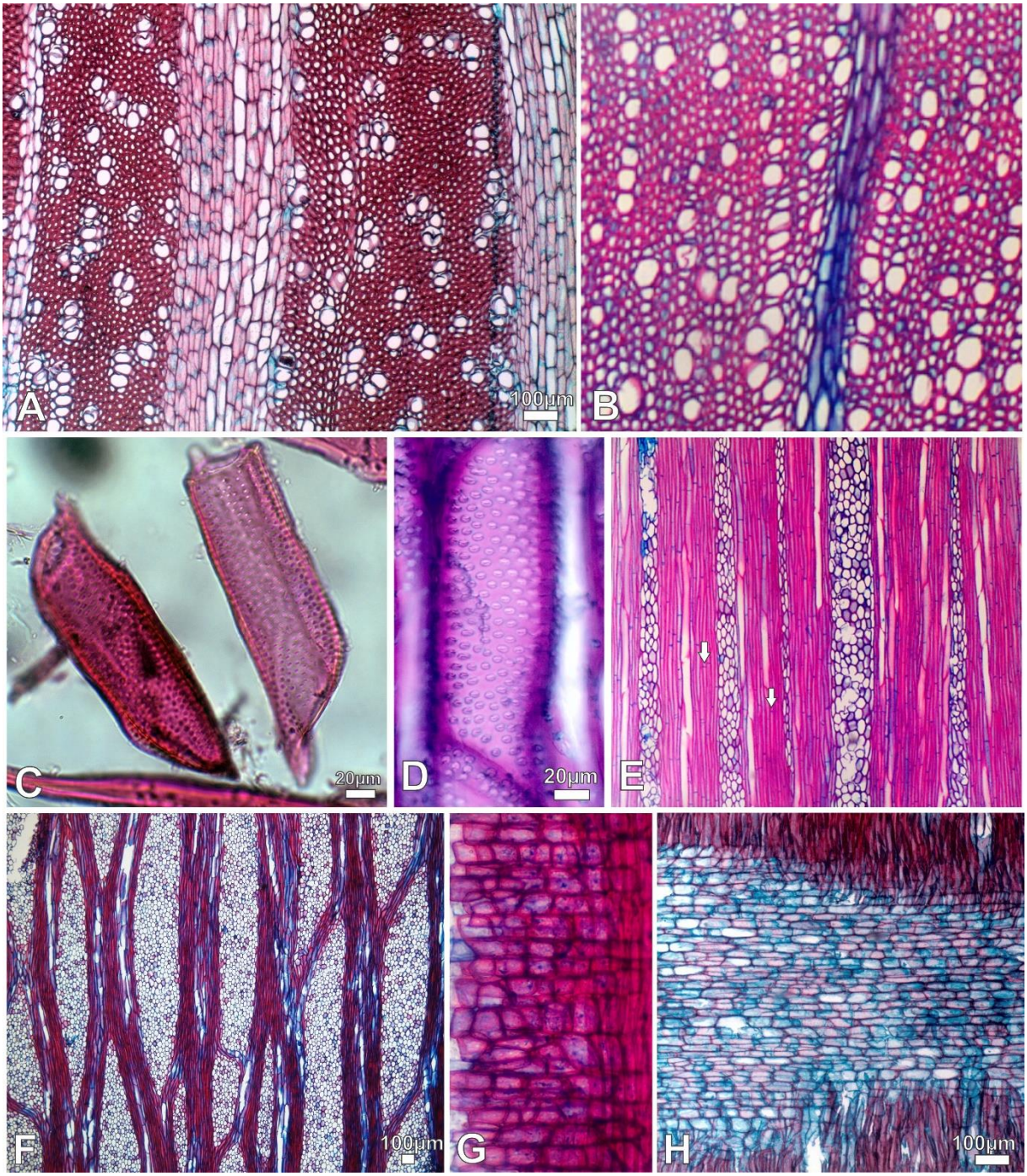

Figure 5 – Wood anatomy of *Claviija* (B, E and G) and *Jacquinia* (A, C, D, F and H). A-H – Light microscopy. A and B – Wood diffuse-porous. C – Vessel elements with simple perforation plates. D – Intervessel pits alternate. E – Multiseriate rays < 10 cells width and septate fibres (arrows). F - Multiseriate rays > 10 cells width. G and H – Square and procumbent ray cells.

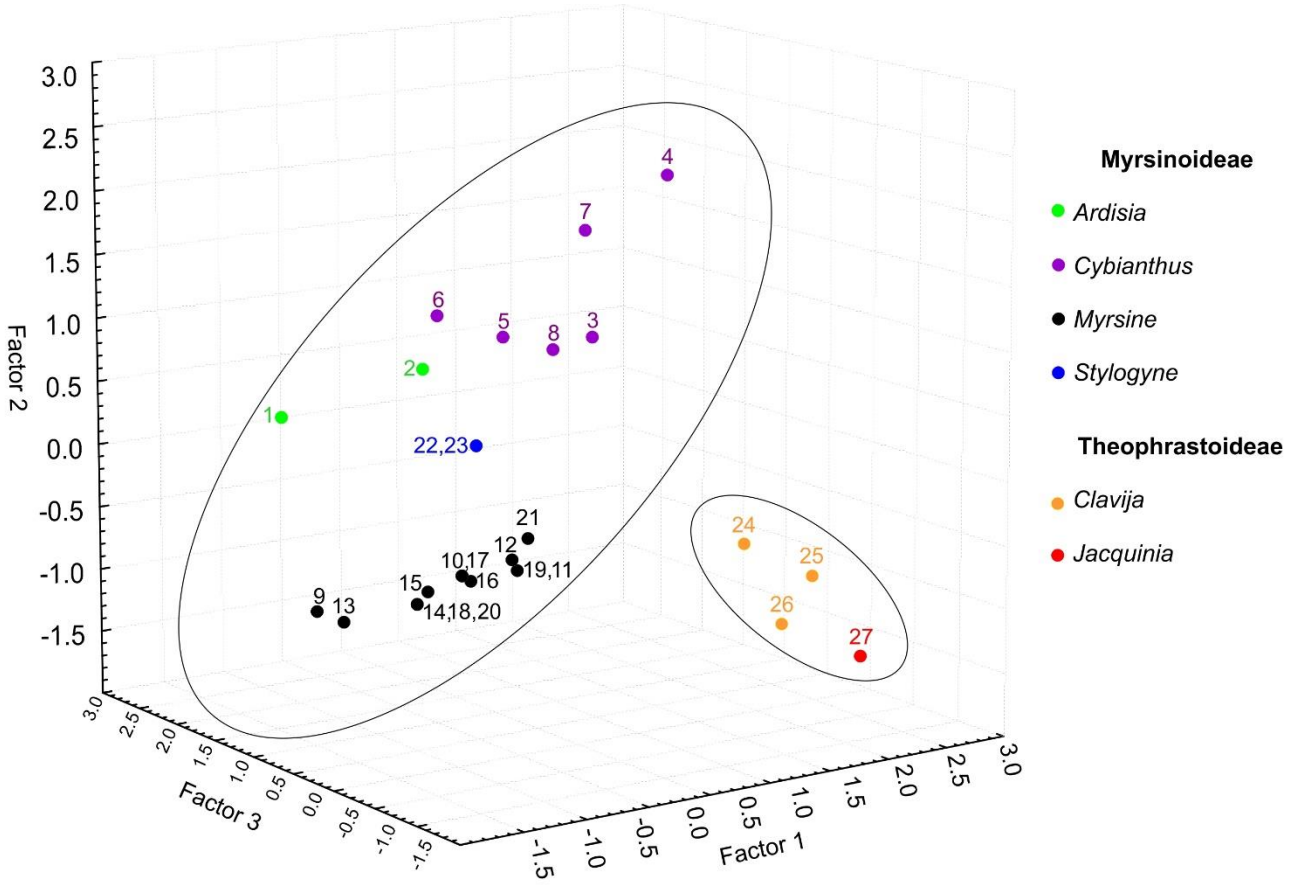

Figure 6 – 3D plot of three factor from principal components analysis of the Neotropical Primulaceae. Factor 1 – 23%, Factor 2 – 14%, Factor 3 – 12%.1 – *Ardisia humilis*, 2 – *A. solanacea*, 3 –*Cybianthus brasiliensis*, 4 – *C. densiflorus*, 5 – *C. glaber*, 6 – *C. guyanensis*, 7 – *C. nemoralis*, 8 – *C. venezuelanus*, 9 – *Jacquinia armillaris*, 10 – *Clavija nutans*, 11 – *C. spinosa*, 12 – *C. weberbaueri*, 13 – *Myrsine coriacea*, 14 – *M. emarginella*, 15 – *M. gardneriana*, 16 – *M. glazioviana*, 17 – *M. guianensis*, 18 – *M. lancifolia*, 19 – *M. lineata*, 20 – *M. parvifolia*, 21 – *M. parvula*, 22 – *M. rubra*, 23 – *M. squarrosa*, 24 – *M. umbellata*, 25 – *M. villosissima*, 26 – *Stylogyne atra,* 27 – *S. pauciflora*.

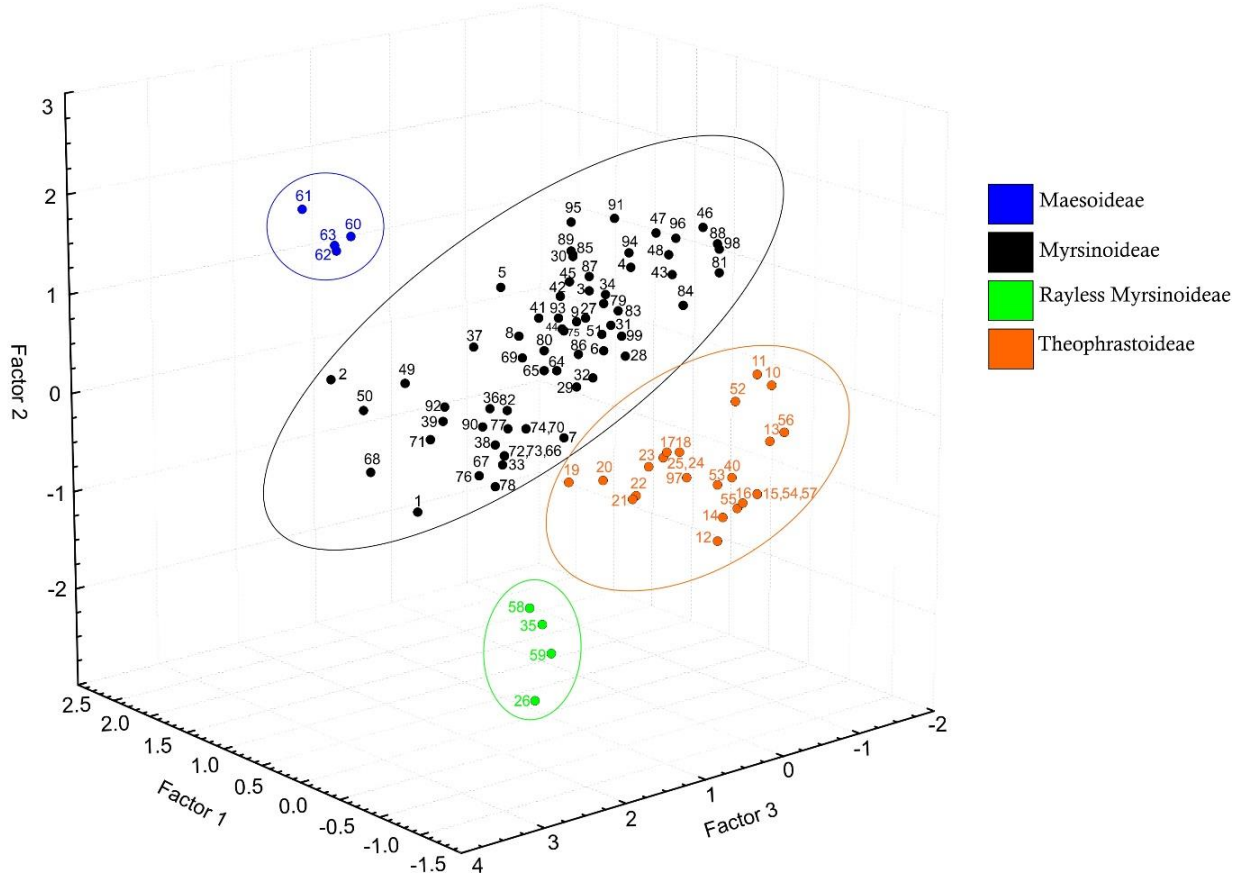

Figure 7 – 3D plot of three factor from principal components analysis merging data from current work and those from Lens et al. (2005). Species are indicated as numbers, they can be assessed on Supplementary data 1.

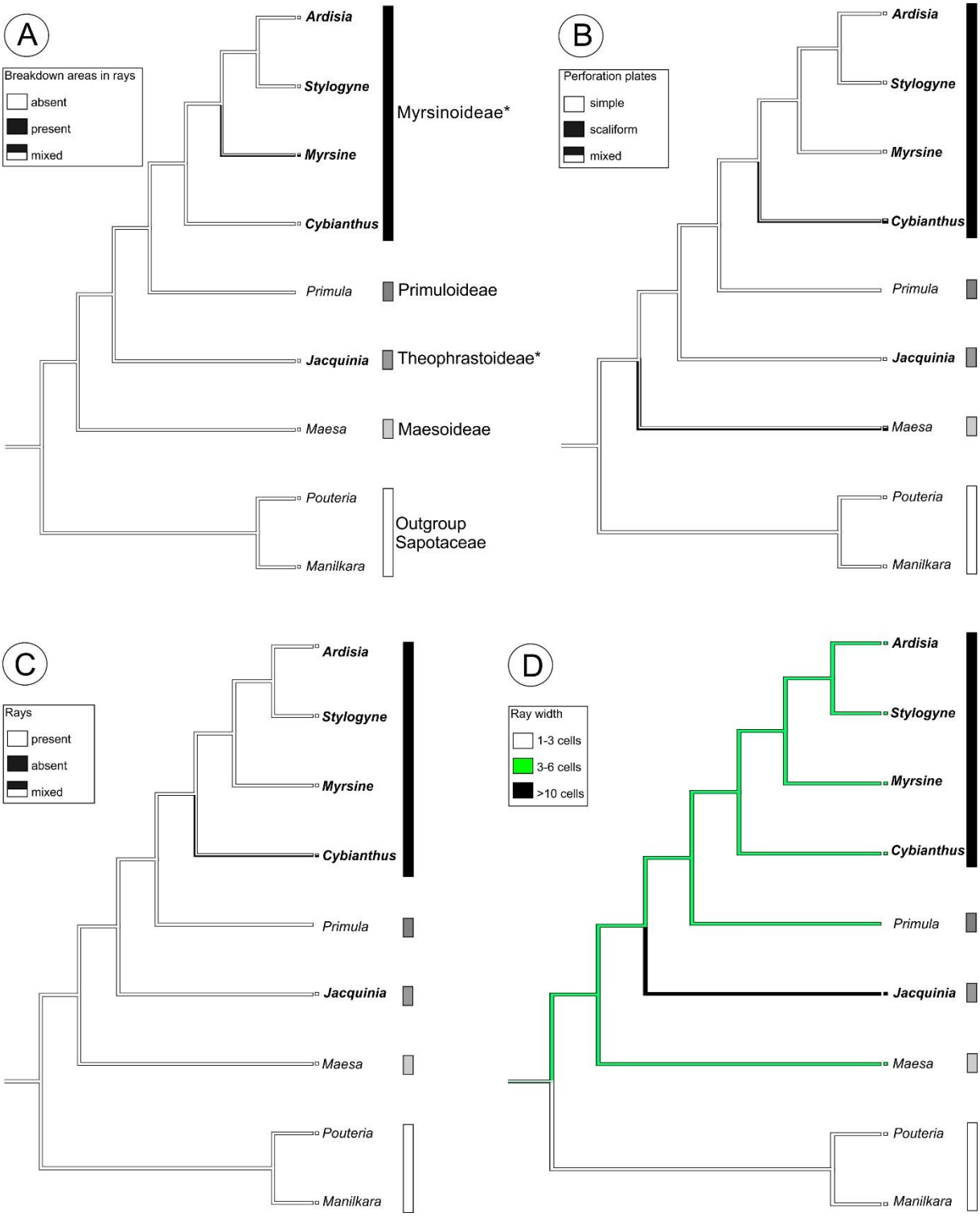

Figure 8 - Wood anatomical characters optimized on the strict consensus cladogram from Luna et al. (2017 – chapter 1). Characters and their states are indicated in the boxes.

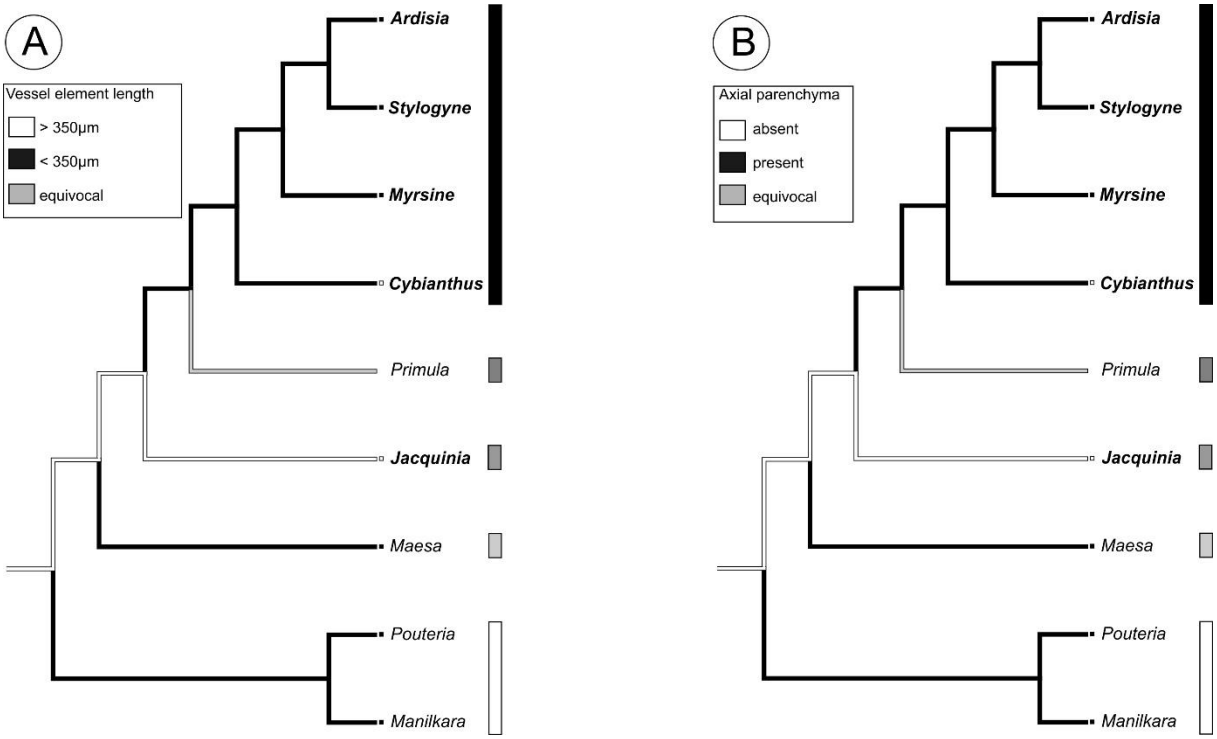

Figure 9 – Wood anatomical characters optimized on the strict consensus cladogram from Luna et al. (2017 – chapter 1). Characters and their states are indicated in the boxes.

Supplementary data 1

Table 1 – Presence and absence matrix of wood anatomical characters.

