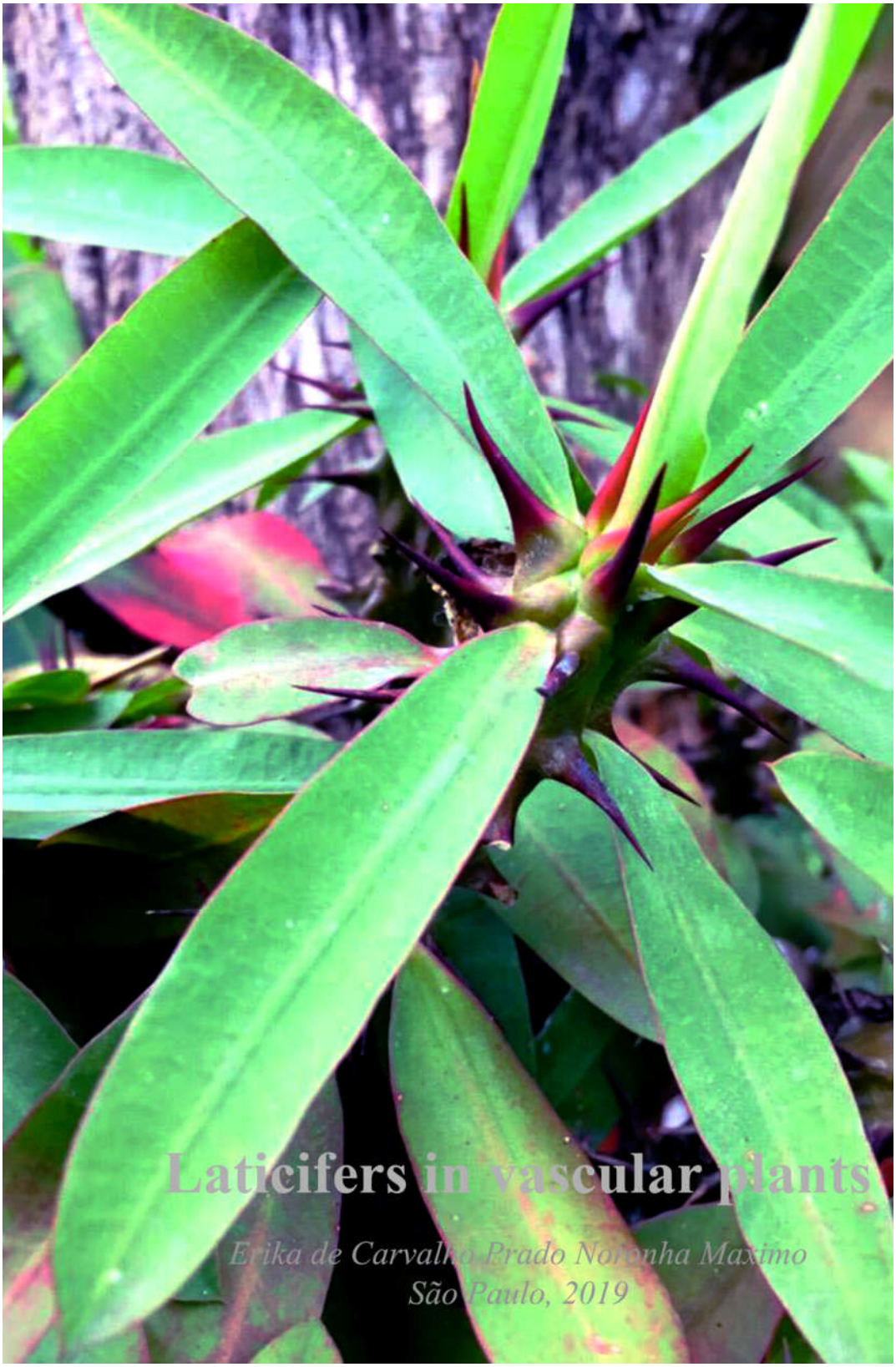

Laticifers in vascular plants

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São Paulo, 2019

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Laticíferos nas plantas vasculares

Laticifers in vascular plants

Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção do título de Mestre em Ciências, área de concentração em Botânica

Orientador: Prof. Dr. Diego Demarco

São Paulo
2019

Prado, Erika

Laticifers in vascular plants
209 páginas

Dissertação (Mestrado) – Instituto de Biociências da Universidade de São Paulo. Departamento de Botânica.