Number Figure 7	Species	Characters (Notes)																															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	*Aegiceras majus*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	1	1	0	1	1	0	0
2	*Afrardisia staudtii*	0	0	1	0	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	1	0	0	0	1	0	1	1	1	1	1	0	0
3	*Ardisia cauliflora*	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	0
4	*Ardisia copelandii*	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0
5	*Ardisia humilis*	0	1	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
6	*Ardisia manglillo*	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	1
7	*Ardisia obovata*	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	1
8	*Ardisia solanaceae*	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1
9	*Badula barthesia*	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1
10	*Bonellia frutescens*	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	1
11	*Bonellia frutescens*	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1
12	*Bonellia macrocarpa*	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
13	*Bonellia shaferi*	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
14	*Bonellia stenophylla*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1
15	*Bonellia umbellata*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
16	*Clavija lancifolia*	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	0
17	*Clavija longifolia*	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1
18	*Clavija nutans*	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0
19	*Clavija nutans*	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0
20	*Clavija procera*	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0
21	*Clavija spinosa*	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	1
22	*Clavija tarapotana*	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	0
23	*Clavija umbrosa1*	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1
24	*Clavija weberbaueri*	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1
25	*Clavija weberbaueri*	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1
26	*Coris monspeliensis*	1	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
27	*Ctenardisia stenobotrys*	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
28	*Cybianthus magniifolia*	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1
29	*Cybianthus multiflorus*	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	0
30	*Cybianthus peruvianus*	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	0
31	*Cybianthus prieurei*	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1
32	*Cybianthus psychotriaefolius*	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1
33	*Cybianthus brasiliensis*	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	1
34	*Cybianthus comperuvianus*	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	0
35	*Cybianthus densiflorus*	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0
36	*Cybianthus glaber*	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	1
37	*Cybianthus guyanensis*	0	1	1	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
38	*Cybianthus nemoralis*	0	1	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	1	0	0	1
39	*Cybianthus venezuelanus*	0	1	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	0	0	1
40	*Deherainia smaragdina*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0
41	*Discocalyx insignis*	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	1	1	0	1
42	*Discocalyx megacarpa*	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	1	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1
43	*Embelia kilimandscharica*	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1
44	*Embelia multiflora*	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	1	1	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0
45	*Embelia schimperi*	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1
46	*Embelia upembensis*	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1
47	*Geissanthus angustiflorus*	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1
48	*Geissanthus quindiensis1*	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1
49	*Grammadenia lineata*	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	1	1	1	1	0	0	1
50	*Grammadenia parasitica*	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	1	1	1	1	0	0	0
51	*Heberdenia bahamensis*	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1
52	*Jacquinia arborea*	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1
53	*Jacquinia armillaris*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	1
54	*Jacquinia berterii*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1
55	*Jacquinia berterii*	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
56	*Jacquinia cf. armillaris*	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1
57	*Jacquinia keyensis*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1
58	*Lysimachia kalalauensis*	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
59	*Lysimachia vulgaris*	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
60	*Maesa indica*	0	1	1	0	1	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	1	1	0	1	1	1	1	0	0	1
61	*Maesa lanceolata*	0	1	1	1	0	0	0	0	0	0	1	0	0	1	1	1	1	1	0	0	0	0	1	1	0	1	1	1	1	0	0	0
62	*Maesa macrothyrsa*	0	1	1	0	1	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	1	0	1	0	1	1	1	1	0	0	0
63	*Maesa ramentacea*	0	1	1	0	1	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	1	1	0	1	1	1	1	0	0	0
64	*Myrsine angustifolia*	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1
65	*Myrsine coriacea*	1	0	0	0	1	0	0	0	1	1	1	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1
66	*Myrsine emarginella*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1
67	*Myrsine gardneriana*	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1
68	*Myrsine glazioviana*	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1	0	0	1
69	*Myrsine guianensis*	1	0	0	0	1	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1
70	*Myrsine lancifolia*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1
71	*Myrsine lineata*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	1	1	1	1	0	0	1
72	*Myrsine parvifolia*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1
73	*Myrsine parvula*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1
74	*Myrsine rubra*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1
75	*Myrsine sandwicensis*	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	0
76	*Myrsine squarrosa*	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1
77	*Myrsine umbellata*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1
78	*Myrsine villosissima*	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	0	0	1
79	*Oncostemum botryoides*	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
80	*Oncostemum cauliflorum*	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
81	*Oncostemum leprosum*	0	0	1	0	1	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1
82	*Oncostemum venulosum*	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1
83	*Parathesis chiapensis*	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1
84	*Parathesis chrysophylla*	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	0	0

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
85	*Parathesis crenulata*	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	1	0	0
86	*Parathesis cubana*	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0
87	*Parathesis leptopa*	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
88	*Parathesis rekoi*	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	1	1	1	0	1
89	*Stylogyne amplifolia*	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
90	*Stylogyne atra*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	1	1	1	0	0	1
91	*Stylogyne latifolia*	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1
92	*Stylogyne pauciflora*	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	1	1	1	0	0	1
93	*Stylogyne standleyi*	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1
94	*Stylogyne venezuelana*	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	1	1	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0
95	*Synardisia venosa*	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	1	0	0	0	0	1	1	1	1	0	1
96	*Tapeinosperma nectandroides*	0	0	1	1	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	0	0	1	0	1	0	0	1	1	1	0	0
97	*Theophrasta americana*	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1
98	*Wallenia grisebachii*	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1	1	0	1
99	*Wallenia laurifolia*	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1

Notes. Characters from 1 to 32 are: 1. Distinct growth rings; 2. Growth rings slightly distinct; 3. Growth rings indistinct; 4. Vessel frequency: <30 vessels/mm²; 5. Vessel frequency: 30-50 vessels/mm²; 6. Vessel frequency: 50-100 vessels/mm²; 7. Vessel frequency: 100-200 vessels/mm²; 8. Vessel frequency: >200 vessels/mm²; 9. Vessel length < 350 μm; 10. Vessel length 350-500 μm; 11. Vessel length 500-650 μm; 12. Vessel length > 650μm; 13. Vessel tangential diameter 20-50 μm; 14. Vessel tangential diameter >50 μm; 15. Sclariform perforation plates; 16. Simple perforation plates; 17. Scalarifom pits; 18. Septate fibres; 19. Fibre lenght 300-500 μm; 20. Fibre lenght 500-700 μm; 21. Fibre lenght 700-900 μm; 22. Fibre lenght 900-1100 μm; 23. Fibre lenght >1100 μm; 24. Unisseriate rays; 25. Rays with > 10 cells width; 26. Rays of two distinct sizes; 27. < 4 rays/mm; 28. Rays > 1 mm height; 29. Rays integrated by square, procumbent and upright cells; 30. Breakdown areas in rays; 31. Rayless wood; 32. Prysmatic crystals.

Supplementary data 2 – Additional images of each analyzed species.

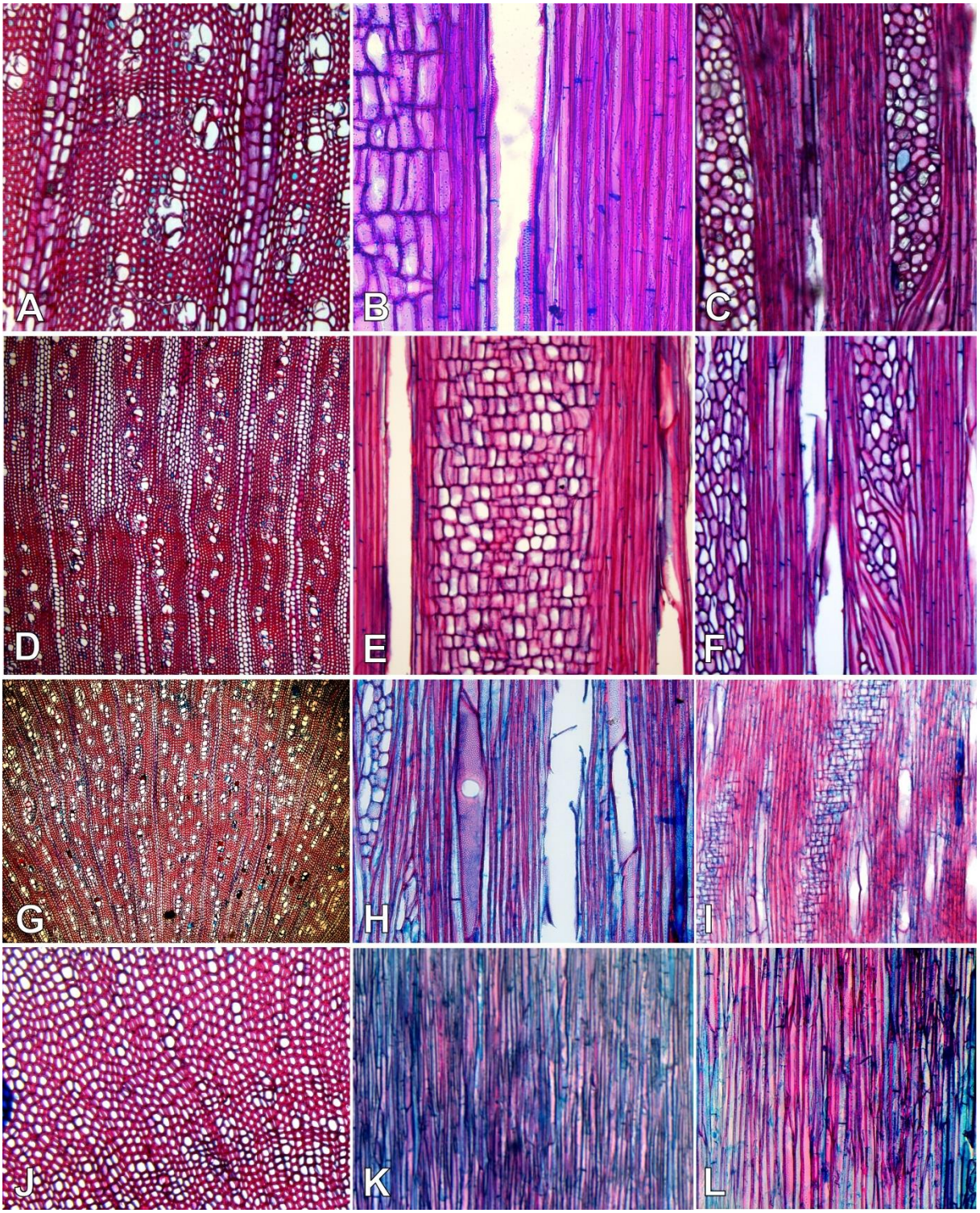

Figure 1 – A, D, G and J – Transversal section. B, E, H and K – Radial section. C, F, I and L – Tangential section. A-C – *Ardisia humilis.* D-F – *A. solanacea*. G – I – *Cybianthus brasiliensis*. J – L – *C. densiflorus.* A-L – Light microscopy.

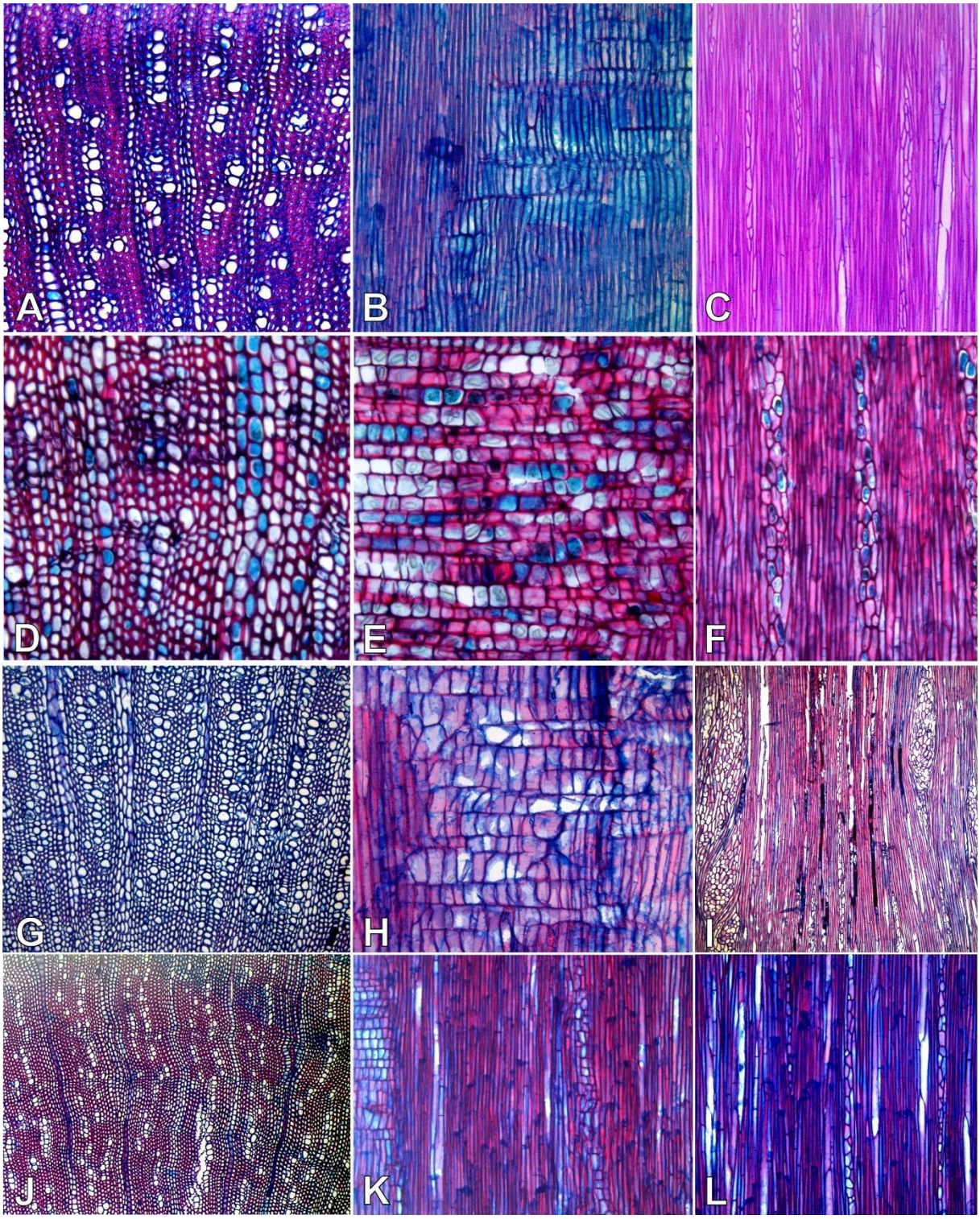

Figure 2 – A, D, G and J – Transversal section. B, E, H and K – Radial section. C, F, I and L – Tangential section. A – C – *Cybianthus guyanensis*. D – F – *C. nemoralis*. G – I – *C. venezuelanus*. J – M – *C. verticilatus*. A-L – Light microscopy.

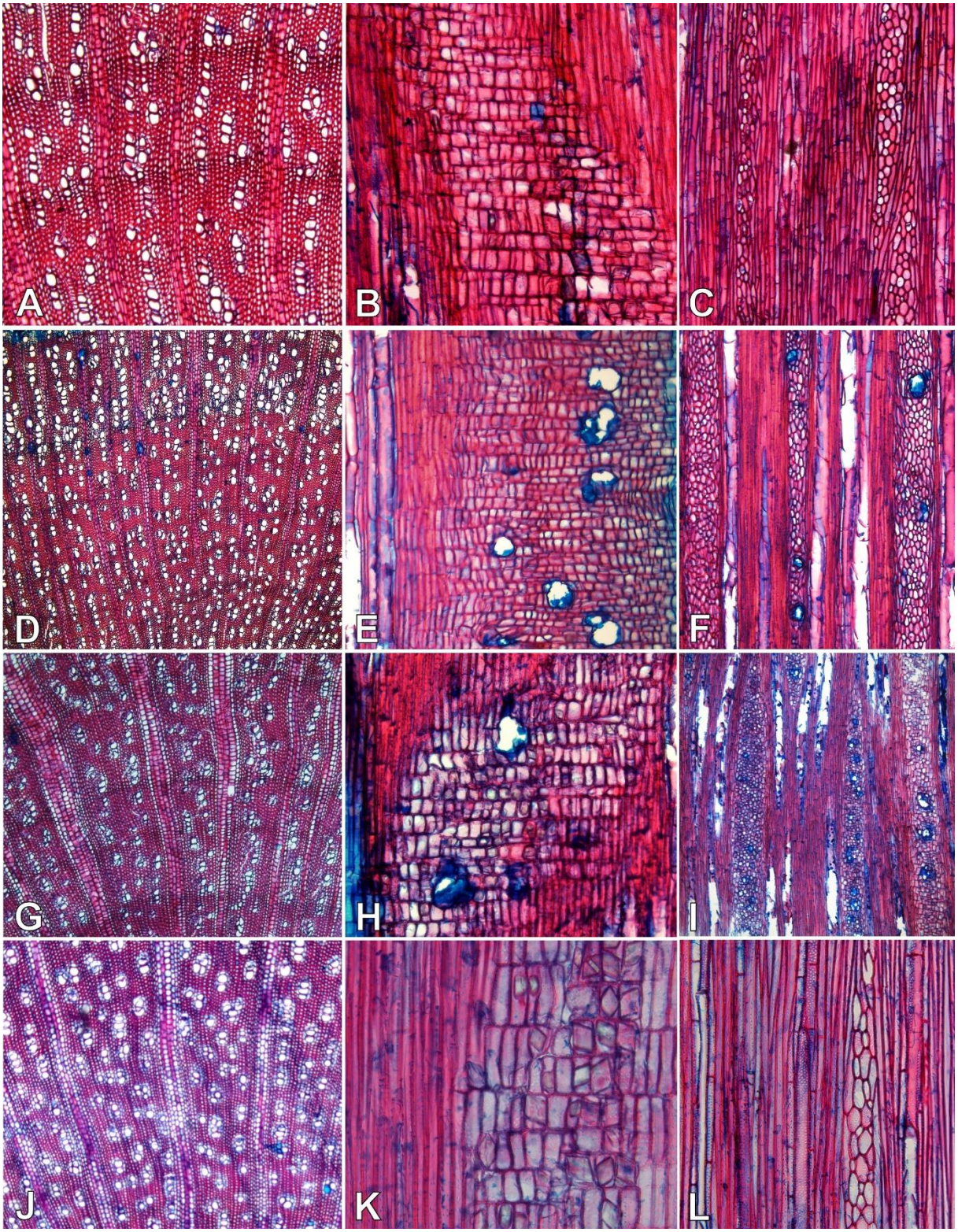

Figure 3 – A, D, G and J – Transversal section. B, E, H and K – Radial section. C, F, I and L – Tangential section. A – C – *Myrsine coriacea*. D – F – *M. emarginella*. G – I – *M. gardneriana*. J – L – *M. glazioviana*. A-L – Light microscopy.

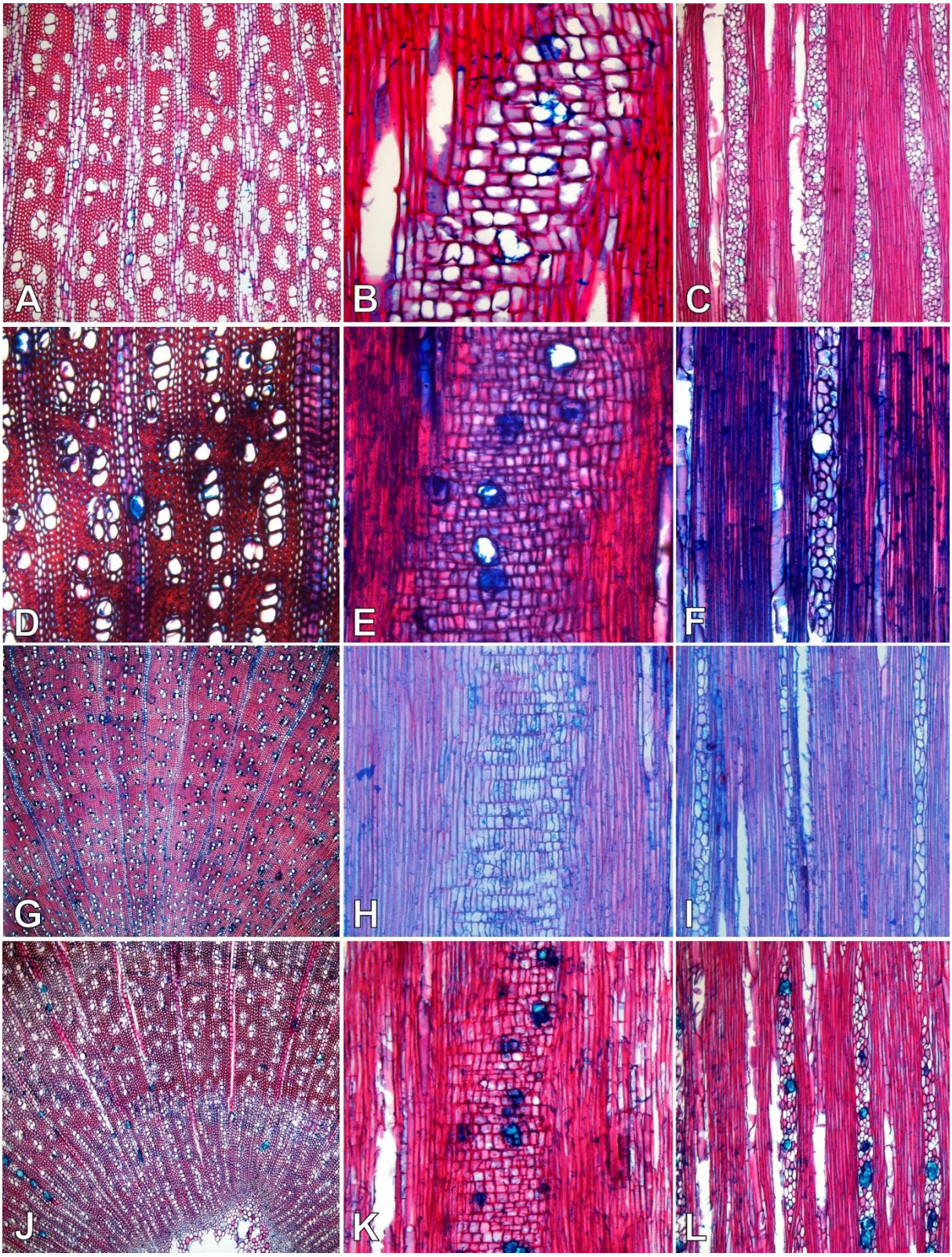

Figure 4 – A, D, G and J – Transversal section. B, E, H and K – Radial section. C, F, I and L – Tangential section. A – C – *Myrsine guianensis*. D – F – *M. lancifolia*. G – I – *M. lineata*. J – L – *M. parvifolia*. A-L – Light microscopy.

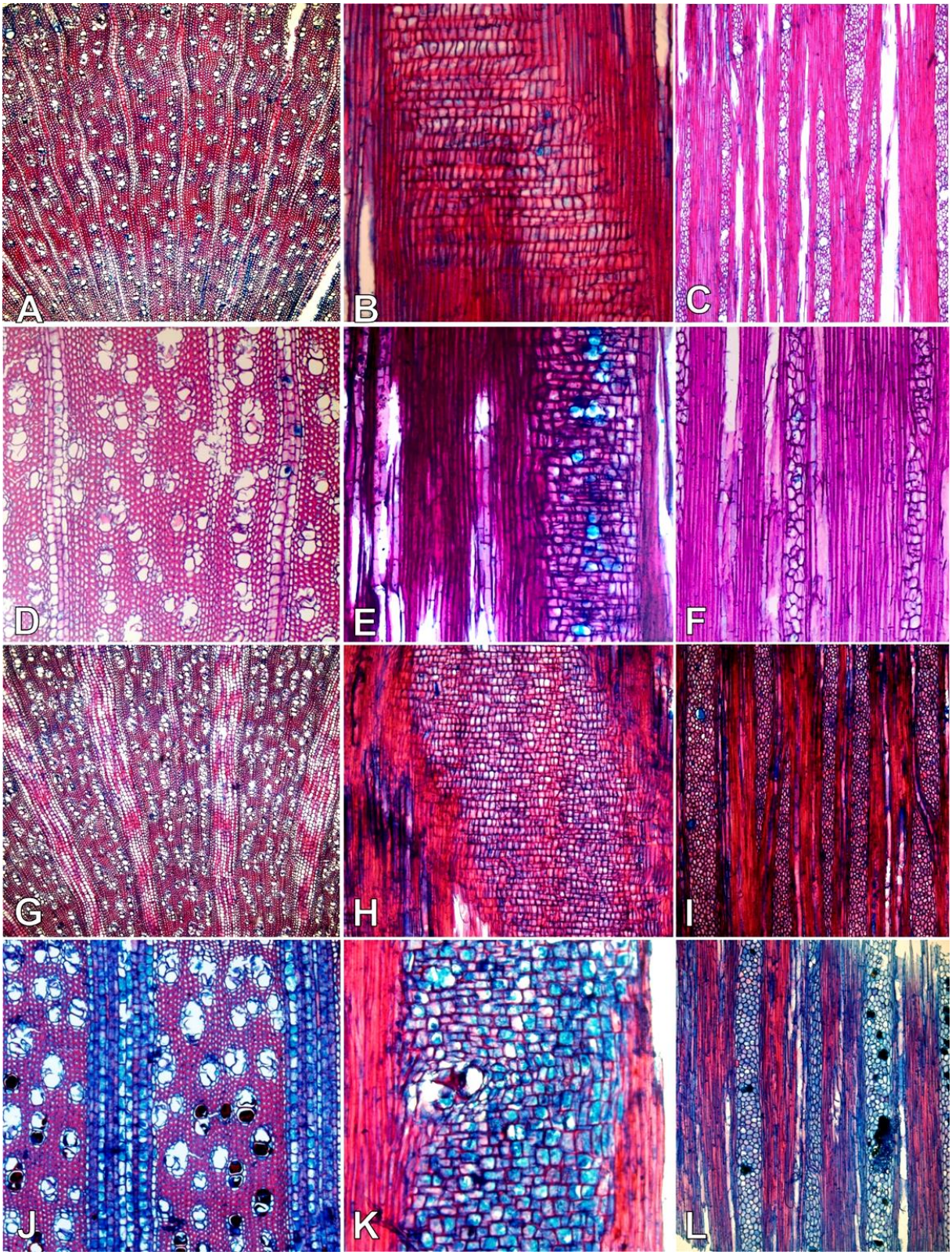

Figure 5 – A, D, G and J – Transversal section. B, E, H and K – Radial section. C, F, I and L – Tangential section. A – C – *Myrsine parvula*. D – F – *M. rubra*. G – I – *M. squarrosa*. J – L – *M. umbellata*. A-L – Light microscopy.

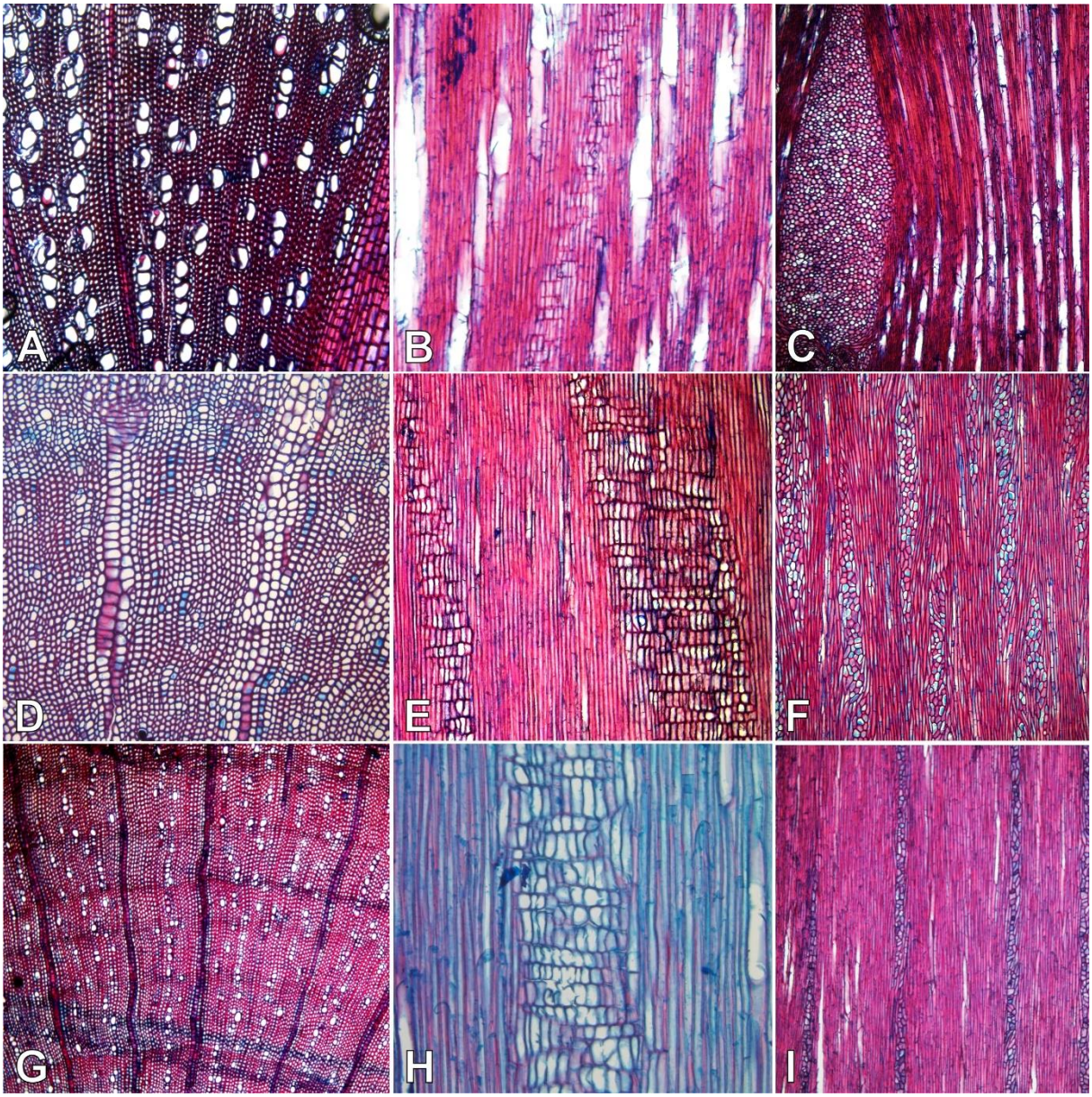

Figure 6 – A, D and G – Transversal section. B, E and H – Radial section. C, F and I – Tangential section. A – C – *Myrsine villosissima*. D – F – *Stylogyne atra*. G – I – *S. pauciflora*. A- I – Light microscopy.

Capítulo 3

SECRETORY STRUCTURES IN THE LEAVES OF PRIMULACEAE

O manuscrito foi redigido de acordo com as normas do periódico *Plant Biology*

Title: Secretory structures in the leaves of Primulaceae

Abstract

Many Primulaceae species have chemical or medicinal importance, which are directly related to the presence of a great set of secretory structures distributed along all plant organs. Besides the pharmaceutical importance, studies regarding secretory structures in Primulaceae are still scarce and little is known about how evolution has worked in the formation of those structures with a high degree of morphological differentiation. We intended to identify and classify the diversity of secretory structures in leaves of five Neotropical genera of Primulaceae, from Myrsinoideae and Theophrastoideae subfamilies, following the standard protocols in light, confocal and epifluorescence microscopy. There were identified the following secretory structures in the leaves: mucilaginous idioblasts, phenolic-content idioblasts, crystalliferous idioblasts, glandular trichomes, hydathodes and secretory cavities; in the xylem: breakdown areas in rays and crystalliferous idioblasts. The development of the secretory cavities/ducts and trichomes is asynchronous and starts before the whole tissue and leaf maturation. Secretory cavities/ducts derives from a ground meristem cell and follows a schizogenous type of development. The first steps of development are the same for all differen types of glandular trichomes, which starts from a protodermal cell, which undergoes two additional anticlinal divisions. In *Cybianthus* subgenus *Conomorpha* there are scale-like trichomes that cover all leaf bud and are shed at maturity. In summary, there were identified and detailed described the secretory structures found in the leaf and xylem of Primulaceae and provides new findings, as the presence of hydathodes in *Myrsine* and mucilaginous idioblasts in *Ardisia*, *Cybianthus* and *Stylogyne*.

Keywords: Idioblasts, hydathodes, secretory cavities, glandular trichomes; epifluorescence.

Introduction

Among the eudicotyledons (APG IV 2016) there are circumscribed species, families or clades that contains at least one type of secretory structure (e.g.: Vieira et al. 2001, Klein et al. 20014, Marinho et al. 2016, Silva et al. 2016). It is known, therefore, that similar types of secretory structures can occur in different orders and may not be phylogenetically related. For example, the secretory cavities found in *Copaifera langsdorffii* Desf. and *Copaifera trapezifolia* Hayne (Leguminosae-Caesalpinoideae- Fabales, respectively, Rodrigues et al. 2011 and Milani et al. 2012), and those from *Myrsine laetevirens* (Mez) Arechav. (Primulaceae-Myrsinoideae- Ericales, Otegui et al. 1998a). In both cases, the ontogenetic process and the morphology of these structures are similar; however, Fabales is circumscribed in the Rosids clade, while Ericales is grouped among the Asterids (Judd et al 2009).

Primulaceae, order Ericales, comprises ca. 2500 species and 58 genera distributed in four subfamilies: Myrsinoideae, Primuloideae, Theophrastoideae and Maesoideae (Stevens *onwards* 2001). Many representatives of this family secrete hydroxibenzoquinone derivatives (Otegui et al. 1998), essential oils (Luna et al. 2014; Corrêa et al. 2017) and other natural products with a broad spectrum of biological and pharmacological activities, e.g., antinociceptive (Hess et al. 2010), anti-inflammatory (Ahmad 2011), anti-leishmaniasis (Vermeersch et al. 2009), antihelminthic (Challam et al. 2010), and antioxidant (Mostafa et al. 2014). In hydroethanolic stem extracts from *Myrsine coriacea*, Baccarin et al. (2011) isolated myrsinoic acid B, a compound used by the pharmaceutical industry for its anti-inflammatory and antinociceptive properties (Hess et al. 2010).

This above-mentioned pharmacological and medicinal importance, in general, is related to the presence of a great set of secretory structures found in different organs in Primulaceae: glandular trichomes (Solereder, 1908; Metcalfe & Chalk, 1950; Luna et al., 2014); extrafloral nectaries (Oliveira & Leitão Filho, 1987; Vogel, 1997); salt glands (Aegiceras,

Solereder, 1908; Cardale & Field, 1971); modified hydathodes (Lersten & Horner, 1976); Hydathodes (Stylogyne, Carrijo et al., 2011); idioblasts (Große, 1908; Solereder, 1908; Metcalf & Chalk, 1950, Luna et al., 2014); and secretory cavities and ducts (Große, 1908; Solereder, 1908; Metcalfe & Chalk, 1950; Otegui et al., 1998b; Luna et al., 2013; Luna et al., 2014).

The presence of such outstanding diversity of secretory structures makes Primulaceae an excellent model to understand how evolution has worked in the formation of these structures with a high degree of morphological differentiation. The most recent study elucidates the ontogeny and chemical composition of the essential oils from two *Myrsine* L. species: *Myrsine coriacea* (Sw.) R. Br. ex Roem. & Schult. and *M. venosa* A.DC. (Luna et al. 2014). In this study, we intended to identify and classify the secretory structures found in the leaves of the woody Neotropical genera of Primulaceae, also to understand the development of the secretory cavities and trichomes.

Methodology

Plant material

All the procedures were carried out at the Laboratório de Botânica Estrutural from Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (Rio de Janeiro – Brazil). Species were selected after a previous leaf anatomical analysis (Luna, B.N. et al. 2017, chapter 1). Samples of five different genera, representing the two subfamilies from the Neotropic, were collected at different sites in Brazil. Parque Estadual da Costa do Sol (22°48′0″ S and 41°55′51″ W), Parque Nacional do Itatiaia (22°25′33″ S and 44°37′15″ W), Parque Estadual do Ibitipoca (21°41′48″ S and 43°53′50″ W), Reserva Ducke (59°58′ 38″ S and 2°55′46″ W) and in the arboretum of Jardim Botânico do Rio de Janeiro (22°58′0″ S and 43°13′28″ W). Selected species, voucher information and procedures taken with each specie are presented in Table 1.

Light microscopy

For the ontogenesis analysis of the secretory structures, meristematic apex, leaves at different developmental stages and fully expanded leaves from the 4th and 5th were collected from at least three specimens from each species.

After collection, samples were fixed for 48 h in a solution of 2.5 % glutaraldehyde and 4.0 % formaldehyde buffered with 0.05 $mol^{-1}L$ sodium cacodylate buffer, pH 7.2, at room temperature (Barros & Miguens, 1998). The samples were dehydrated in a graded ethanol series and embedded in methacrylate resin (Historesin, Leica, Nussloch, Heidelberg, Germany). Cross sections of 3-5 μm were made in a Leica RM2245 semi-automated rotary microtome, stained with toluidine blue 0.05 % in 0.1 $mol^{-1}L$ phosphate buffer (Feder & O'Brien, 1968), and sealed with Entellan (Merck).

Histochemical tests

Histochemical tests were performed on freehanded sections of mature fresh leaves. Nile blue (Cain 1947) was used for the detection of neutral and acid lipids, Sudan III (Johansen 1940) and neutral red (Jensen 1962) for total lipids, ferric chloride for phenolic compounds (Johansen 1940), and ruthenium red for mucilage (Johansen 1940).

All slides were examined and documented with an *Olympus* BX 50 light microscope equipped with an *Olympus* DP73 digital camera.

Epifluorescence microscopy

For epifluorescent and laser confocal scanning microscopy (LCSM), fragments of cuticular macerations were used. Auramin O 0.05% (Ex – 450-480 nm/ Em – 515 nm) was used for cuticular membrane (Considine & Knox, 1979), and DAPI (Ex - 360–370 nm/ Em - 420 nm) was used for the detection of nuclear DNA (Kapuscinski 1995). Epifluorescence

images were taken with an *Olympus* DP73 camera attached to an *Olympus* BX50 epifluorescence microscope. The LCSM images were taken using the LAS AF LITE (v. 2.6.0) software (Leica Microsystems) in a Leica *TCS SPE* laser scanning confocal microscope.

Results

Secretory structures in Primulaceae

Among the analyzed species there were identified the following secretory structures in the leaves: idioblasts (Figs. 1A-C), hydathodes (Figs. 1D-E), secretory cavities (Figs. 1A, 1E-F) and glandular trichomes (Figs. 1G-J).

Summarized details on the presence and absence of each secretory structures is presented in Table 2.

Idioblasts

There were identified different types of idioblasts among the studied species: mucilaginous (Fig. 1A-B), and phenolic-producing idioblasts (Fig.1C). The chemical nature of the secretion stored by each type of idioblast was confirmed by histochemical tests. In the leaves, crystalliferous idioblasts contain calcium oxalate druses (Fig. 1B.) and are dispersed throughout the mesophyll.

The mucilaginous idioblasts occur in both sides of the epidermis and can be distinguished from the other epidermal cells by their large size (Fig. 1A). The common epidermal cells are procumbent and the mucilaginous idioblasts have a voluminous vacuole filled with a purple content when stained with toluidine blue (Fig. 1B). The phenolic-producing idioblasts can be found in the epidermis, mesophyll and cortex of the petiole and midrib, they stain green whith toluidine blue (Fig. 1C).

It is possible to note that the synthesis of the content within the mucilaginous and phenolic idioblasts starts before their fully development (Fig. 5D). The development of the mucilaginous and phenolic-content idioblasts starts with the development of small vacuoles (Fig. 1C)

Hydathodes

Hydathodes were visible to the naked eye as white dots in the leaf margin of *Myrsine umbellata*, *M. lancifolia* and M. *lineata* (Fig. 1D). Anatomically, they are characterized by the presence of conspicuous xylem cells arriving in the epithem, and the water pore (Fig. 1E-F). The pore is formed by a modified stomata, generally placed in the margin of the adaxial epidermis (Fig. 1F).

Glandular trichomes

Peltate glandular trichomes are found in all species, they are observed sparsally distributed in both sides of the epidermis, at the same level of the other epidermal cells or in sunken areas (Fig. 1G and 1H). In *J. armillaris* they are seen as dark or brownish glandular dots in the epidermis when seen in frontal view (Fig. 2I). They are formed by a basal cell, a stalk cell and a multicellular secretory head cell covered by a thick cuticle.

The development of the trichomes is asynchronous and premature in the leaf primordium, they can be found at different maturation stages on a single leaf primordium, and before the differentiation of the tissues in leaf, the trichomes are already formed (Fig. 3A).

Ontogenetically, the initial stages of the development of the glandular trichomes is similar in all species. First, occur a protusion of a protodermal cell (Fig. 2A and 3B), which undergoes a periclinal division. As a result of this division, there are formed two daughter cells: the basal and the apical cell (Fig. 2B and 3C). After that, the apical cell undergoes a new division, originating the stalk and the mother head cell (Fig. 2C and 3D-E).

Subsequentlty, the mother head cell divides anticlinally (Fig. 3E) several times to form the glandular secretory head (Fig. 2D-G and 3F-G).

The trichomes walls are thin during first stages of development, but occur a parietal thickening in the stalk cell in a later moment of development (Fig. 2H). It was not possible to detect the histochemical nature of the secretion, as it is stored in the subcuticular space of mature trichomes and does not persist when the leaf is cut.

In the leaves of *C. guyanensis* and *C. brasiliensis*, besides the peltate type, also occur scale-like trichomes (Fig. 1I and 1J). The development of the scale-like trichomes follows the above mentioned steps for peltate trichomes, however, they undergoes additional subsequent steps of development (Fig. 3K-N). The first formed trichomes of this type present a long basal cell (Fig. 3L-M), in which occur a thickening in the cells of their secretory head. These trichomes cover and protect all the leaf primordium while younger trichomes are formed (Fig. 3K), but do not persist to leaf maturity.

Secretory cavities

Secretory cavities and ducts are randomly distributed throughout the mesophyll, midrib and petiole of *Ardisia, Cybianthus, Myrsine* and *Stylogyne* (Fig. 1A), and are absent in *Jacquinia*. In the petiole and midrib they occur in the cortical portion and in the inner portion of the vascular system. When fully developed, these structures are formed by a secretory epithelium and by the lumen, where the secretion is stored (Fig. 1A and 1E).

Within the same leaf primordium there were observed secretory cavities at different development stages, characterizing an asynchronous type of development (Fig. 4A and 5A) and from a development point of view, these structures derives from ground meristem cells and follows the same ontogenetical pattern between the different genera: *Cybianthus* (Fig. 4), *Ardisia* (Fig. 5) and *Stylogyne*. First, a ground meristem cell undergoes a periclinal division (Fig. 4B), then these cells divides anticlinally resulting in a four-celled cluster (Fig.

4D and 5B). At this point, the secretory epithelium is already distinguished from the other ground meristem cells (Fig. 4C-D and 5B). Gradually, the middle lamelae facing the inner portion of the epithelial cells, starts to desintegrate originating a space between them (Fig. 4E), which will later form the lumen. While the secretory epithelium undergoes subsequent divisions (Fig. 4F), the size of the lumen increases (Fig. 4G-I and 5C-G). During the maturation of the structure, there are small vacuoles within the ephitelial cells that gets filled with phenolic compound (Fig. 4G and 5D). Subsequently, occur the merge of these small vacuoles (Fig. 4 H-I and 5C). When the epithelial cells are completely formed, the secretion process starts, and the content is stored within the lumen (Fig. 4J and 5D-E). There were detected a mix of secondary metabolites in the secretion: acid lipids (Fig. 4K), polysacharides (Fig. 4L), lipids (Fig. 4M) and phenolic compounds (Fig. 4N). The secretion in *Ardisia* is naturally red (Fig. 5D-E).

Discussion

The importance of secretory structures in Primulaceae - It is well known that many Primulaceae species displays a great variety of medicinal application (Cabanillas et al. 2015; Charneau et al. 2015; Van et al. 2015). This variety is directly related to the diversity of secretory structures found in this family, as previously reported by many authors (e.g: Metcalfe & Chalk 1950; Otegui et al. 1998; Luna et al. 2014), and confirmed in the present work. There were observed different types of secretory structures in the leaves, trichomes, idioblasts, secretory cavities/ducts, and in the xylem, breakdown areas in rays.

Secretion in plants is related to several ecological process. Roshchina & Roshchina (1993) stated that the secretory activity in plants is one way to express the ability to exchange substances and energy with the environment. The presence of different types of secretory structures secreting, for example, phenolic compounds one of the compounds identified in the secretion of the analyzed species, can be related to the plant defense. In addition, in

Primulaceae, there are other natural products that have been identified in the secretion, as the hydroxybenzoquinone derivatives which were reported in Primuloideae (Nestler 1904), Myrsinoideae (Manguro et al. 2003) and Maesoideae (Kuruvilla et al. 2010); mono and sesquiterpenes found in the leaves of several *Myrsine* species (Luna et al. 2014, França 2014, Cabral 2016, Corrêa et al. 2017) and in *Ardisia* (França 2014).

Secretory cavities, a synapomorphy of Myrsinoideae and Maesoideae - The secretory cavities and ducts of *Ardisia*, *Cybianthus* and *Stylogyne* follows the same pathway of development observed in *Myrsine coriacea* and *M. venosa* (Luna et al. 2014) and in *Lysimachia vulgaris* L. (Lersten 1986), species from related genera placed in the same subfamily within Primulaceae, Myrsinoideae. This pattern consists in the development of a secretory cavity/duct from a ground meristem cell, following a schizogenous process, in which there is no programmed cell death but only occur the dissolution of the middle lamelae. Such structures are formed during the leaf development and starts their secretory process before the fully development of the leaves, which reinforces their protective role against pathogens and herbivores. The pathway of the secretory process still lacks further informations, specially those related to the ultrastructure of the the epithelial cells.

Secretory cavities/ducts are found in both Myrsinoideae and in Maesoideae subfamilies (Utteridge 1998) and are absent in *Jacquinia armillaris* (present study) and in other Theophrastoidae (Stahl 1987; 1989) and in Primuloideae (Metcalfe & Chalk 1950).

The significance of hydathodes and idioblasts - Idioblasts secreting mucilaginous and phenolic compounds were found in *Myrsine laetevirens* (Otegui et al. 1998a), *M. coriacea* and *M. venosa* (Luna et al. 2014) and here we reported their presence in *Ardisia*, *Cybianthus* and *Stylogyne*. The mucilaginous idioblasts occur in both sides of the epidermis and are morphologically distinct from the other cells due to their large volume and their purple content, and the

phenolic ones can be found in the epidermis or distributed throughout the mesophyll. There are no specific function reported to these structures in Primulaceae, but it is known for other species that mucilaginous idioblasts are related to water retention in leaves from other species (Trachtenberg & Fahn 1981).

To the best of our knowledge, passive hydathodes in *Myrsine* species were reported herein for the first time. They are located in the leaf margin and present the classical structure with epithem, and modified stomata (Metcalfe & Chalk 1979). Such structures are known in Primulaceae, especially in *Ardisia*, since modified hydathodes are mutualistic associated with bacterias (Lersten & Horner 1976). Further investigations on the ultrastructure of the hydathodes in *Myrsine* are needed to assess if they present similar bacterial associations as those observed in *Ardisia*.

The importance of glandular trichomes in the initial phases of leaf development - Glandular trichomes, specially the peltate type found in the species studied in this research, have been reported in almost all Primulaceae species (Metcalfe & Chalk 1950; Luna et al. 2013, Luna et al. 2014). The secretory activity of the glandular trichomes in Primulaceae starts before the whole maturation of the leaf. It suggests that they act protecting leaf buds against pathogens and phytophagous organisms, as was also observed in other taxa (e.g.: Solanaceae - Munien et al. 2015). Also, they can be present only in the first stages of leaf development protecting the leaves and shed at maturity, as the scale-like trichomes with a long basal cell observed during the first stages of development in *Cybianthus brasiliensis* and *C. guyanensis* leaf primordium.