Laticíferos, látex, distribuição, ductos resiníferos, idioblastos fenólicos, plantas vasculares

Comissão Julgadora:

Prof (a). Dr (a).

Prof (a). Dr (a).

Prof. Dr. *Diego Demarco*
Orientador

Despedi-me de um sonho, Uma louca fantasia,

Revestida quimera de terna poesia

Acalentado ao embalo de sutil encanto

E vivido apenas entre embriaguez e espanto

Construí um mundo em distante paragem,

Ideal, perfeito,

Verdadeira miragem

E despertei ao ver que tinha em mãos vazias

E meu ser perseguido, uma doida utopia

Jandira Mazzariello de Carvalho

Dedico

A minha amada avó Jandira, minha querida mãe Edna, minha Bruna e para meu amor Edu. Para vocês com todo amor.

Agradecimentos

Meu mestrado foi uma etapa muito importante para mim, e só foi possível através de muito estudo, dedicação e pessoas que me ajudaram muito.

Aos órgãos de fomento que tornaram possível esta pesquisa, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (processo nº 8882.332992/2019/1) e Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (processo número: 2017/23882-0), pelo auxilio no financiamento da pesquisa.

À Universidade de São Paulo e ao Instituto de Biociências pela infraestrutura.

Com carinho e eterno agradecimento ao meu orientador Dr. Diego Demarco. Agradeço imensamente pela oportunidade dada a mim e confiar em meu trabalho. Sem o senhor não seria possível realizar esse sonho. Não há palavras que possa descrever a minha infinita gratidão.

Aos professores do Instituto de Botânica, no qual tive o imenso prazer de conhecer: Dra. Gladys Flávia, Dra. Verônica Angyalossy, Dr. Gregório Camargo e Dra. Nanuza Luíza de Menezes.

Um agradecimento especial a Joelma Oliveira Prado, que com sua competência e amor ao seu trabalho foi possível fazer a tradução desta dissertação.

As queridas amigas que tive o prazer de conhecer: Ellenhise Ribeiro, Gisele Alves, Juliana Castelo, Leyde Nunes, Mariana Monteiro, Natalie Capelli e Rebeca Laino. E a Maria Camila Medina que sempre se disponibilizou a me ajudar, me ensinando com boa vontade e carinho.

Agradeço à Mariana Victorio que através da Bolsa de Treinamento Técnico pela FAPESP foi possível finalizar com muito empenho e habilidade, os cortes das espécies.

Agradeço também as técnicas do laboratório de anatomia: Gisele Costa, Tássia Cristina dos Santos.

Com muito amor agradeço a minha querida mãe, Edna. Obrigada por estar sempre ao meu lado e por sempre acreditar em mim. Pela nossa linda amizade, amor e seu carinho.

Ao meu marido Eduardo, meu companheiro, meu amigo e meu grande amor. Obrigada por caminhar comigo nessa jornada e sempre acreditar em mim.