Moreover, it is known that the function of glandular trichomes is not restricted to the young leaves, as that are indicatives that they act as extrafloral nectaries in mature leaves of *Myrsine guianensis* Aubl. and *M. lancifolia* Mez from the Cerrado (Oliveira & Leitão-Filho 1987; Machado et al. 2008). Althought we did not observed visitants in the leaves during

collections, further investigations are needed to confirm the role of this type of trichome in other species.

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Legends

Tables

Table 1. Selected species, voucher information and procedure taken with each of them. LA = leaf anatomy; O = ontogeny.

Table 2. Presence (+) and absence (-) of the secretory structures in mature leaves and xylem of Primulaceae. HY = Hydathodes; CI = Crystalyferous idioblasts; MI = mucilaginous idioblasts; PI = Phenolic-content idioblasts; SC = Secretory cavities; PT = peltate trichomes; ST = Scale-like trichomes.

Figures

Figure 1 – Secretory structures found in the leaves of Primulaceae. A-C; E-I – light microscopy. J, epifluorescence microscopy. A – C, E - J, transversal section of the leaf. A – Leaf of *Myrsine lancifolia*, evidencing the presence of mucilaginous idioblasts in the epidermis (mi) and secretory cavity in the mesophyll (*).B – Detail of the mucilaginous idioblast in the adaxial surface of the epidermis. C – Phenolic-content idioblasts. D – Leaf margin of *M. lineata*, showing hydathodes (arrow). E and F – Anatomical observation of the hydathode in the leaf margin of *M. lineata*, showing the irrigation by xylem cells (xy), the modified parenchyma (ep) and the modified stomata (arrow). G – Transversal section of the sunken glandular trichome in *Jacquinia armillaris*. H – Transversal section of the peltate trichome in *M. lineata*. Transversal *(I)* and frontal *(J)* view of the scale-like trichome in *Cybianthus guyanensis*.

Figure 2 – Successive stages of development of glandular trichomes in *Jacquinia armillaris*. A-I – Light microscopy. A – First stage of development with a projection of a protodermal cell. B – Periclinal division of the protodermal cell resulting in a basal cell (bc) and an apical cell (ac). C – Division of the apical cell, resulting in a stalk cell (sc) and mother head cell. D and E –Anticlinal division of the mother head cell. F and G – Subsequent divisions of the head cell, resulting in the development of the glandular head. H – Fully developed trichome located in a sunken area of the epidermis. I – Frontal view of the abaxial epidermis, showing the trichomes distribution (◄).

Figure 3 – Leaf trichome ontogeny of *Cybianthus verticillatus* (A-J) and *C. guyanensis* (K-N). B, E, H, I, L and N – Light microscopy. C, D, F, G, stained with DAPI and A, J, K, M stained with Auramin O – Epifluorescence microscopy. A – General aspect of the leaf primordium showing glandular trichomes under different developmental stages (◄). First step of the development with a protrusion of a protodermal cell *(B)* followed by a periclinal division *(C)*, resulting in the basal and apical cell. D- Division of the apical cell, resulting in a stalk and mother head cell. E – Nuclei stained with DAPI, evidencing the organization of the chromosomes at the metaphase plate. F and G – Division of the mother head cell, resulting in two head cells. After that, the head cell undergoes several divisions *(H and I).* J – Transversal section of the peltate trichome in a mature leaf. K – Frontal view of the full-developed trichome. L – Trichomes at different developmental stages stained with Auramin O, before the head thickening (◄) and before (arrow). M – Detail of the scale-like trichomes in the primordium, with an elongated basal cell and a short stalk cell (◄) covering the other under development (yellow arrow head). Note that the head cells stay with their orange color. N – Fully developed scale-like trichome, note that the head cell do not fluoresce under any spectrum of emission. O – Transversal section of the peltate trichome in a mature leaf.

Figure 4 – Ontogenesis of the leaf secretory cavities in *C. verticilatus*. A-E, G and I, J-M – Light microscopy. F and H – Epifluorescence microscopy. A – General aspect of the leaf primordium showing the secretory cavities under different developmental stages (◄). B – First step of the secretory cavity development, with an anticlinal division of a ground meristem cell. C and D – Sequential steps of the secretory cavity development with the division of the first two cells. E – Dissection of the middle lamellae and formation of the lumen (arrow). F – Epithelial cells with the stained nuclei. Note the process of division. G and H – Sequential step of the secretory cavity/duct development, evidencing a wider lumen (*) after the division of the epithelial cells. I – Maturation of the epithelial cells. Note the vacuole filled with phenolic compounds. Secretion stained with *(J)* Nile Blue, for acid lipids; *(K)* ruthenium red for mucilage; *(L)*; neutral red for total lipids *(N)*; and ferric chloride *(N)* for phenolic compounds.

Figure 5 – Ontogenesis of the leaf secretory cavities in *Ardisia solanacea*. A – E, Light microscopy. A – Transversal section of the leaf primordium (ca. 5 mm), showing secretory cavities at different development stages (yellow arrows). B – Cluster of cells in the initial step of development, with the anticlinal division of a single ground meristem cell, followed by several division at different planes. C – Secretory cavity with the lumen formation. D and E – Fully developed cavity. Bars – A = 100 μm; B-E = 20 μm.

Table 1. Selected species, voucher information and procedure taken with each of them.

Species	Voucher information	Procedure
Myrsinoideae		
Ardisia solanacea Roxb.	RBv 2094	LA/O
Cybianthus brasiliensis (Mez) G.Agostini	RB 605169	LA/O
Cybianthus guyanensis (Mez) G.Agostini	INPA 178624	LA/O
Cybianthus verticillatus (Vell.) G. Agostini	RB 586284	LA/O
Myrsine lineata (Mez) Imkhan.	RB 605196	LA
Myrsine lancifolia Mart.	UEC 140880	LA
Myrsine umbellata Mart.	RB 605182	LA
Stylogyne depauperata Mez	RB 444752	LA/O
Theophrastoideae		
Jacquinia armillaris Jacq.	RB 369866	LA/O

Notes: LA = leaf anatomy; O = leaf ontogeny.

Table 2. Presence (+) and absence (-) of the secretory structures in mature leaves of Primulaceae.

Species	Leaves					
	Hy	**MI**	**PI**	**SC**	**PT**	**ST**
Myrsinoideae						
Ardisia solanacea	-	-	+	+	+	-
Cybianthus brasiliensis	-	+	+	+	+	+
C. guyanensis	-	+	+	+	+	+
C. verticillatus	-	-	-	+	+	-
Myrsine lineata	+	-	+	+	+	-
M. lancifolia	+	+	+	+	+	-
M. umbellata	+	+	+	+	+	-
Stylogyne depauperata	-	+	+	+	+	-
Theophrastoideae						
Jacquinia armillaris	-	-	-	-	+	-

Notes: HY = Hydathodes; MI = mucilaginous idioblasts; PI = Phenolic-content idioblasts; SC = Secretory cavities; PT = peltate trichomes; ST = Scale-like trichomes.

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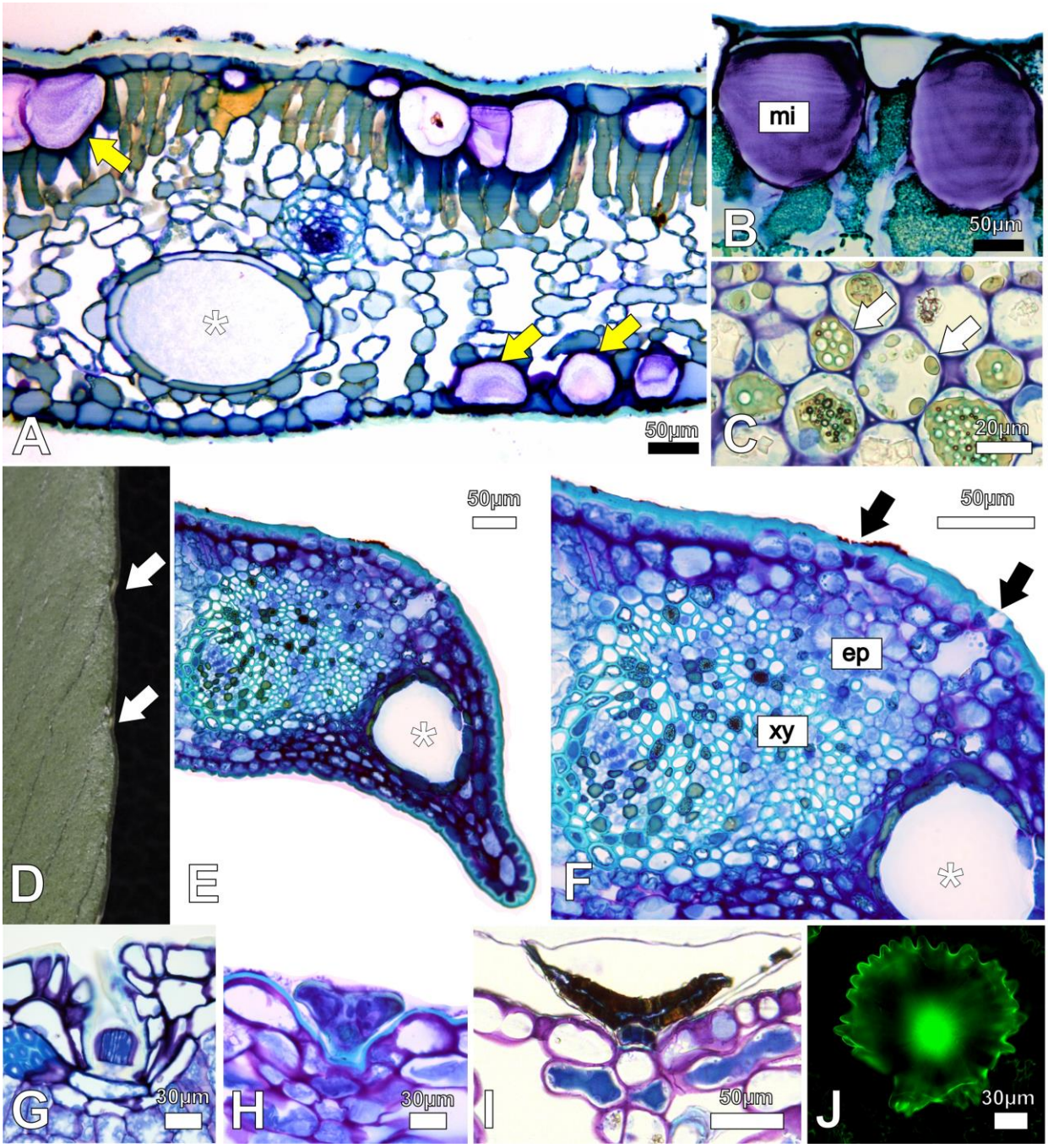
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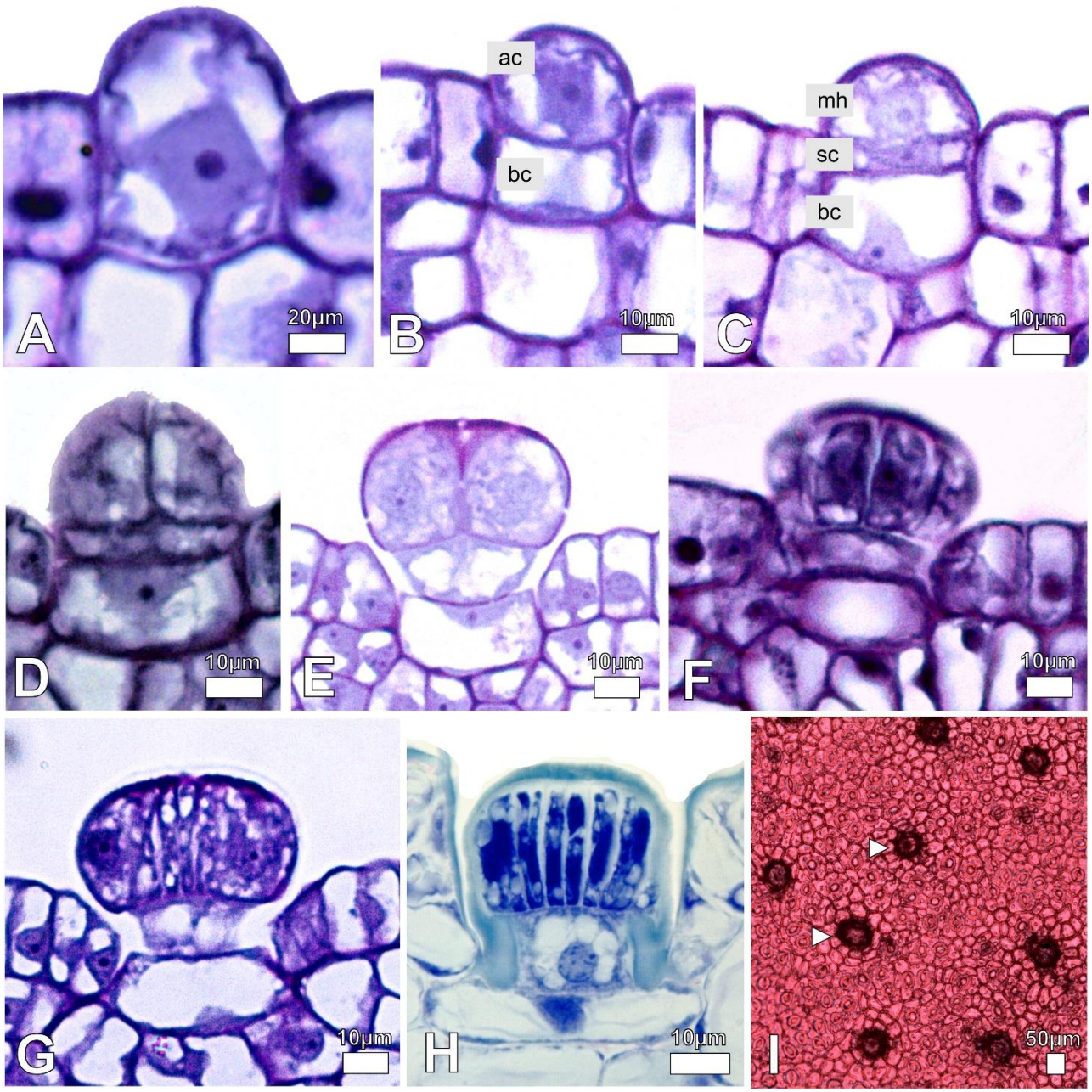

Figure 2 – Successive stages of development of glandular trichomes in *Jacquinia armillaris*. A-I – Light microscopy. A – First stage of development with a projection of a protodermal cell. B – Periclinal division of the protodermal cell resulting in a basal cell (bc) and an apical cell (ac). C – Division of the apical cell, resulting in a stalk cell (sc) and mother head cell. D and E – Anticlinal division of the mother head cell. F and G – Subsequent divisions of the head cell, resulting in the development of the glandular head. H – Fully developed trichome located in a sunken area of the epidermis. I – Frontal view of the abaxial epidermis, showing the trichomes distribution (◄).

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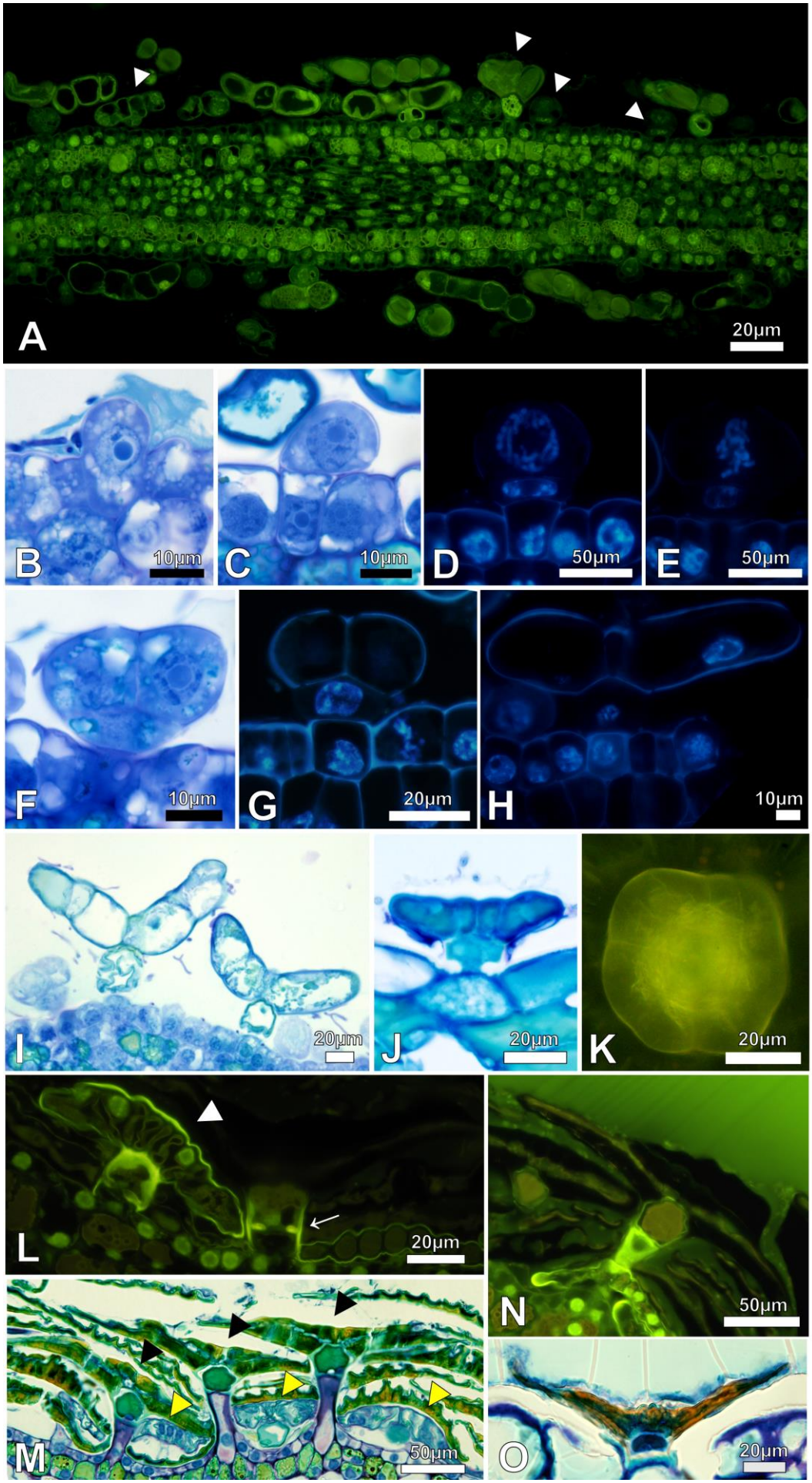
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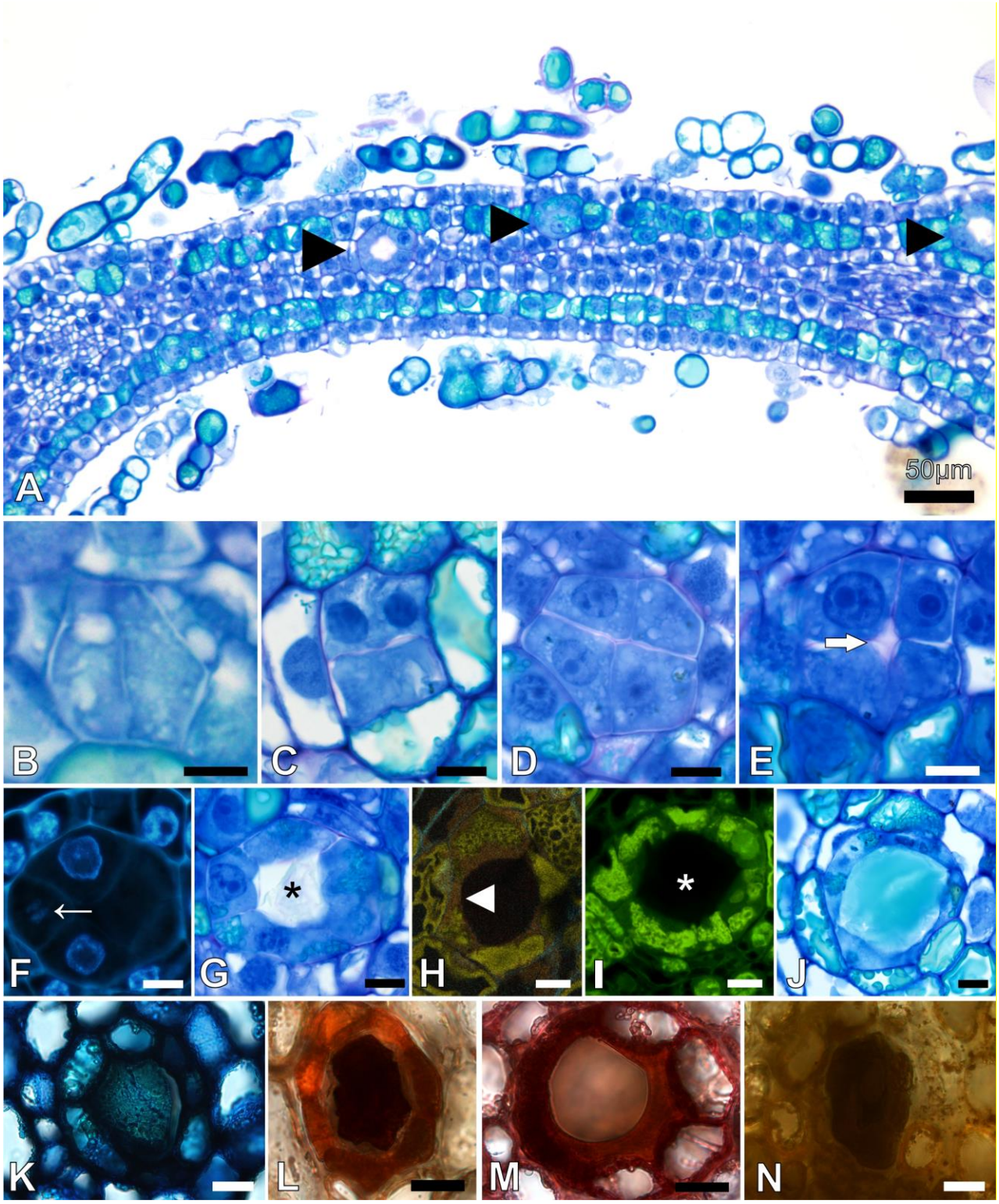
Figure 4 – Ontogenesis of the leaf secretory cavities in *C. verticilatus*. A-E, G and I, J-M – Light microscopy. F, stained with DAPI and H, stained with Calcofluor and Auramin O – Epifluorescence microscopy. A – General aspect of the leaf primordium showing the secretory cavities under different developmental stages (◄). B – First step of the secretory cavity development, with an anticlinal division of a ground meristem cell. C and D – Sequential steps of the secretory cavity development with the division of the first two cells. E – Dissection of the middle lamellae and formation of the lumen (arrow). F – Epithelial cells with the stained nuclei. Note the process of division. G and H – Sequential step of the secretory cavity/duct development, evidencing a wider lumen (*) after the division of the epithelial cells. I – Maturation of the epithelial cells. Note the vacuole filled with phenolic compounds. Secretion stained with *(J)* Nile Blue, for acid lipids; *(K)* ruthenium red for mucilage; *(L)*; neutral red for total lipids *(N)*; and ferric chloride *(N)* for phenolic compounds.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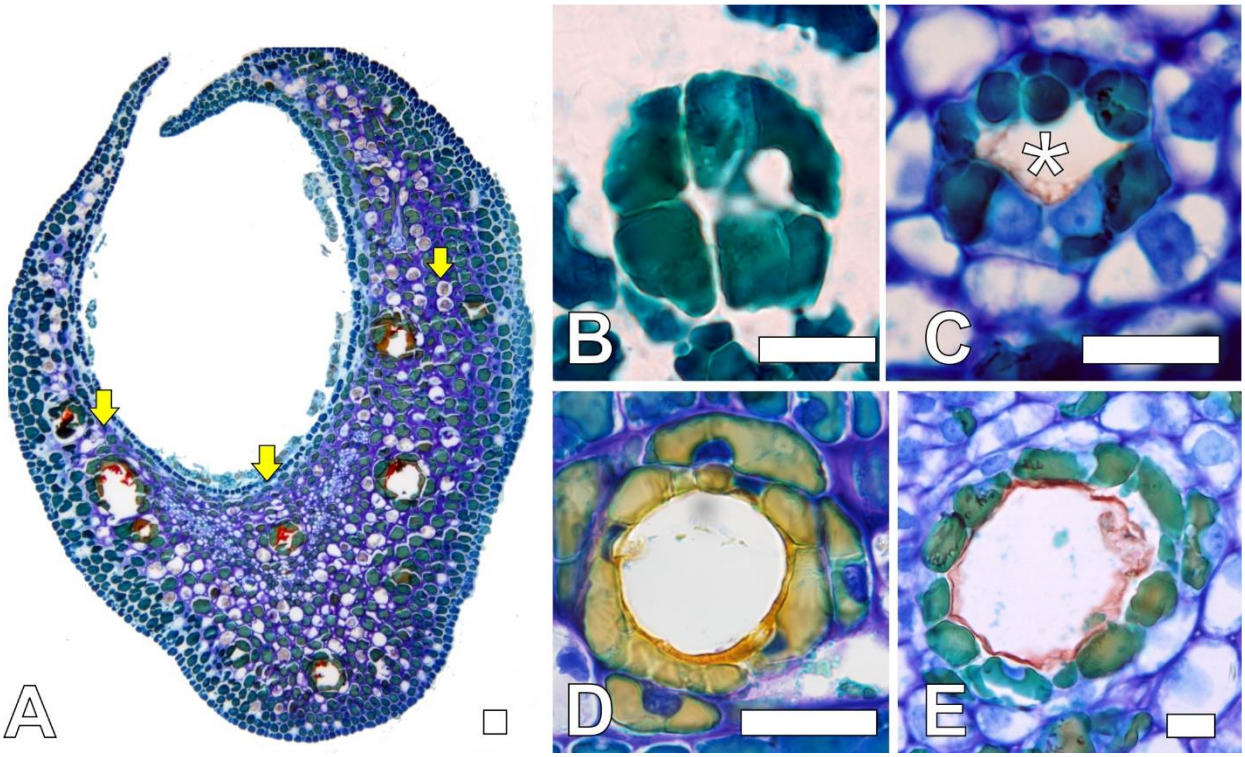

Figure 5 – Ontogenesis of the leaf secretory cavities in *Ardisia solanacea*. A – E, Light microscopy. A – Transversal section of the leaf primordium (ca. 5 mm), showing secretory cavities at different development stages (yellow arrows). B – Cluster of cells in the initial step of development, with the anticlinal division of a single ground meristem cell, followed by several division at different planes. C – Secretory cavity with the lumen formation. D and E – Fully developed cavity. Bars – A = 100 µm; B-E = 20 µm.

Capítulo 4

A ANATOMIA DE PRIMULACEAE E SEU SIGNIFICADO TAXONÔMICO E FILOGENÉTICO

Resumo

Primulaceae, circunscrita na ordem Ericales, é uma família de distribuição pantropical, que abrange cerca de 2.500 espécies. Durante as últimas duas décadas, as relações filogenéticas em Ericales, especialmente no clado formado pelas famílias Myrsinaceae, Theophrastaceae, Maesaceae e Primulaceae, foram alvo de estudo de muitos grupos de pesquisa que utilizaram dados morfológicos e moleculares combinados. Entretanto, as delimitações infragenéricas na família ainda carecem de estudos. Além disso, estas hipóteses filogenéticas estão baseadas em grande parte na análise de espécies Paleotropicais, negligenciando as Neotropicais. Desta maneira, conduzir estudos filogenéticos, utilizando espécies do Neotrópico e associando dados anatômicos é um passo significativo na resolução da delimitação dos gêneros. Neste trabalho foram realizados ensaios filogenéticos para os gêneros lenhosos de Primulaceae no Brasil, a partir de dados anatômicos foliares e da madeira. Além disso, reconstruiu-se a evolução das estruturas secretoras presentes nesta subfamília, a partir de análises ontogenéticas. A hipótese filogenética obtida apresentou IC = 0.36 e recuperou o monofiletismo das subfamílias analisadas.

Palavras-chave: anatomia vegetal, Myrsinaceae, Theophrastaceae; anatomia da madeira, anatomia foliar

Introdução

Ericales está inserida no clado das asterídeas e apresenta monofiletismo bem suportado (Bremer *et al.* 2002; Schönenberger *et al.* 2005). A ordem circunscreve 25 famílias e 346 gêneros (APG III 2009). Em Ericales, muitos estudos validam a significância dos caracteres anatômicos da madeira para a sistemática do grupo, uma vez que fornecem informações que estão em concordância com as filogenias baseadas em dados moleculares (em Ericales - Lens *et al.* 2007, em Primuloideae – Lens *et al.* 2005a, em Balsaminoideae – Lens *et al.* 2005b). Lens *et al.* (2007) reúnem dados sobre a anatomia da madeira de 52 espécies de Ericales, e os combinam com os dados moleculares da Ordem gerados por Schönenberger *et al.* (2005), a fim de demonstrar o valor sistemático de caracteres da madeira, destacando a importância de se conduzir estudos nessa área, e concluem que os caracteres da madeira foram informativos quando combinados aos moleculares.

A família Primulaceae, subordinada à ordem Ericales (APG III 2009 e APG IV 2016), apresenta distribuição pantropical e é encontrada em regiões temperadas e tropicais. Seus representantes, cerca de 2500 espécies, apresentam porte arbóreo, arbustivo ou herbáceo. As relações filogenéticas na família tem sido foco de estudo de muitos grupos de pesquisa, que utilizam dados morfológicos e/ou moleculares, destes, sequências de genes plastidiais (*atp*B, *ndh*F, *rbcl, mat*K e t*rnL-F*), nucleares (ITS) e mitocondriais (*atp1* e *mat*R) (Anderberg & Ståhl 1995; Anderberg *et al.* 1998, 2000, 2002; Caris & Smets 2004; Oh *et al.* 2008).

Com base nesses estudos, foram reconhecidas quatro famílias pertencentes ao clado primulóide: Primulaceae, Myrsinaceae, Theophrastaceae e Maesaceae (Källersjö *et al.* 2000). Devido à proximidade filogenética, a partir do APG III (2009) estas famílias passaram à categoria de subfamílias de Primulaceae, ficando estabelecida a família Primulaceae *sensu lato* e as subfamílias: Maesoideae, Myrsinoideae, Primuloideae e Theophrastoideae. Entretanto, apesar dos inúmeros trabalhos acerca da filogenia de Primulaceae, ainda são incipientes os que tratam as delimitações infragenéricas na família.

A subfamília Primuloideae tem distribuição restrita às regiões temperadas, Myrsinoideae tem distribuição pantropical, Theophrastoideae ocorre no Neotrópico e Maesoideae é restrita às regiões Paleotropicais (Stevens *onwards* 2001)

A utilização de caracteres morfo-anatômicos individualmente ou combinados à dados moleculares são promissores para a compreensão das relações entre gêneros e famílias de Angiospermas, p. ex. em Bromeliaceae (Gomes-da-Silva *et al.* 2012; Monteiro *et al.* 2015), Leguminosae (Redden *et al.* 2010), Myrtaceae (Soh & Parnell, 2011), e em Primulaceae (grupo primulóide - Anderberg & Ståhl 1995).

Apesar do esforço em compreender as relações filogenéticas de todo o grupo, no clado Primulóide, até o momento nenhuma classificação foi conduzida unindo todos os gêneros estudados neste trabalho. Face ao exposto, levantam-se as seguintes questões: 1) é possível recuperar o monofiletismo dos gêneros Neotropicais de Primulaceae utilizando os dados anatômicos? e 2) quais são as sinapormofias anatômicas de cada clado formado?

Para responder as perguntas formuladas, neste capítulo é apresentada uma hipótese filogenética baseada em dados anatômicos da folha e da madeira abrangendo os táxons Neotropicais de Primulaceae. Além disso, o presente estudo explora as tendências evolutivas de algumas características.

Material e métodos

A fim de realizar uma análise combinada de todos os dados levantados nos três primeiros capítulos, foram conduzidos: *(i)* ensaios filogenéticos a partir de matrizes preparadas com dados anatômicos da folha e da madeira; e *(ii)* análise dos componentes principais combinando os dados da folha e da madeira.

Ensaio filogenético

Terminais

Para a análise cladística foram incluídos 37 terminais (Apêndice 1), dos quais 36 espécies, 32 de Myrsinoideae e 4 de Theophrastoideae como grupos internos e 1 espécie de Sapotaceae, grupo irmão de Primulaceae (Anderberg *et al.* 2002), utilizada para o enraizamento da árvore (Nixon & Carpenter 1993).

Caracteres

Os dados anatômicos utilizados para a confecção da matriz de caracteres foram obtidos nos dois primeiros capítulos do presente estudo. Os dados anatômicos foliares das espécies de *Clavija* foram extraídos de Ståhl (1991) e os de *Pouteria* de Monteiro *et al.* (2007). Para os dados anatômicos da madeira de *Pouteria* foram utilizados dados do Inside Wood Sendo assim, foram selecionados 34 caracteres filogeneticamente informativos (discretos e contínuos), excluindo as autapomorfias, totalizando 20 referentes à anatomia foliar, 12 à anatomia da madeira e 2 sobre o arranjo das folhas e o hábito. Os caracteres contínuos foram convertidos em discretos a partir da criação de classes para os estados numéricos.

Foram utilizados caracteres neomórficos e transformacionais baseados na observação da diversidade morfológica encontrada nos órgãos vegetativos e reprodutivos. Os caracteres levantados serão analisados quanto ao seu estado, de acordo com os quatro componentes propostos por Sereno (2007). Os componentes são: localizador (L1) (estrutura morfológica), variável (V) (aspecto que varia), qualidade da variável (q) (modificação da variável) e o estado do caractere (Vn) (condições exclusivas do caráter) (Tabela 3). A partir desses quatro componentes podem-se definir dois padrões fundamentais de caractere: o neomórfico (presença e ausência de um caractere) e o transformacional (qualidade do caractere) (Sereno 2007).

Tabela 1- Exemplo de estrutura lógica para os caracteres morfológicos. Dois tipos fundamentais são reconhecidos. Legenda: Ln- estrutura morfológica; V- variável; q- qualidade da variável; vn- estado do caractere. (Extraído e modificado de: Sereno, 2007).

Tipo de caractere	Exemplo	Notação simbólica
Neomórfico	L1 V v0 v1 Folha, bainha: ausente (0); presente (1)	L1; V; v0; v1
Neomórfico	L1 v0 v1 Tricomas glandulares: ausente (0); presente (1)	L1; v0; v1
Transformacional	L1 V q v0 v1 v2 Folha, corte transversal, forma: dorsiventral (0); homogênea (1); isolateral (2)	L1; V; q; v0; v1; v2

Análise cladística

As análises foram realizadas através do critério da máxima parcimônia, conduzindo uma busca heurística com 1000 replicações (Nreps=1000), adição randômica de terminal (AddSeq = Random), mantendo 10 árvores por replicação (hold=10), com rearranjo dos ramos por meio da utilização algoritmo *branch swapping* com o método *tree bisection-reconnection* (TBR) (Hennig86; Farris, 1988), a partir do método de otimização dos caracteres ACCTRAN (*accelerated transformation optimization*), desordenados e sem peso, e retenção de múltiplas árvores mais parcimoniosas (MAXTREE), utilizando o programa PAUP* 4.0 para Windows (Swofford, 2002). Os valores de suporte dos ramos foram avaliados através da replicação "*bootstrap*" de busca heurística com 1000 replicações (Felsenstein, 1985).

Para apresentação, editoração e mapeamento dos caracteres nos cladogramas foi utilizado o programa Mesquite v 3.10 (Maddison & Maddison, 2016). O *software* Winclada v. 1.00.08 (Nixon 2002) também foi utilizado como uma ferramenta complementar para a otimização dos caracteres.

Análise estatística

Com os dados obtidos nos capítulos 1 e 2, foi realizada uma análise combinada dos componentes principais para testar a validade dos caracteres anatômicos na segregação das espécies e gêneros de Primulaceae (Manly, 1994). Os dados anatômicos foliares das espécies de *Clavija* não foram analisados, portanto, não foram incluídos na análise. A análise dos componentes principais (ACP) foi realizada com o *software* Statistica v. 7.0 (paraWindows).

Resultados

Ensaio filogenético

Foram realizadas análises com os dados anatômicos foliares separados e com o acréscimo de outros dados morfológicos (forma do ápice e da base das folhas, pubescência dos ramos), entretanto, a última análise não recuperou o monofiletismo da maioria dos ramos. Desta maneira, optou-se por priorizar a apresentação dos dados anatômicos da folha e da madeira combinados, os quais resolveram melhor as delimitações infragenéricas.

A análise de parcimônia realizada a partir da matriz de caracteres anatômicos da folha e da madeira gerou 33 árvores igualmente parcimoniosas com 144 passos, índice de consistência (IC) = 0,36 e índice de retenção (IR) = 0,63. Dos 33 caracteres utilizados, somente 1 não foi filogeneticamente informativo. O mapeamento dos estados de caráter foi realizado na primeira árvore mais parcimoniosa resultante da análise (Fig. 1). O baixo IC está relacionado com a quantidade de caracteres homoplásticos (círculos brancos – Fig. 1). Os clados/grados formados receberam letras nos ramos para facilitar descrição e posterior discussão.

No consenso estrito (Figura 2) as duas subfamílias amostradas na presente análise emergem monofiléticas, sendo o clado A (Theophrastoideae) com 60% de suporte *bootstrap* e o clado B (Myrsinoideae) com 91% de suporte.

As sinapormorfias não homoplásticas que caracterizam a subfamília Theophrastoideae são: a presença de feixes de fibras ao longo do mesofilo (caracter 17 | Fig. 3C) e os raios do xilema compostos por células quadradas e procumbentes (29), e a sinapomorfia homplástica é o arranjo pseudoverticilado das folhas no ramo (32). Quatro sinapomorfias anatômicas não homoplásticas caracterizam o clado formado pelos gêneros subordinados à subfamília Myrsinoideae, a presença de estômatos anisocíticos (5 | Figura 3A), a presença de cavidades secretoras (14 | Fig. 5), a ausência de esclerênquima na margem das folhas (20 | Figura 3D) e a presença de pontoações coalescentes (28).

A anatomia não recupera o monofiletismo dos gêneros analisados de Theophrastoideae (Figs. 1 e 2). Dos gêneros subordinados à subfamília Myrsinoideae, *Myrsine* (Clado F) emerge como grupo irmão do clado C do qual emergem os gêneros *Cybianthus* (monofilético), *Ardisia* e *Stylogyne* (parafiléticos) e é sustentado pela forma do sistema vascular na nervura, característica não homoplástica. O clado D é formado pelas espécies de *Cybianthus* e apresenta duas sinapomorfias homoplásticas: o contorno levemente sinuoso da parede anticlinal em vista frontal na face adaxial (8) e na face abaxial (9); e uma sinapomorfia não homoplástica: o tipo de estômato (5).

O Clado D^1 é formado por *Cybianthus brasiliensis* e *C. guyanensis* e é caracterizado por duas sinapormofias homoplásticas, pelo arranjo em "V" do sistema vascular no pecíolo (2) e a presença de células mucilaginosas na epiderme (12). O Clado D^2 é formado por *Cybianthus densiflorus* e *Cybianthus nemoralis*, sendo sustentado pela seguinte característica homoplástica: o arranjo em "V" do sistema vascular na nervura (3). Além disso, uma autapomorfia para *C. densiflorus* é a ausência de raios no xilema e para *C. nemoralis* é a presença de elementos de vaso com placas de perfuração escalariformes. O Clado D^3 é formado por *Cybianthus venezuelanus*, *C. glaber* e *C. verticillatus* e é sustentado pelo arranjo em arco do sistema vascular no pecíolo (2), pela presença de braquiesclereides na nervura e no pecíolo (19) e pelo arranjo pseudoverticilado das folhas nos ramos (32).

O Clado E abrange os gêneros *Ardisia* e *Stylogyne*, os quais são reunidos pelas características homoplásticas: presença do mesofilo fracamente dorsiventral (13); e pelas características não-homoplásticas: presença de células com invaginações no mesofilo (21) e arranjo escalariforme das pontoações raio-vasculares (30). Este clado é formado por dois grupos-irmãos formados: 1) *Stylogyne warmingii*, *S. atra* e *S. pauciflora*, que emergem monofiléticas devido ao arranjo em "V" do sistema vascular no pecíolo (característica homoplástica, 2); e 2) *Ardisia guianensis*, *A. solanacea*, *A. humilis, S. depauperata* e *S. sordida*,

que compartilham a frequência de 30-50 elementos de vaso/mm² no xilema (característica homoplástica, 23 | Figura 1).

O gênero *Myrsine* emerge monofilético (Clado F), e é sustentado por uma sinapormofia homoplástica: o arranjo do sistema vascular em arco com extremidades fechadas na nervura (2); e uma não-homoplástica: a presença de camadas de crescimento distintas (22). O Clado F^1 é constituído pelas espécies, *Myrsine glazioviana, M. venosa* e *M. vilosissima*. As características que sustentam o ramo são o contorno biconvexo do pecíolo (1) e a superfície adaxial ondulada (11). O clado F^2 é formado pelas demais espécies de *Myrsine* e é sustentado por duas características homoplásticas: a presença de feixe adicional sobre o sistema vascular da nervura mediana (4) e pelo arranjo em arco com extremidades convolutas do sistema vascular na nervura mediana. Além destas características, o Clado F^2 abrange as espécies de *Myrsine* nas quais ocorrem as estruturas secretoras nos raios do xilema (*breakdown areas in rays*). A figura 4 sumariza todas sinapomorfias anatômicas, apresentadas sobre cada ramo.

Evolução dos caracteres

Os caracteres não-homoplásticos que tiveram a evolução mapeada sobre a hipótese filogenética gerada foram: o tipo de estômato (Fig. 3A), a ausência ou presença de cavidades secretoras (Fig. 3B), a ausência ou presença de feixes de fibras ao longo do mesofilo (Fig. 3C), a ausência ou presença de esclerênquima na margem da folha (Fig. 3D).

A reconstrução da condição ancestral para o tipo de estômato revela que a condição plesiomórfica são os estômatos anomocíticos (Fig. 3A-c), mas esta característica evoluiu independentemente duas vezes dentro do grupo estudado, sendo encontrados estômatos anisocíticos em *Ardisia, Myrsine* e *Stylogyne* (Fig. 3A-a) e estômatos paracíticos em *Cybianthus* e *M. glazioviana* (Fig. 3A-b).

As cavidades secretoras constituem um ganho evolutivo da subfamília Myrsinoideae, como apresentado na Figura 3B. A presença de feixes de fibras (Fig. 3C) e de esclerênquima na margem da folha (Fig. 3D) é reconstruída como um caráter ancestral, estando presente em Theophrastoideae e ausente em Myrsinoideae.

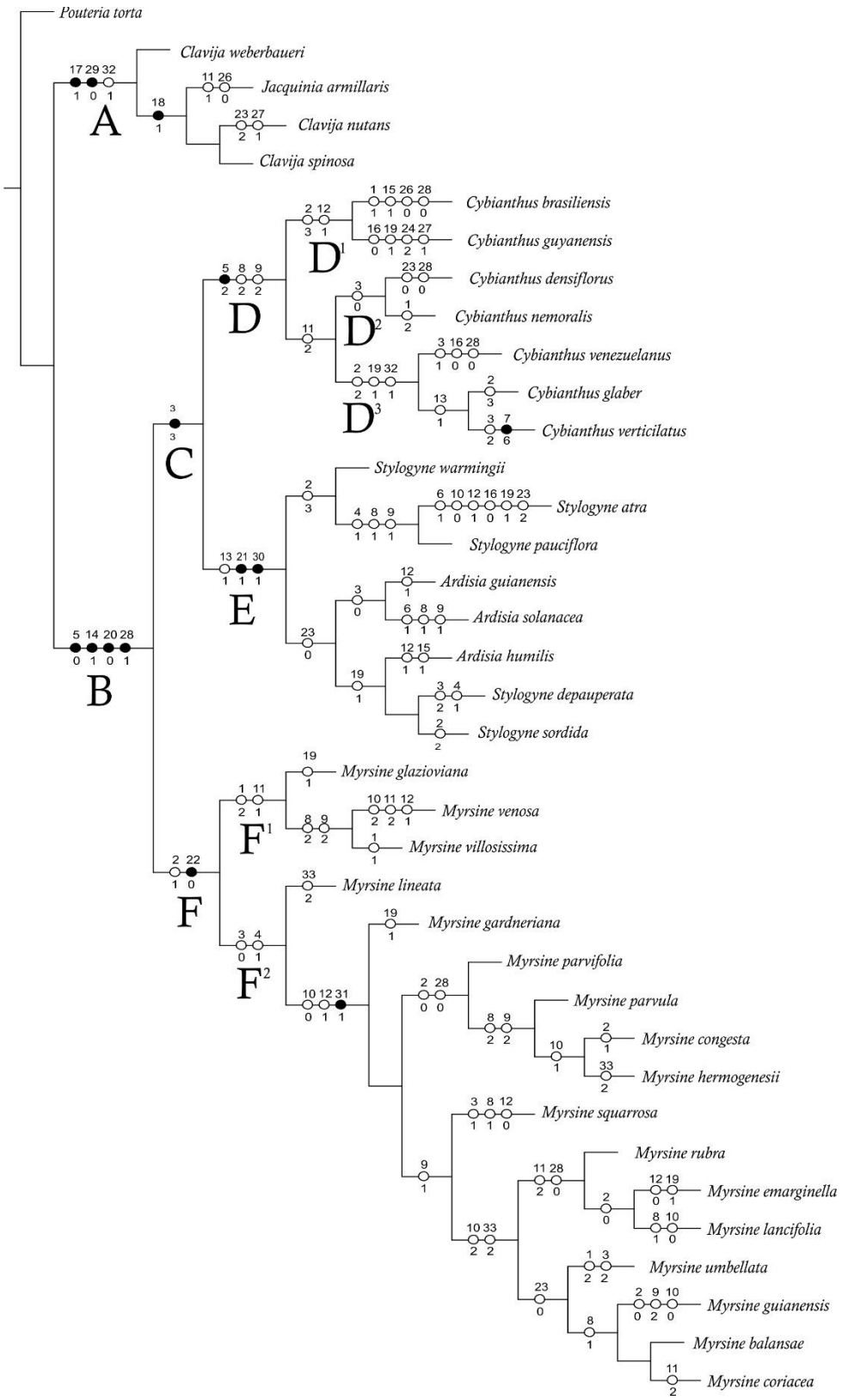

Figura 1 – Mapeamento dos caracteres em uma das árvores igualmente parcimoniosas (realizado no *software* Winclada). Os círculos brancos representam os caracteres homoplásticos, enquanto os pretos são os não homoplásticos. Os números acima dos ramos indicam os caracteres e abaixo dos ramos são os estados dos caracteres.

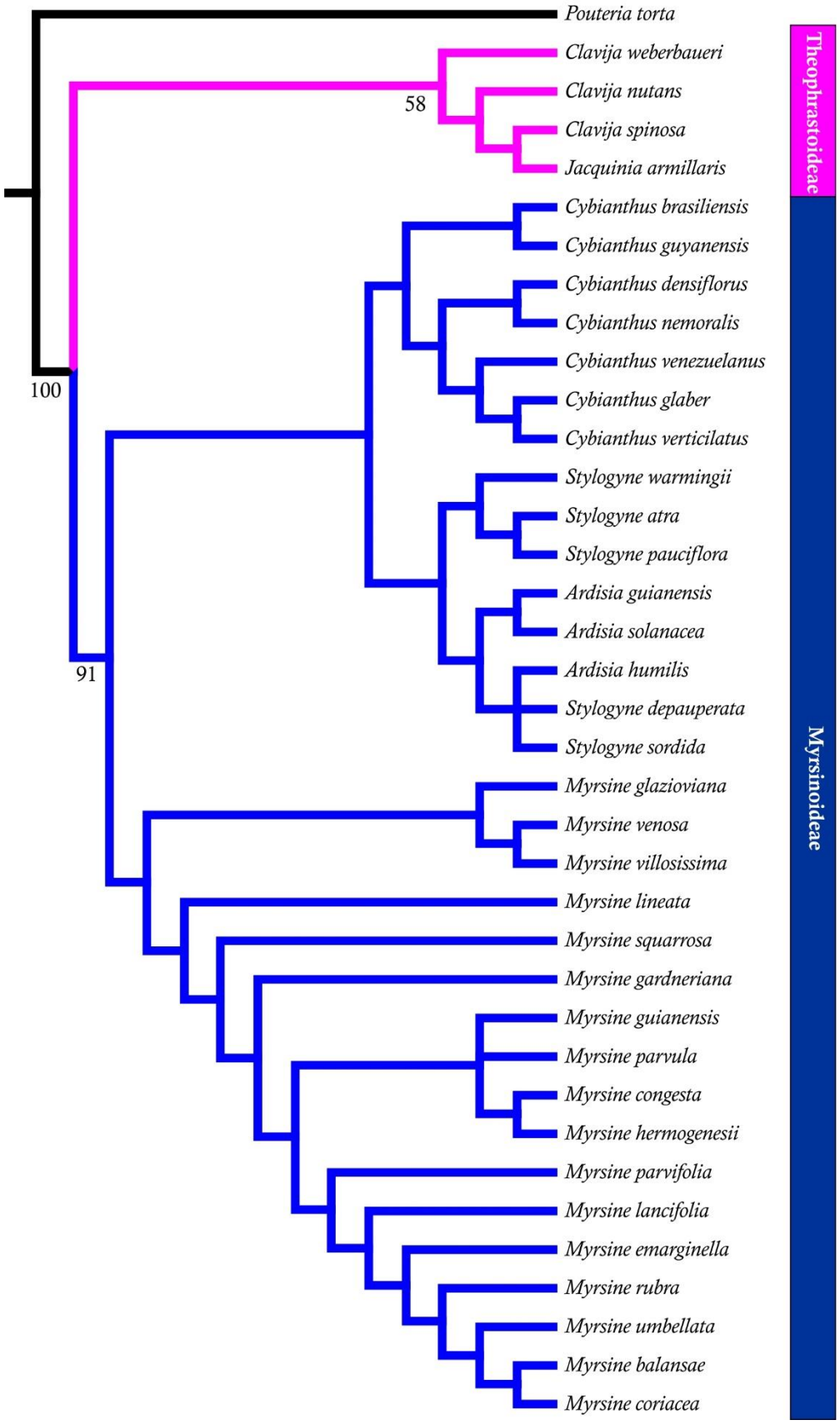

Figura 2 – Consenso estrito de dez árvores igualmente parcimoniosas. Análise de parcimônia com base em dados anatômicos da folha e da madeira. Os números nos ramos indicam os valores de *bootstrap* maiores que 50%.

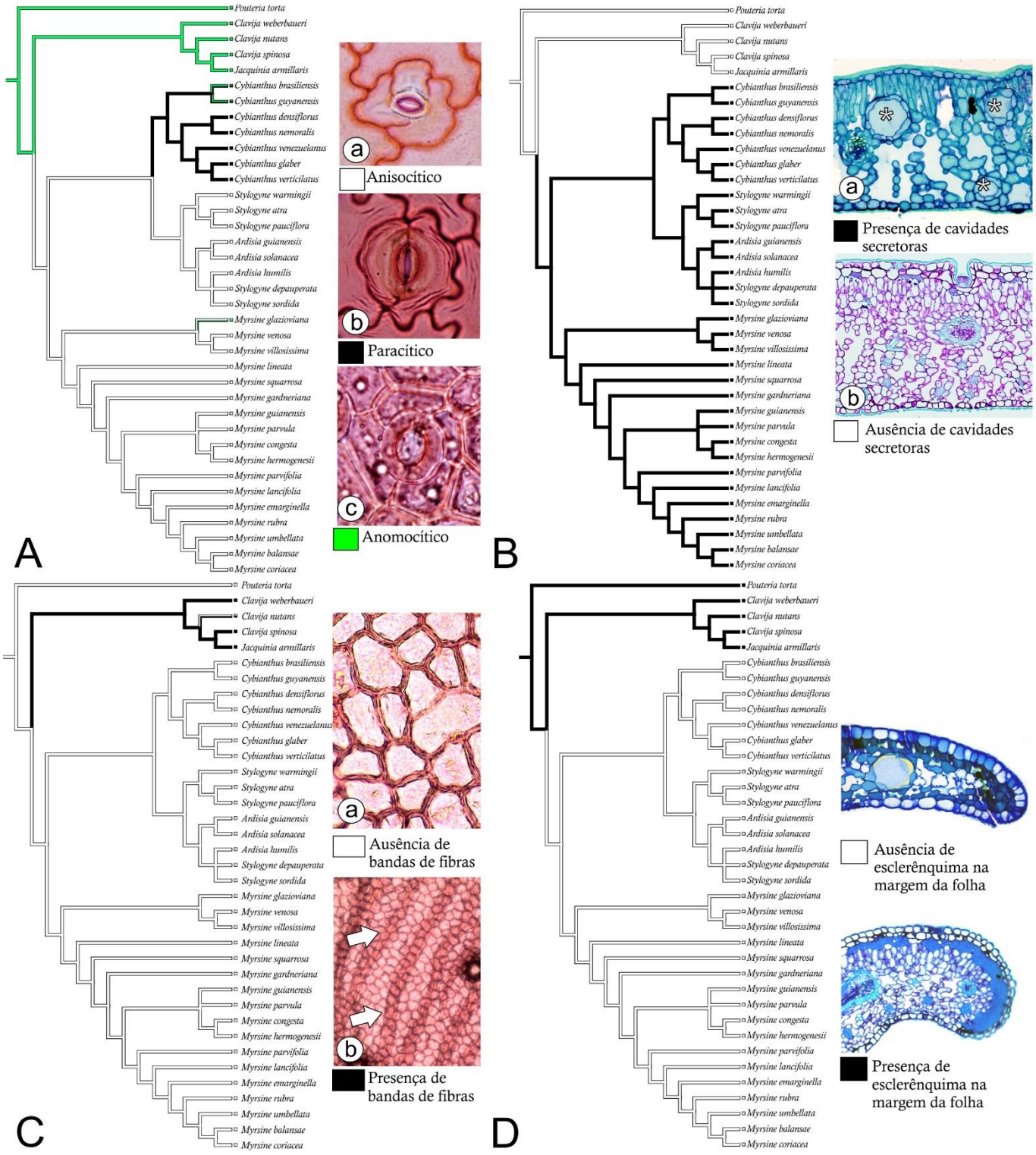

Figura 3 – **A -** Mapeamento dos tipos de estômatos. a – c – Vista frontal (VF). a – Estômato anisocítico em *Stylogyne atra*. b – Estômato paracítico em *Cybianthus*. c – Estômato anomocítico em *Jacquinia armillaris.* **B -** Mapeamento da presença cavidades secretoras nas folhas. a e b – Secção transversal (ST). a – Presença de cavidades secretoras no mesofilo de *Myrsine emarginella.* b – Ausência de cavidades secretoras em *Jacquinia armillaris.* **C** – Mapeamento da ausência (a) ou presença (b) de fibras ao longo do mesofilo. **D** – Mapeamento da presença de esclerênquima na margem das folhas. a – Secção transversal da folha de *Ardisia guianensis* evidenciando a ausência de esclerênquima na margem da folha. b - ST da folha de *Jacquinia armillaris*

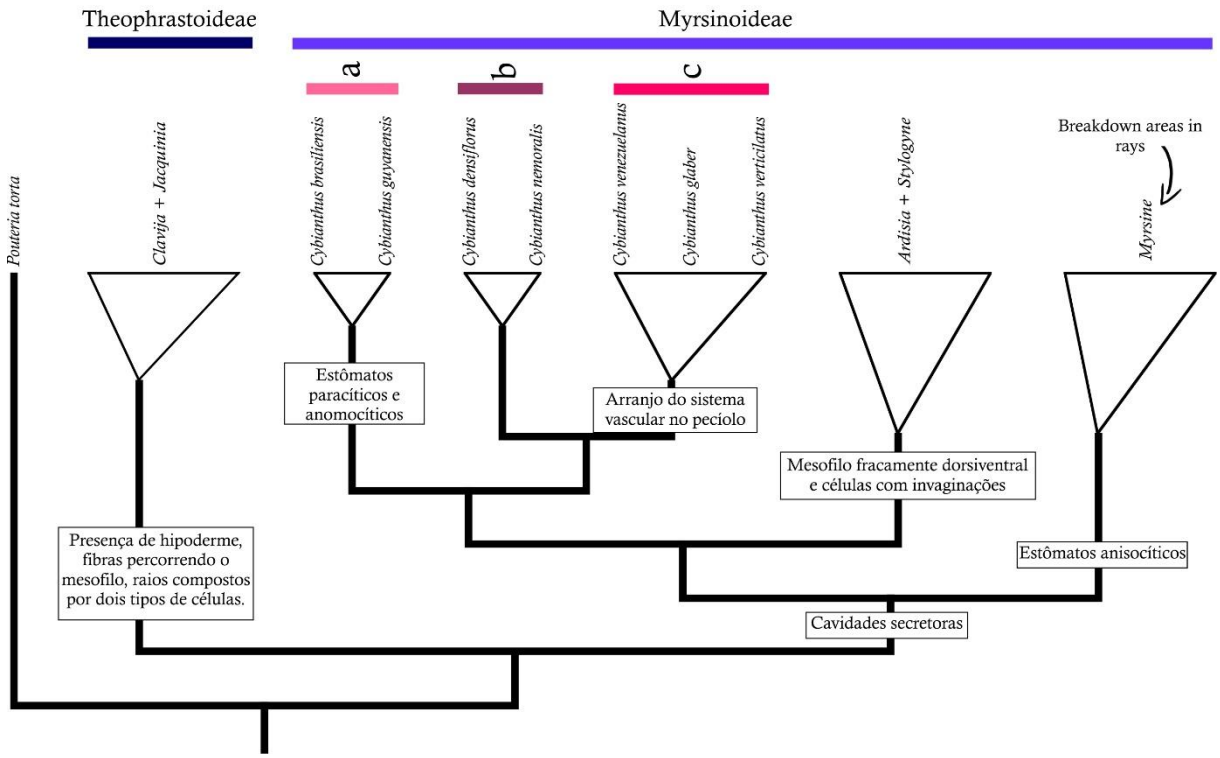

Figura 4 – Mapeamento dos caracteres. As barras em tons de rosa indicam os agrupamentos das espécies de *Cybianthus* de acordo com os subgêneros propostos por Agostini (1980). A – Subgênero *Conomorpha*. B – Subgênero *Weilgetia*. C – Subgênero *Cybianthus*.

Combinação dos dados anatômicos da folha e da madeira

Através da análise dos componentes principais com os dados anatômicos da folha e da madeira observou-se a separação das subfamílias Myrsinoideae e Theophrastoideae e de cada gênero (Fig. 5). A única espécie que se distanciou do gênero ao qual é circunscrita foi *Cybianthus densiflorus*, devido à ausência de raios no xilema. O Fator 1 respondeu por 18% da variância total, o Fator 2 por 15% e o Fator 3 por 11%, totalizando 44%. No Fator 1 os caracteres com maiores valores positivos foram o parênquima paratraqueal escasso (0.87), as estruturas secretoras no raio (0.83), os raios compostos por células eretas, quadradas e procumbentes, e as cavidades secretoras (0.87); e os com maiores valores negativos foram o parênquima raro ou ausente (-0.87), os raios com mais de 10 células de largura (-0.87), raios compostos por células quadradas e procumbentes (-0.87), os três feixes separados no sistema vascular do pecíolo (-0.87), o tricoma tipo 7 (-

0.87), os feixes de fibras extra-xilemáticas percorrendo o mesofilo (-0.87), a hipoderme (-0.87) e a presença de esclerênquima na margem da folha (-0.87). No Fator 2, os caracteres que apresentaram maiores scores positivos foram as camadas de crescimento pouco distintas (0.72) e indistintas (0.71), e os estômatos paracíticos (0.86). Os caracteres com maiores scores negativos foram as camadas de crescimento distintas (-0.71). No Fator 3 o caracter com maior valor positivo foi o mesofilo fracamente dorsiventral (0.67) e o maior score negativo foi o caracter "madeira sem raios" (-0.62).

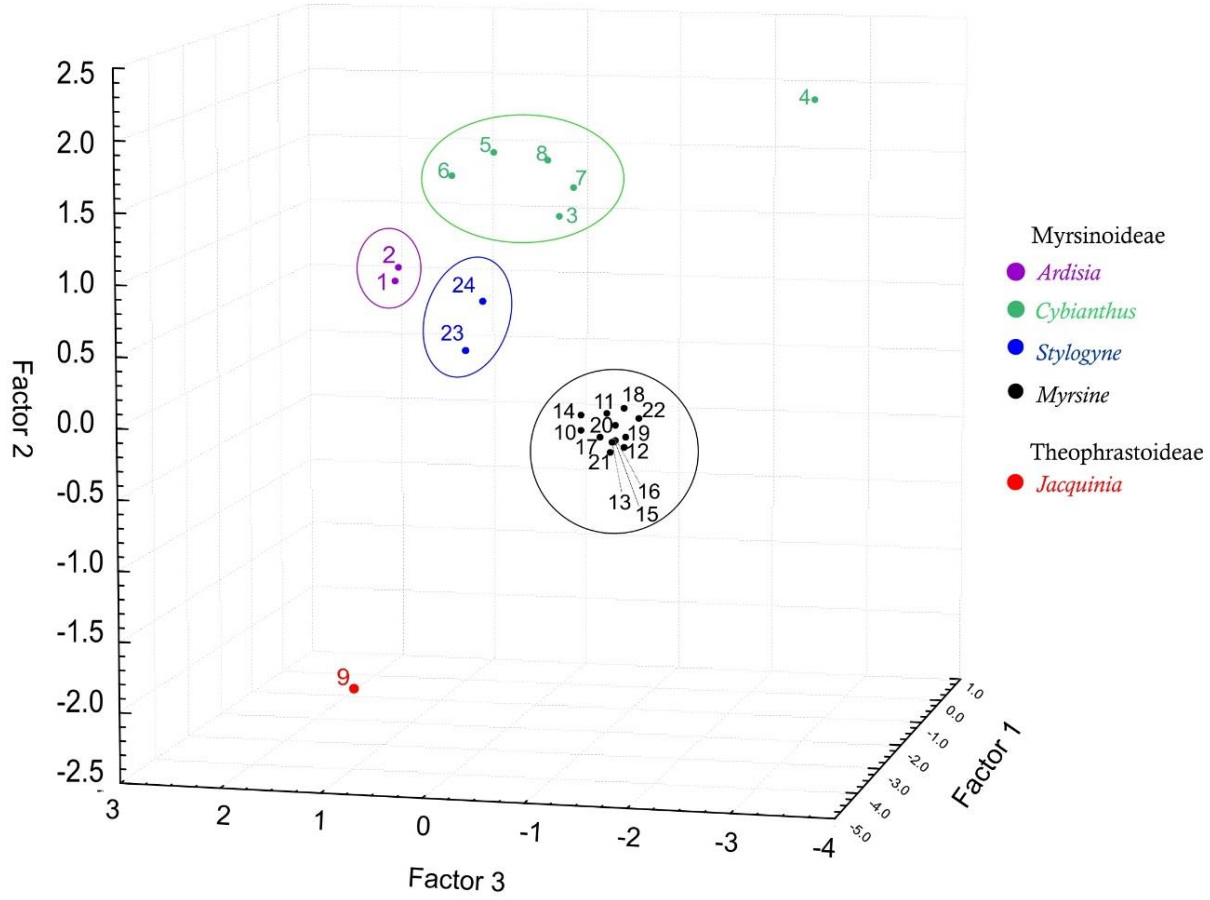

Figura 5 – Análise dos componentes principais com os dados anatômicos da folha e da madeira combinados. 1 – *Ardisia humilis*, 2 – *A. solanacea*, 3 –*Cybianthus brasiliensis*, 4 – *C. densiflorus*, 5 – *C. glaber*, 6 – *C. guyanensis*, 7 – *C. nemoralis*, 8 – *C. venezuelanus*, 9 – *Jacquinia armillaris*, 10 – *Myrsine coriacea*, 11 – *M. emarginella*, 12 – *M. gardneriana*, 13 – *M. glazioviana*, 14 – *M. guianensis*, 15 – *M. lancifolia*, 16 – *M. lineata*, 17 – *M. parvifolia*, 18 – *M. parvula*, 19 – *M. rubra*, 20 – *M. squarrosa*, 21 – *M. umbellata*, 22 – *M. villosissima*, 23 – *Stylogyne atra,* 24 – *S. pauciflora*.

Discussão

A família Primulaceae é reconhecidamente monofilética (APG III e APG IV), entretanto as relações entre os gêneros ainda carecem de maiores esclarecimentos. As primeiras análises que buscaram elucidar como os gêneros se organizavam nas famílias que antes formavam o clado "primulóide" (Ordem Primulales – Cronquist, 1981; Takhtajan, 1987) foram baseadas em dados morfológicos, palinológicos e embriológicos, detalhadamente tratados por Anderberg & Ståhl (1995). Os mesmos autores atentaram para a necessidade do rearranjo de alguns gêneros para que se estabelecesse o monofiletismo das subfamílias. Subsequentemente, as análises moleculares confirmaram a hipótese inicialmente obtida através dos dados morfo-anatômicos (Anderberg & Ståhl 1995). A hipótese filogenética apresentada neste trabalho abrange táxons que não haviam sido incluídos nas hipóteses previamente levantadas para a família. E, apesar do caráter homoplástico de muitos caracteres, a anatomia da folha e da madeira foi capaz de recuperar o monofiletismo das subfamílias Myrsinoideae e Theophrastoideae e dos gêneros *Cybianthus* e *Myrsine*.

O monofiletismo de Theophrastoideae é recuperado em todas as hipóteses filogenéticas já desenvolvidas que incluíram representantes do grupo (Anderberg *et al.* 1998; Mast *et al.* 2001; Anderberg *et al.* 2002; Källersjö & Ståhl, 2003), e as sinapomorfias anatômicas que sustentam a subfamília são: a ausência de cavidades secretoras e a presença de feixe de fibras percorrendo todo o mesofilo, características apontadas por Källersjö & Ståhl (2003), e também observadas nas espécies analisadas no presente trabalho. Entretanto, a hipótese filogenética aqui apresentada, não recupera o monofiletismo dos gêneros *Clavija* e *Jacquinia* devido à ausência de hipoderme em *C. weberbaueri*. Neste sentido, uma análise mais detalhada reunindo mais espécies de *Clavija* poderia auxiliar no esclarecimento da relação entre estes gêneros.

Além disso, de acordo com a reconstrução dos caracteres anatômicos, pode-se concluir que Theophrastoideae constitui um grupo mais próximo ao ancestral da família Primulaceae, por reunir maior número de caracteres plesiomórficos. Em contrapartida, Myrsinoideae abarca características que representam ganhos evolutivos para a subfamília. Tal arranjo, foi igualmente reportado por Schönenberger *et al.* (2005).

A subfamília Myrsinoideae tornou-se monofilética a partir do arranjo proposto por Anderberg *et al.* (1998) e, a partir de então, emerge monofilética em todas as análises propostas (Anderberg *et al.* 2002; Källersjö & Ståhl, 2003), incluindo também a presente análise. Dos quatro gêneros amostrados de Myrsinoideae, *Cybianthus* (Clado D) e *Myrsine* (Clado F) são monofiléticos O clado E, formado por *Ardisia* e *Stylogyne* não apresentou resolução, formando um agrupamento parafilético. Tradicionalmente, estes gêneros são proximamente relacionados devido à sobreposição dos caracteres morfológicos (Carrijo, 2011), como também foi observado neste trabalho (Capítulo 1). Esta condição deve estar relacionada à recente diversificação destes gêneros. Novas análises filogenéticas, combinando dados moleculares aos morfológicos aqui obtidos, podem oferecer maior resolução e novas informações às relações entre os gêneros e as espécies estudadas.

No clado D, as espécies de *Cybianthus* refletiram a classificação dos subgêneros proposta por Agostini (1980), a qual baseou-se especialmente em características morfológicas reprodutivas e vegetativas, sem considerar caracteres anatômicos. No clado D^1 *Cybianthus brasiliensis* e *C. guyanensis*, pertencentes ao subgênero *Conomorpha*, em D^2 as espécies do subgênero *Cybianthus*: *C. glaber*, *C. venezuelanus* e *C. verticilatus*; e em D^3, emergiram *C. densiflorus* e *C. nemoralis* do subgênero *Weilgetia*.

Dentre os subgêneros de *Cybianthus, Weilgetia* destaca-se por reunir espécies que apresentam características únicas se comparadas às demais espécies do gênero e da subfamília Myrsinoideae. Em *Cybianthus densiflorus* não ocorrem raios, uma característica que pode ser considerada uma evidência adicional à origem herbácea de um táxon

(Carlquist 1992), enquanto *C. nemoralis* apresenta placas de perfuração escalariformes, característica considerada mais basal em comparação às placas de perfuração simples (*sensu* Bailey 1944).

Hao *et al.* (2004) inferiu que a presença de cavidades secretoras (chamadas *colored glands*) em *Lysimachia* L. pode não ser uma condição apomórfica do gênero, uma vez que observaram que esta estrutura não está presente em todas as espécies. No mapeamento dos caracteres (Figura 4) observa-se que a presença de cavidades secretoras é uma sinapomorfia para Myrsinoideae. Entretanto, para compreender a evolução das cavidades/ductos secretores em Primulaceae, é necessário entender o contexto de classificação dos seus gêneros e subfamílias. Anteriormente às alterações taxonômicas que foram necessárias para garantir o monofiletismo das subfamílias que compõem Primulaceae (Anderberg *et al.* 1995; 1998), o gênero *Maesa* era circunscrito em Myrsinaceae (*sensu* APG II 2003 - atual Myrsinoideae *sensu* APG III 2009 e IV 2016), especialmente devido à presença de cavidades secretoras distribuídas por todos os órgãos da planta (Utteridge, 1998). Contudo, as filogenias morfológicas e moleculares não sustentavam o posicionamento deste gênero em Myrsinaceae (Anderberg *et al.* 1998). Assim, foi proposta a criação de uma família monogenérica, Maesaceae, a qual apresenta posicionamento filogenético basal em relação às demais subfamílias (Anderberg *et al.* 2000). Sendo assim, há dois caminhos possíveis para compreender a presença de cavidades/ductos secretores nestas duas subfamílias: *(i)* eram estruturas presentes no ancestral comum, portanto são estruturas homólogas, que foram perdidas nas espécies de Primuloideae e Theophrastoideae; *(ii)* representam um tipo de evolução paralela (convergência). Para confirmar esta relação e origem, são necessários estudos ontogenéticos comparando o desenvolvimento dessas estruturas nas duas subfamílias.

A análise dos componentes principais revelou que, quando combinados, os dados anatômicos da folha e da madeira contribuíram ainda mais para a segregação das

subfamílias e dos gêneros estudados. Havendo a formação de grupos distintos de cada gênero analisado, à exceção de *C. densiflorus*, que se distanciou das demais espécies de *Cybianthus* devido à ausência de raios no xilema. A segregação de *C. densiflorus* na presente análise reflete o que foi também observado no Capítulo 2 tanto na análise utilizando somente os dados anatômicos da madeira dos gêneros Neotropicais, quanto naquela realizada com os dados anatômicos combinados com os de Lens *et al*. (2005a).

De maneira geral, pode-se concluir que os dados anatômicos em Primulaceae apresentam peso taxonômico e filogenético e contribuem tanto para a segregação de gêneros e espécies, quanto para o estabelecimento das relações filogenéticas entre os táxons. Análises futuras combinando dados morfo-anatômicos e moleculares poderão fornecer informações ainda mais robustas a respeito das relações entre os gêneros e espécies Neotropicais da família.

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Tabela 2 - Matriz de dados morfológicos para análise filogenética dos gêneros Neotropicais de Primulaceae. Estados de caráter sem informação (-).

Espécies	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
Ardisia guianensis	0	0	0	0	0	0	0	0	0	2	0	1	1	1	0	1	0	0	0	0	1	-	-	-	-	-	-	-	-	-	-	0	1
A. humilis	0	0	3	0	0	0	0.4	0	0	2	0	1	1	1	1	1	0	0	1	0	1	2	0	2	1	1	1	1	1	1	0	0	1
A. solanacea	0	0	0	0	0	1	0	1	1	2	0	0	1	1	0	1	0	0	0	0	1	2	0	3	0	1	2	1	1	1	0	0	1
Cybianthus brasiliensis	1	3	3	0	1.2	0	1	2	2	2	0	1	0	1	1	1	0	0	0	0	0	2	1	1	0	0	0	0	1	0	0	0	1
C. densiflorus	0	0	0	0	2	0	0.2.3	2	2	2	2	0	0	1	0	1	0	0	0	0	0	2	0	1	0	1	0	0	1	0	0	0	0
C. glaber	0	3	3	0	2	0	0.2.3	2	2	2	2	0	1	1	0	1	0	0	1	0	0	-	-	-	-	-	-	-	-	-	-	1	1
C. guyanensis	0	3	3	0	1.2	0	1	2	2	2	0	1	0	1	0	0	0	0	1	0	0	2	1	2	0	1	1	1	1	0	0	0	1.2
C. nemoralis	2	0	0	0	2	0	2	2	2	2	2	0	0	1	0	1	0	0	0	0	0	2	1	1	0	1	0	1	1	0	0	0	1
C. venezuelanus	0	2	1	0	2	0	3.4	2	2	2	2	0	0	1	0	0	0	0	1	0	0	2	2	1	0	1	0	0	1	0	0	1	0
C. verticilatus	0	2	2	0	2	0	6	2	2	2	2	0	1	1	0	1	0	0	1	0	0	2	1.2	2	0	0	0	1	1	0	0	1	1
Clavija nutans	-	-	-	-	1	0	0	-	-	-	-	0	0	0	-	-	0.1	1	0	1	0	2	2	0	0	1	1	0	0	0	0	1	1
C. spinosa	-	-	-	-	1	0	0	-	-	-	-	0	0	0	-	-	1	1	0	1	0	2	1	0	0	1	0	0	0	0	0	1	1
C. weberbaueri	-	-	-	-	1	0	0.9	-	-	-	-	0	0	0	-	-	1	0	0	1	0	2	1	0	0	1	0	0	0	0	0	1	1
Jacquinia armillaris	0	4	2	0	1	0	0.7	0	0	1	1	0	0	0	0	1	1	1	0	1	0	2	0.1	0.1	0	0	0	0	0	0	0	1	1
Myrsine balansae	0	1	0	1	0	0	0	1	1	2	0	1	0	1	0	1	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	0	1
M. congesta	0	1	0	1	0	0	0	2	2	1	0	1	0	1	0	1	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	0	1
M. coriacea	0	1	0	1	0	0	0.5	1	1	2	2	1	0	1	0	1	0	0	0	0	0	0	0	0.1.2	1	1	0	1	1	0	0	0	2
M. emarginella	0	0	0	1	0	0	0	0	1	2	2	0	0	1	0	1	0	0	1	0	0	0	1	1	0	1	0	0	1	0	1	0	2
M. gardneriana	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0.1	0.1	0	1	0	1	1	0	1	0	2
M.glazioviana	2	1	2	0	0.1	0	0.6	0	0	1	1	0	0	1	0	1	0	0	1	0	0	0	1.2	0.1	0	1	0	1	1	0	0	0	1
M. guianensis	0	0	0	1	0	0	0	1	2	0	0	1	0	1	0	1	0	0	0	0	0	0	0	2	0.1	1	0	1	1	0	1	0	2
M. hermogenesii	0	0	0	1	0	0	0	2	2	1	0	1	0	1	0	1	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	0	2
M. lancifolia	0	0	0	1	0	0	0	1	1	0	2	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	2
M. lineata	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	1	1	0	0	0	2
M. parvifolia	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	1
M. parvula	0	0	0	1	0	0	0	2	2	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	2
M. rubra	0	1	0	1	0	0	0	0	1	2	2	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	2
M. squarrosa	0	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	1	1	0	1	0	1
M. umbellata	2	1	2	1	0	0	0	0	1	2	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	1	0	1.2
M. venosa	2	1	2	0	0	0	0	2	2	2	2	1	0	1	0	1	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	0	2
M. villosissima	1	1	2	0	0	0	0.6	2	2	1	1	0	0	1	0	1	0	0	0	0	0	0	2	1	0	1	0	0	1	0	0	0	1
Stylogyne atra	0	3	3	1	0	1	0	1	1	0	0	1	1	1	0	0	0	0	1	0	1	1	2	1	0	1	0	1	1	1	0	0	1
S.depauperata	0	0	2	1	0	0	0	0	0	2	0	0	1	1	0	1	0	0	1	0	1	-	-	-	-	-	-	-	-	-	-	0	1
S. pauciflora	0	3	3	1	0	0	0	1	1	2	2	0	1	1	0	1	0	0	0	0	1	1	1	1	0	1	0	1	1	1	0	0	1
S. sordida	0	2	3	0	0	0	0	0	0	2	0	0	1	1	0	1	0	0	1	0	1	-	-	-	-	-	-	-	-	-	-	0	1
S. warmingii	0	3	3	0	0	0	0	0	0	2	2	0	1	1	0	1	0	0	0	0	1	-	-	-	-	-	-	-	-	-	-	0	1
Pouteria torta	2	0	1	0	1	0	8	0	0	2	2	0	0	0	0	0	0	0	0	1	0	2	3	2	1	0	2	0	1	0	0	2	2

Lista 1 - Caracteres anatômicos e respectiva codificação

1. Anatomia foliar | Pecíolo | Contorno
(0) plano-convexo (1) côncavo-convexo (2) biconvexo

2. Anatomia foliar | Pecíolo | Sistema vascular
(0) arco com extremidades convolutas (1) arco com extremidades fechadas
(2) arco (3) "V" (4) Três feixes (Separated bundles)

3. Anatomia foliar | Nervura mediana | Sistema vascular
(0) arco com extremidades convolutas (1) arco com extremidades fechadas
(2) arco (3) "V"

4. Anatomia foliar | Nervura mediana | Feixe adicional

(0) ausente (1) presente

5. Anatomia foliar | Epiderme | estômatos
(0) anisocíticos (1) anomocíticos (2) paracíticos

6. Anatomia foliar | Epiderme |posição dos estômatos
(0) hipoestomática (1) anfiestomática

7. Anatomia foliar | Epiderme |Tricomas
(0) tipo 0 (1) tipo 1 (2) tipo 2 (3) tipo 3 (4) tipo 4 (5) tipo 5 (6) tipo 6 (7) tipo 7
(8) tipo 8 (9) tipo 9

8. Anatomia foliar | Epiderme |Contorno da parede anticlinal – face adaxial
(0) reta (1) levemente sinuosa (2) sinuosa

9. Anatomia foliar | Epiderme | Contorno da parede anticlinal – face abaxial
(0) reta (1) levemente sinuosa (2) sinuosa

10. Anatomia foliar | Epiderme | Superfície abaxial
(0) lisa (1) ondulada (2) estriada

11. Anatomia foliar | Epiderme | Superfície adaxial
(0) lisa (1) ondulada (2) estriada

12. Anatomia foliar | Epiderme |Células mucilaginosas
(0) ausente (1) presente

13. Anatomia foliar | Mesofilo|Arranjo
(0) dorsiventral (1) fracamente dorsiventral

14. Anatomia foliar | Cavidades secretoras
(0) ausente (1) presente

15. Anatomia foliar | Cristais prismáticos
(0) ausente (1) presente

16. Anatomia foliar | Drusas
(0) ausente (1) presente

17. Anatomia foliar | Feixes de fibras
(0) ausente (1) presente

18. Anatomia foliar | Hipoderme
(0) ausente (1) presente

19. Anatomia foliar | Braquiesclereídes
(0) ausente (1) presente

20. Anatomia foliar | Esclerênquima na margem da folha
(0) ausente (1) presente

21. Anatomia foliar | Células com invaginações no mesofilo
(0) ausente (1) presente

22. Anatomia da madeira | Camadas de crescimento
(0) disitinta (0) pouco distinta (1) indistinta

23. Anatomia da madeira | Elementos de vaso | Frequência
(0) 30-50/mm² (1) 50-100/mm² (2) 100-200/mm² (3) >200/mm²

24. Anatomia da madeira | Elementos de vaso | Comprimento
(0) <350 μm (1) 300-500 μm (2) 500-650 μm (3) >650 μm

25. Anatomia da madeira | Elementos de vaso | Diâmetro
(0) 20-50 μm (1)>50 μm

26. Anatomia da madeira | Fibras | Fibras septadas
(0) ausente (1) presente

27. Anatomia da madeira |Fibras | Comprimento
(0) 300-500 μm (1) 500-700 μm (2) 700-900 μm (3) 900-1100 μm (4) >1100 μm

28. Anatomia da Madeira | Pontoações coalescentes
(0) ausente (1) presente

29. Anatomia da madeira | Raios | Composição celular
(0) células quadradas e procumbentes (1) células eretas, quadradas e procumbentes

30. Anatomia da madeira | Arranjo das pontoações
(0) alternas (1) escalariformes

31. Anatomia da madeira | Breakdown areas in rays
(0) ausente (1) presente

32. Morfologia externa | Arranjo das folhas
(0) alternas (1) pseudoverticiladas

33. Hábito
(0) sub-arbusto (1) arbusto (2) árvore

Tabela 3 - Factor loadings

Caracteres			Fator 1	Fator 2	Fator 3
Anatomia da madeira		Camadas de crescimento distintas	0.52397	**-0.71176**	-0.36317
		Camadas de crescimento pouco distintas	-0.09409	**0.72664**	0.493312
		Camadas de crescimento indistintas	-0.52397	**0.71176**	0.363171
	Elementos de vaso	30-50 vasos/mm²	-0.42663	-0.39252	0.094279
		50-100 vasos/mm2	0.38352	-0.0515	0.321683
		100-200 vasos/mm2	-0.10593	0.28116	-0.41064
		> 200 vasos/mm2	-0.01495	0.12203	-0.04938
		Comprimento do vaso < 350 µm	-0.64989	0.09778	-0.27454
		Comprimento do vaso entre 350 a 500 µm	0.49207	-0.43786	-0.27564
		Comprimento do vaso entre 500 e 650 µm	0.06278	0.22998	0.356024
		Comprimento do vaso > 650µm	0.07353	0.12182	0.390404
		Diâmetro tangencial do vaso entre 20 e 50 µm	-0.08919	0.01959	-0.2652
		Diâmetro tangencial do vaso >50 µm	0.13418	-0.08352	0.211631
		Placa de perfuração escalariforme	-0.1349	0.28134	-0.06241
		Placas de perfuração simples	0.1349	-0.28134	0.062412
		Pontoações coalescentes	0.33695	0.0817	0.457632
	Fibras	Fibras septadas	0.61395	-0.08802	-0.19335
		Comprimento fibras 300-500	-0.73358	-0.09143	0.097115
		Comprimento fibras 500-700	0.14295	0.06316	-0.35091
		Comprimento fibras 700-900	0.24585	-0.23681	-0.12183
		Comprimento fibras 900-1100	0.01749	0.27854	0.455796
		Comprimento fibras >1100	0.07353	0.12182	0.390404
		Parênquima raro ou ausente	**-0.87974**	-0.40779	0.196734
		Parênquima paratraqueal escasso	**0.87974**	0.40779	-0.19673
	Raios	Raios > 10 células de largura	**-0.87974**	-0.40779	0.196734
		< 4 raios/mm	0.27236	-0.4395	**0.622194**
		Raios > 1 mm de altura	0.27236	-0.4395	**0.622194**
		Raios agregados	0.27236	-0.4395	**0.622194**
		Raios com células quadradas e procumbentes	**-0.87974**	-0.40779	0.196734
		Raios com procumbentes, quadradas e eretas misturadas	**0.83296**	-0.02292	0.307607
		Breakdown areas in rays	0.41949	-0.50495	-0.30625
		Células envolventes	0.27236	-0.4395	0.622194
		Madeira sem raios	-0.27236	0.4395	-0.62219
		Cristais prismáticos	0.27236	-0.4395	0.622194
Anatomia foliar	Pecíolo	Contorno plano-convexo	0.05728	-0.0516	0.219897
		Côncavo-convexo	-0.0844	0.09859	-0.14292
		Contorno Circular	0.00019	-0.01903	-0.15059
		Arco com extremidade convoluta	0.17001	0.04147	0.287177
		Arco com extremidade fechada	0.13346	-0.28162	-0.59069
		Arco	-0.04046	0.31096	0.040418
		Sistema vascular em V	0.06516	0.35428	0.445631
		Três feixes separados no sistema vascular	**-0.87974**	-0.40779	0.196734
		Bainha esclerênquimática	0.03699	-0.60829	-0.04606

	Nervura mediana	Feixe adicional no sistema da NM	0.56024	-0.58121	-0.16074
		Arco com extremidade convoluta	0.18948	-0.00107	-0.38714
		V	0.08362	0.37609	0.601306
		Arco com extremidade fechada	0.05503	0.13216	-0.03763
		Arco	-0.35435	-0.46471	-0.09906
	Estômatos	Estômatos anisocíticos	0.6542	-0.64148	0.037245
		Estômatos anomocíticos	-0.50954	-0.02081	0.169922
		Estômatos paracíticos	-0.28073	**0.86155**	-0.12989
		Folhas hipoestomáticas	-0.07353	-0.12182	-0.3904
		Folhas anfiestomáticas	0.15626	0.07623	0.469106
	Tricomas	Tipo 0	0.14248	-0.59166	-0.11094
		Tipo 1	-0.06533	0.36956	0.16549
		Tipo 2	-0.28852	0.63129	-0.33278
		Tipo 3	-0.23146	0.64919	-0.27065
		Tipo 4	0.00575	0.29973	0.311883
		Tipo 5	0.07495	-0.13069	-0.02415
		Tipo 6	-0.02227	-0.17013	-0.21388
		Tipo 7	**-0.87974**	-0.40779	0.196734
	Epiderme	Parede anticlinal/adaxial reta	-0.06714	-0.54809	-0.0204
		Parede anticlinal/adaxial levemente sinuosa	0.31381	-0.15328	0.27692
		Parede anticlinal/adaxial sinuosa	-0.23363	0.71067	-0.24606
		Parede anticlinal/ abaxial reta	-0.23218	-0.41769	0.099338
		Parede anticlinal/ abaxial levemente sinuosa	0.39442	-0.27253	0.157578
		Parede anticlinal/ abaxial sinuosa	-0.18675	0.64613	-0.24643
		Superfície abaxial / Estriada	0.00096	0.64584	0.16461
		Superfície abaxial / Ondulada	-0.67263	-0.36217	0.006961
		Superfície abaxial / Lisa	0.35983	-0.35917	-0.09621
		Superfície adaxial / Estriada	-0.00435	0.3669	-0.25049
		Superfície adaxial / Ondulada	-0.67263	-0.36217	0.006961
		Superfície adaxial / Lisa	0.29699	0.00635	0.330345
		Células Mucilaginosas	0.38646	-0.20767	0.110591
	Mesofilo	Mesofilo dorsiventral	-0.13172	-0.29744	-0.67557
		Mesofilo fracamente dorsiventral	0.13172	0.29744	**0.675572**
		Cavidades secretoras	**0.87974**	0.40779	-0.19673
		Cristais prismáticos	-0.06613	0.2295	-0.01057
		Drusas	-0.04707	-0.34817	-0.32526
		Fibras extra-xilemáticas	**-0.87974**	-0.40779	0.196734
		Hipoderme	**-0.87974**	-0.40779	0.196734
		Esclerênquima na margem da folha	**-0.87974**	-0.40779	0.196734
		Células com invaginações no mesofilo	0.1812	0.15042	0.664424
		Hábito arbóreo	0.34829	-0.28835	-0.19146

CONCLUSÕES GERAIS

- ✓ As duas subfamílias analisadas apresentam caracteres anatômicos distintivos na folha. Enquanto as espécies subordinadas à Myrsinoideae apresentam cavidades secretoras distribuídas por toda a folha, essas estruturas estão ausentes em Theophrastoideae. Em contrapartida, as espécies de Theophrastoideae apresentam feixes de fibras extra-xilemáticas, que ocorrem ao longo de toda a folha. Tal característica não se apresenta nas espécies de Myrsinoideae;

- ✓ Além dos caracteres distintivos entre as subfamílias, também foi possível identicar caracteres diagnósticos a maioria dos gêneros: *Cybianthus* apresenta estômatos do tipo paracítico; *Myrsine* apresenta um feixe adicional sobre a nervura, que apresenta arranjo oposto; e *Jacquinia* contem as fibras extra-xilemática e hipoderme. *Ardisia* e *Stylogyne* apresentam mesofilo fracamente dorsiventral e células com invaginações no mesofilo, esses dois gêneros não poder ser separados um do outro;

- ✓ Através da análise anatômica da madeira também foi possível identificar caracteres anatômicos distintivos às duas subfamílias Neotropicais. Theophrastoideae apresenta elementos de vaso pequenos (até 300 μm) raios largos (>10 células), enquanto em Myrsinoideae são observadas estruturas secretoras nos raios (*breakdown areas in rays*) e raios longos (>300 - < 1200);

- ✓ Além disso, pode-se observar: a presença de estruturas secretoras nos raios exclusivamente em espécies de *Myrsine*, os raios compostos por células procumbentes e quadradas em *Jacquinia* e *Clavija*, a ausência de raios em *Cybianthus densiflorus*, a presença de placas de perfuração escalariformes em *C. nemoralis*;

- ✓ Entre as espécies de Primulaceae, foram observados diferentes tipos de estruturas secretoras: cavidades e ductos, tricomas glandulares, idioblastos mucilaginosos, idioblastos cristalíferos, idioblastos com conteúdo fenólico;

- ✓ O desenvolvimento das estruturas secretoras (tricomas e cavidades/ductos) é assincrônico e se inicia primariamente à diferenciação dos demais tecidos foliares;

- ✓ Em todos os gêneros analisados, as cavidades/ductos secretores têm origem de uma célula do meristema fundamental, a qual sofre divisões anticlinais e periclinais para a formação do epitélio secretor, e, através de um processo esquizógeno, ocorre a formação do lúmen;

- ✓ Os tricomas têm origem de uma célula protodérmica, e o processo inicial de formação é o mesmo para os diferentes tipos morfológicos;

- ✓ Apesar da baixa resolução em alguns clados, a hipótese filogenética aqui apresentada recupera o monofiletismo das duas subfamílias Neotropicais de Primulaceae: Theophrastoideae e Myrsinoideae.

- ✓ O consenso estrito não conferiu resolução ao clado formado por *Ardisia* e *Stylogyne*, mas está de acordo com o que é indicado na literatura, a qual apresenta os dois gêneros intimamente relacionados devido à uma série de sobreposição de caracteres morfológicos;

- ✓ Os gêneros *Cybianthus* e *Myrsine* emergiram monofiléticos;

- ✓ Em *Cybianthus* cada subgênero emergiu monofilético de acordo com a classificação proposta por Agostini (1980). Subgênero *Conomorpha*: *Cybianthus guyanensis* e *C. brasiliensis*; subgênero *Cybianthus*: *C. glaber, C. verticilatus* e *C. venezuelanus*; subgênero *Weilgetia*: *C. densiflorus* e *C. nemoralis*.

- ✓ A análise dos componentes principais com os dados anatômicos das folhas e madeira conferiu maior resolução à segregação dos gêneros;

- ✓ De maneira geral, pode-se concluir que os dados anatômicos em Primulaceae apresentam peso taxonômico e filogenético e contribuem tanto para a segregação de gêneros e espécies, quanto para o estabelecimento das relações filogenéticas entre os táxons.

PERSPECTIVAS FUTURAS

1. Identificar, caracterizar e descrever a anatomia das estruturas secretoras em espécies Neotropicais de Primulaceae e traçar sua evolução a partir da hipótese filogenética proposta por Luna (2017).

2. Caracterizar ultraestruturalmente os tricomas glandulares em *Jacquinia armillaris* Jacq.;

3. Verificar a ocorrência da associação com bactérias nos hidatódios observados na margem das folhas em *Myrsine*;

4. Verificar a presença de bainha ao redor das células epiteliais das cavidades secretoras em espécies de Myrsinoideae;

5. Descrever a ontogenia das estruturas secretoras nos raios do xilema em espécies de Myrsinoideae;

6. Descrever a ontogenia das estruturas secretoras dos grupos irmãos de Primulaceae, a saber, Sapotaceae e Ebenaceae

7. Levantar informações sobre o registro fóssil de Primulaceae para mapear a ocorrência de estruturas secretoras;

8. Estabelecer características existentes entre as células secretoras, o material secretado e o seu papel fisiológico;

ANEXOS

I) Anexo 1 – Informações sobre as coletas realizadas em 2013-2016. As demais espécies foram coletadas pela Dra. Maria de Fátima Freitas.

Estado	Localidade	Coletores	Período	Espécies coletadas
RJ	Armação dos Búzios, praia de Tucuns	Bruna Luna, Claudia Barros, Tahysa Macedo, Warlen Costa	25-26/03/2013	*Jacquinia armillaris*
RJ	Quatis - Vale do Paraíba	Bruna Luna, Mássimo Bovini, José F. Baumgratz, Lucas Jordão	06-10/05/2013	*M. coriacea*
RJ	Rio de Janeiro. Restinga de Grumari, Parque Estadual Costa do Sol.	Bruna Luna, Claudia Barros, Fátima Freitas	6/28/2013	*M. guianensis*
RJ	Arraial do Cabo, Pontal do Atalaia, Parque Estadual Costa do Sol	Bruna Luna, Fátima Freitas	03-04/07/2013	*M. guianensis, M. coriacea* e *J. armillaris*
RJ	Paraty	Bruna Luna, Fátima Freitas, Fernanda Masullo, Mássimo Bovini, Wellignton	28-29/08/2013	*M.coriacea*
RJ	Rio de Janeiro. Restinga de Grumari.	Bruna Luna, Alice Sato, Anna Carina	9/26/2013	*M. guianensis*
RJ	Parque Nacional do Itatiaia	Bruna Luna, Fátima Freitas, Karen de Toni, Fernanda Masullo	29-30/10/2013	*M. lineata, M. coriacea, M. umbellata e C. glaber*
AM	Manaus, Reserva Ducke	Bruna Luna, Fátima Freitas, Mike Hopkins, Elton Lírio, Antônio Tavares	16-22/03/2014	*Cybianthus fuscus, C. densiflorus, C. guyanensis, C. subg. Weigeltia sp.nova, Stylogyne sp.*
MG	Conceição do Ibitipoca, Parque Nacional do Ibitipoca	Bruna Luna, Maria de Fátima Freitas, Ronaldo Marquete, Diego Gonzaga	12-16/05/2014	*M. glazioviana, M. parvula, M. squarrosa, M. emarginella, M. lancifolia, M. lineata, M. gardneriana, C. brasiliensis*
RJ	Bosque da Barra. Rio de Janeiro	Bruna Luna, Maria de Fátima Freitas, Anna Carina Antunes e Defaveri, Mateus Lombardi	29/01/2015	*M. rubra e M. coriacea*
RJ	Parque Nacional do Itatiaia	Bruna Luna, Maria de Fátima Freitas	06/2016	*Cybianthus verticilatus*

II) Trabalho em desenvolvimento:

A) Title: Trends of the wood anatomy of a widespread Neotropical Primulaceae specie

Authors: Bruna Nunes de Luna, Glaucia Crispim, Alessandra Regina Aguilar Voigt, Maria de Fátima Freitas & Claudia Franca Barros.

B) Título: Flora da Serra dos Carajás: Primulaceae.

Autores: Maria de Fátima Freitas & Bruna Nunes de Luna

C) Title: Essential oil composition of two *Myrsine* (Primulaceae) species.

Authors: Joana Bion Cabral, Bruna Nunes de Luna, Anna Carina Antunes e Defaveri, Maria de Fátima Freitas & Alice Sato.

III) Produção durante o doutorado, relacionada ao tema de pesquisa:

A - Comparative leaf anatomy of neotropical *Stylogyne* species (Myrsinoideae – Primulaceae). Publicado no periódico *Rodriguésia* em 2013.

Rodriguésia 64(4): 717-726. 2013
http://rodriguesia.jbrj.gov.br

Comparative leaf anatomy of neotropical *Stylogyne* species (Myrsinoideae – Primulaceae)

Anatomia foliar comparada de espécies neotropicais de Stylogyne (*Myrsinoideae – Primulaceae*)

Bruna Nunes da Luna[1,3], *Tatiana Tavares Carrijo*[2], *Maria da Fátima Freitas*[1] & *Claudia Franca Barros*[1]

Abstract

Anatomical studies were performed here in order to provide diagnostic characteristics to differentiate the species *Stylogyne depauperata*, *S. pauciflora*, *S. sordida* and *S. warmingii*. Fully expanded leaves were processed by the usual techniques of optical microscopy and scanning electron microscopy. Traits common to all species were observed, such as dorsiventral mesophyll, unistratified epidermis, anisocytic stomata, druses and secretory cavities distributed throughout the mesophyll. Cuticular ornamentation, configuration of the vascular system in the petiole and shape of the secretory cavities provide diagnostic characteristics. Variance analysis proved that these characters are potentially efficient to differentiate these species.

Key words: *Ardisia*, secretory structures, taxonomy, Myrsinaceae.

Resumo

Estudos anatômicos foram realizados com o objetivo de buscar caracteres diagnósticos para diferenciar *Stylogyne depauperata*, *S. pauciflora*, *S. sordida* and *S. warmingii*. Folhas totalmente expandidas foram submetidas aos procedimentos usuais em microscopia óptica e eletrônica de varredura. As espécies analisadas apresentam mesofilo dorsiventral, epiderme uniestratificada, estômatos anisocíticos, idioblastos com cristais em drusas e cavidades secretoras dispersas pelo mesofilo. Caracteres diagnósticos são a ornamentação cuticular, disposição do sistema vascular no pecíolo e forma das cavidades secretoras. As análises de variância reforçam a eficiência desses caracteres para a segregação das espécies.

Palavras-chave: *Ardisia*, estruturas secretoras, taxonomia, Myrsinaceae.

Introduction

The Neotropical *Stylogyne* A.DC. comprises 18 species in Brazil (Carrijo *et al.* 2012) distributed in Amazon and Atlantic Rain Forests. The nine members of Atlantic Rain Forest are shrubs with leaves generally punctuated, small 4(5)-merous flowers, and brightly colored fruits (Carrijo & Freitas 2008). The six species with 4-merous flowers seems to be a natural group, characterized by a high frequency of local endemism and low tolerance to environmental disturbance (Carrijo & Freitas 2008, 2009; Carrijo *et al.* 2011). Some of these species are circumscribed by fine characters (e.g. calyx papillose, anthers opening by short or long slits) or by a set of shared features (e.g. inflorescence racemose or fasciculate, petals punctuate), which sometimes makes it difficult to distinguish related taxa from extremes of infraspecific variation. Anatomical traits proved to be a value tool to delimit taxonomically related species, to provide a consistent foundation for phylogenetic studies, and other ecological applications of species from the Atlantic Rain Forest (Barros & Callado 1997). However, some data on anatomical aspects are available in the literature for Myrsinoideae species, especially for the genus *Stylogyne* (Grose 1908; Otegui 1986; Carrijo *et al.* 2011).

Grose's monograph (1908) provides an overview of the anatomic traits in Myrsinoideae, in order to characterize the genera. More recent contributions have dealt with the description of the trichomes and crystals of *Ardisia* Sw. species (Lersten 1977), and the development of secretory cavities of *Lysimachia nummularia* L. (Lersten 1986). Other

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B – Leaf secretory structures of *M. coriacea* and *M. venosa* (Primulaceae): ontogeny, morphology and chemical composition of essential oils. Publicado no periódico *Botany* em 2014.

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ARTICLE

Leaf secretory tissues in *Myrsine coriacea* and *Myrsine venosa* (Primulaceae): ontogeny, morphology, and chemical composition of essential oils

Bruna Nunes de Luna, Anna Carina Antunes e Defaveri, Alice Sato, Humberto Ribeiro Bizzo, Maria de Fátima Freitas, and Claudia Franca Barros

Abstract: Secretory structures are an outstanding feature in Primulaceae (Ericales). Such structures are known for their taxonomical and medicinal importance. However, a detailed morphological study of the secretory structures in Primulaceae has been neglected. Selected species for this study belong to *Myrsine*, a widely distributed genus in Brazil, popularly known as "capororoca". In this study, we aimed to elucidate the ontogenesis of the secretory structures in the leaves of *Myrsine coriacea* (Sw.) R. Br. ex Roem & Schult. and *Myrsine venosa* A.DC. and report, for the first time, on the composition of their essential oils. The following secretory structures are found in *M. coriacea* and *M. venosa*: idioblasts, glandular trichomes, and secretory cavities. The development of all secretory structures, which is asynchronous, occurs during leaf expansion and differentiation; therefore, in leaf primordia, the same type of secretory structure could be observed at different stages of differentiation. By the complete expansion of leaf primordia, all secretory structures have reached their full size. Idioblasts are derived from both protodermal and ground meristem cells and they secrete mucilage or phenolic compounds. The glandular trichomes can be peltate, as found in both species, or branched, as found only in *M. coriacea*. Trichomes are initiated by the enlargement of protodermal cells, followed by their division, and they are completely formed by the end of leaf expansion. Secretory cavities are schizogenous and originated from ground meristem cells. Major components from *M. coriacea* essential oils were β-elemene, γ-muurolene, and α-cadinene, while the major components of *M. venosa* essential oils were β-caryophyllene, γ-muurolene, and δ-cadinene.

Key words: ontogenesis, secretory structures, essential oil, *Myrsine coriacea*, *Myrsine venosa*, Primulaceae.

Résumé : Les structures sécrétoires constituent un trait remarquable chez les Primulaceae (Ericales). Ces structures sont connues pour leur importance taxonomique et médicinale. Cependant, l'étude morphologique détaillée des structures sécrétoires des Primulaceae a été négligée. Les espèces choisies pour cette étude appartiennent aux *Myrsine*, un genre largement distribué au Brésil, connu de manière populaire sous le nom de « capororoca ». Dans cette étude, les auteurs visaient à élucider l'ontogénèse des structures sécrétoires des feuilles de *Myrsine coriacea* (Sw.) R. Br. ex Roem & Schult. et de *Myrsine venosa* A.DC., et à rapporter, pour la première fois, la composition de leurs huiles essentielles. Les structures sécrétoires suivantes ont été trouvées chez *M. coriacea* et *M. venosa*: idioblastes, trichomes glandulaires et cavités sécrétoires. Le développement de toutes les structures sécrétoires, qui est asynchrone, survient lors de l'expansion et la différenciation de la feuille; ainsi, dans le primordium foliaire, le même type de structure sécrétoire pouvait être observé à différents stades de différenciation. Lorsque l'expansion du primordium foliaire était complétée, toutes les structures sécrétoires atteignaient leur pleine taille. Les idioblastes sont dérivés du protoderme et des cellules du méristème fondamental, et ils secrètent du mucilage ou des composés phénoliques. Les trichomes glandulaires peuvent être peltés, tels que trouvés chez les deux espèces, ou branchés, tels que trouvés chez *M. coriacea* seulement. Les trichomes émanent de l'élargissement des cellules du protoderme, suivi de leur division, et ils sont complètement formés à la fin de l'expansion de la feuille. Les cavités sécrétoires sont schizogènes et proviennent des cellules du méristème fondamental. Les composantes majeures des huiles essentielles de *M. coriacea* étaient le β-élémène, le γ-muurolène et le α-cadinène, alors que les composantes majeures des huiles essentielles de *M. venosa* étaient le β-caryophyllène, le γ-muurolène et le δ-cadinène. [Traduit par la Rédaction]

Mots-clés : ontogenèse, structures sécrétoires, huile essentielle, *Myrsine coriacea*, *Myrsine venosa*, Primulaceae.

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C – Rediscovery of *Cybianthus froelichii* (Primulaceae), an endangered species from Brazil. Publicado no Boletim do Museu de Biologia Mello Leitão em 2016.

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Rediscovery of *Cybianthus froelichii* (Primulaceae), an endangered species from Brazil

Maria de Fátima Freitas[1*], Tatiana Tavares Carrijo[2] & Bruna Nunes de Luna[1]

RESUMO: (**Redescoberta de *Cybianthus froelichii* (Primulaceae), uma espécie ameaçada de extinção do Brasil**). Uma espécie redescoberta de *Cybianthus* subgênero *Cybianthus* é descrita e ilustrada. *Cybianthus froelichii* é proximamente relacionado à *C. cuneifolius*, mas se diferencia pelas folhas grandes e flores pistiladas sésseis. Espécie endêmica da Mata Atlântica e considerada ameaçada de extinção. *C. froelichii* é aqui ilustrada pela primeira vez.

Palavras-chave: Mata Atlântica, diversidade, Ericales, Neotrópico, Myrsinoideae.

ABSTRACT: A rediscovered species of *Cybianthus* subgenus *Cybianthus* is described and illustrated. *Cybianthus froelichii* is most closely related to *C. cuneifolius*, but may be distinguished by its large leaves and sessile pistillate flowers. This species is endemic to the Atlantic Forest, Brazil, and is considered endangered. *C. froelichii* is illustrated here for the first time.

Key words: Atlantic Forest, diversity, Ericales, Neotropic, Myrsinoideae.

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D – Volatile Constituents of of three Myrsine L. species from Brazil, publicado no periódico *Records of Natural Products.*

SHORT REPORT

A C G publications

Rec. Nat. Prod. 11:1 (2017) 82-87

records of natural products

Volatile Constituents of Three *Myrsine* L. Species from Brazil

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Abstract: The chemical compositions of the essential oils obtained by hydrodistillation from the aerial parts of *Myrsine rubra*, *Myrsine gardneriana* and *Myrsine parvifolia* and the fruits of *Myrsine parvifolia* were elucidated by a combination of GC and GC-MS analyses. The main constituents of the native *M. parvifolia* were caryophyllene oxide (14.4%), β-caryophyllene (12.6%) and γ-Muurolene (7.9%) of the leaves oil and β-caryophyllene (11.7%), δ-Cadinene (7.1%) of the fruit oil. The volatile oil of the endemic *M. rubra* leaves was dominated by β-caryophyllene (17.2%), γ-Muurolene (11.1%), Germacrene B (10.0%). The essential oil of the native *M. gardneriana* leaves was characterized by β-caryophyllene (18.0%), γ-Muurolene (8.4%). These three *Myrsine* species are similar in the dominance of sesquiterpenes. By contrast, monoterpenes were found only in the volatile oil from the fruits of *M. parvifolia*. To the best of our knowledge, this study is the first report on the volatile constituents of *M. rubra*, *M. gardneriana*, *M. parvifolia*.

Keywords: *Myrsine rubra*; *Myrsine gardneriana*; *Myrsine parvifolia*; essential oil.

1. Plant Source

The fruits and the leaves of *Myrsine parvifolia* A.DC. and the leaves of *Myrsine rubra* M.F.Freitas & Kin.-Gouv. were collected in May 2013 and June 2010, in Restinga de Jurubatiba National Park, Rio de Janeiro State (Brazil), respectively. The plants were identified by Dr. Marcelo Guerra Santos, Universidade do Estado do Rio de Janeiro, Brazil. Voucher specimens, M.G.Santos

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E – Sinopse dos gêneros de Primulaceae no Brasil, publicado no periódico *Rodriguésia*.

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Sinopse dos gêneros de Primulaceae no Brasil

Synopsis of the genera of Primulaceae in Brazil

Maria de Fátima Freitas[1,3], *Tatiana Tavares Carrijo*[2] & *Bruna Nunes de Luna*[1]

Resumo

Primulaceae é representada no Brasil por 12 gêneros e cerca de 140 espécies. As espécies apresentam hábitos de herbáceo a arbóreo e estão distribuídas nos gêneros *Ardisia*, *Clavija*, *Ctenardisia*, *Cybianthus*, *Geissanthus*, *Gentlea*, *Jacquinia*, *Lysimachia*, *Myrsine*, *Parathesis*, *Samolus* e *Stylogyne*. Esta família é amplamente distribuída no Brasil, sendo que espécies exclusivamente amazônicas pertencem aos gêneros *Ctenardisia*, *Gentlea*, e *Parathesis*. Este estudo apresenta o primeiro registro de *Gentlea* para o Brasil. É apresentada uma chave de identificação dos gêneros para o Brasil, descrições, ilustrações e comentários com referência dos materiais de herbário analisados.

Palavras-chave: Ericales, Myrsinaceae, taxonomia, Theophrastraceae.

Abstract

Primulaceae is represented by 12 genera and about 140 species in Brazil. Species varies from herbs to trees and are grouped within the genus: *Ardisia*, *Clavija*, *Ctenardisia*, *Cybianthus*, *Geissanthus*, *Gentlea*, *Jacquinia*, *Lysimachia*, *Myrsine*, *Parathesis*, *Samolus*, and *Stylogyne*. The family is widely distributed, but *Ctenardisia*, *Gentlea* and *Parathesis* are exclusively from the Amazonian forests. This study presents the first record of *Gentlea* in Brazil. An identification key for the Brazilian genera, diagnostics descriptions, illustrations, and comments with reference to herbarium specimens are provided.

Key words: Ericales, Myrsinaceae, taxonomy, Theophrastraceaes.

Introdução

Primulaceae compreende atualmente as subfamílias Maesoideae, Theophrastoideae, Myrsinoideae e Primuloideae (Stevens 2001 onwards). Em sistemas anteriores (Cronquist 1988; APG II 2003) essas subfamílias foram tratadas como famílias independentes (Maesaceae, Theophrastaceae, Myrsinaceae e Primulaceae *s.s.*) e, por serem estreitamente relacionadas, eram designadas como o "grupo primulóide". Este grupo é caracterizado pelas flores haplostêmones, com corola simpétala, estames opositipétalos, placentação central livre, óvulos bitegmáticos, tenuinucelados e endosperma de formação nuclear (Källersjö *et al.* 2000).

A significativa alteração, trazida pelas análises moleculares, foi a transferência de gêneros tradicionalmente subordinados à Primulaceae *s.s.* para Myrsinaceae e Theophrastaceae (Källersjö *et al.* 2000; Stahl & Anderberg 2004). Estudos posteriores ampliaram a circunscrição de Primulaceae, que tem prioridade de nome, e subordinaram as demais famílias como subfamílias (APG IV 2016). Na atual fase do conhecimento, apesar das relações entre as subfamílias, cujos gêneros ainda necessitam de maiores esclarecimentos e estudos, Primulaceae *s.l.* é considerada monofilética (APG IV 2016).

Considerando a circunscrição atual de Primulaceae, são registrados para o Brasil 11 gêneros e 140 espécies (BFG 2015), com ampla distribuição, sendo aqui registrada a primeira ocorrência do gênero *Gentlea*. Registram-se, portanto, 12 gêneros de Primulaceae para o Brasil. Este trabalho apresenta uma sinopse dos gêneros, com chave para identificação, descrições, comentários taxonômicos e distribuição geográfica, com o propósito de auxiliar na sua identificação e subsidiar projetos futuros.

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F – Artigo aceito para publicação no periódico *International Journal of Plant Sciences* (2017): "Leaf anatomy of five the Neotropical of Primulaceae".

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G – Trabalhos desenvolvidos em parceria com outras instituições para a extração dos óleos essenciais de espécies de Primulaceae

Julia Vitor França

Composição do óleo essencial das espécies *Ardisia humilis*, *Ardisia solanacea*, *Jacquinia amilaris* e *Myrsine lineata* (Primulaceae)

Monografia apresentada ao Curso de Ciências Biológicas – Bacharelado, do Instituto de Biociências da Universidade Federal do Estado do Rio de Janeiro, como requisito parcial para a obtenção de título de Bacharel em Ciências Biológicas.

Orientadoras: MSc. Anna Carina Antunes e Defaveri

Profa. Dra. Alice Sato

Rio de Janeiro

2014

H - Trabalhos desenvolvidos em parceria com outras instituições para a extração dos óleos essenciais de espécies de Primulaceae

Composição do óleo essencial das espécies *Myrsine glazioviana* Warm. e *Myrsine squarrosa* (Mez) M.F.Freitas & Kin.-Gouv. (Primulaceae).

Joana Bion Cabral

Monografia apresentada ao Curso de Ciências Biológicas – Bacharelado, do Instituto de Biociências da Universidade Federal do Estado do Rio de Janeiro, como requisito parcial para a obtenção de título de Bacharel em Ciências Biológicas.

Orientadora: Prof. Dra. Alice Sato

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