SUMÁRIO

RESUMO

Os laticíferos são células especializadas que secretam látex, uma complexa mistura de metabólitos que tem como principal função a defesa contra a herbivoria. Os laticíferos estão presentes em diversas famílias de plantas vasculares e sua ocorrência é considerada um indicador taxonômico e, muitas vezes, filogenético. Há inúmeras interpretações equivocadas na literatura, atribuindo a presença de laticíferos em famílias que não os possuem. Desta forma, uma ampla revisão sobre esta estrutura e sua real distribuição e evolução se faz necessária. Os objetivos do presente trabalho são caracterizar e distinguir os laticíferos de outras estruturas secretoras, avaliar comparativamente a composição do látex nas diferentes famílias de plantas latescentes e identificar o tipo de laticífero em cada família com base em sua ontogênese. Verificamos que, embora haja semelhança de cor e diversidade de metabólitos na secreção dos laticíferos e de alguns ductos resiníferos, o látex é uma secreção intracelular que corresponde ao próprio protoplasto dos laticíferos, enquanto a resina dos ductos é uma secreção extracelular que não possui componentes estruturais em sua composição. Além disso, látex é uma secreção específica dos laticíferos e laticíferos são conceitualmente fileiras de células, enquanto ductos são formados por um epitélio que delimita um lume, não se enquadrando em sua classificação. Quanto aos metabólitos presentes no látex, identificamos lipídeos, proteínas, compostos fenólicos, carboidratos e alcalóides nas diferentes famílias de plantas latescentes, evidenciando a grande convergência evolutiva que ocorreu nas diferentes linhagens de plantas vasculares. Em relação à distribuição dos laticíferos, verificamos que das 63 famílias analisadas, apenas 34 possuem látex em pelo menos um de seus representantes. Muitas dessas famílias não têm relação filogenética, demonstrando que os laticíferos surgiram múltiplas vezes na evolução das plantas vasculares. Demonstramos que 18 famílias ditas latescentes, na verdade, possuem ductos resiníferos ou idioblastos secretores. Também verificamos que todos os laticíferos são articulados, podendo ser classificados como anastomosados ou não anastomosados, ramificados ou não ramificados, refutando a existência dos laticíferos classificados como não articulados. Por fim, também refutamos a teoria do crescimento autônomo intrusivo por não haver indícios de sua ocorrência em nenhuma das dezenas de famílias analisadas. Esse estudo traz uma contribuição muito significativa para o entendimento dos laticíferos quanto a sua identificação, composição do látex, distribuição, modo de crescimento e evolução nas plantas vasculares.

Palavras-chave: laticíferos, látex, distribuição, ductos resiníferos, idioblastos fenólicos, plantas vasculares.

ABSTRACT

Laticifers are specialized cells that secrete latex, a complex mixture of metabolites whose main function is defense against herbivory. Laticifers are present in several families of vascular plants and their occurrence is considered as a taxonomic and often phylogenetic indicator. There are many misinterpretations in the literature attributing the presence of laticifers in families that do not have them. Thus, a comprehensive review of this structure and its actual distribution and evolution is necessary. The objectives of the present work are to describe laticifers structurally, distinguishing them from other secretory structures and correcting any description errors; to comparatively evaluate the composition of latex in the different families of latescent plants; to identify the type of laticifer in each family based on their ontogenesis; and to evaluate their actual distribution and evolution in vascular plants. Although the secretions of laticifers and resin ducts are similar in color and the diversity of metabolites, latex is an intracellular secretion that consists of the very protoplast of laticifers, while the resin of ducts is an extracellular secretion that has no structural components in its composition. In addition, latex is a secretion specific to laticifers and laticifers are conceptually rows of cells, while ducts are formed by an epithelium that delimits a lumen; therefore, they do not fall under this classification. As for the metabolites present in latex, we have identified lipids, proteins, phenolic compounds, carbohydrates, and alkaloids in the various families of latescent plants, evidencing the important evolutionary convergence that occurred in the various lines of vascular plants. With relation to the distribution of laticifers, we have found that of the 63 families analyzed, only 34 have latex in at least one of their representatives. Many of these families have no phylogenetic relationship, demonstrating that laticifers emerged multiple times in the evolution of vascular plants. We have demonstrated that 18 so-called latescent families actually have resin ducts or secretory idioblasts instead of laticifers. We have also ascertained that all laticifers are articulated and can be classified as anastomosed or non-anastomosed, branched or unbranched, thereby refuting the existence of laticifers classified as non-articulated. Finally, we also refute the intrusive autonomous growth theory since there is no evidence of its occurrence in any of the latescent families. This study makes a very significant contribution to the understanding of laticifers regarding their identification, latex composition, distribution, growth and evolution in vascular plants.

Keywords: laticifers, latex, distribution, resin ducts, phenolic idioblasts, vascular plants.

A laticifer is a specialized cell or a row of latex-containing cells (Fahn 1979). The definition of this secretion has changed over several decades of study; however, nowadays, the whole protoplast of the laticifer is taken into consideration because when the plant is injured the entire cell content is released (Demarco *et al.* 2006, Demarco 2015, Prado and Demarco 2018, Ramos *et al*, 2019).

When present, laticifers generally occur in all vegetative and reproductive organs of the plant (Metcalfe and Chalk 1950; Demarco et al. 2006; Demarco and Castro 2008); however, there have been cases where their occurrence was restricted to certain organs of certain families (Metcalfe 1967, Lewinsohn 1991).

Latex is a complex mixture of metabolites such as lipids, proteins, carbohydrates, starch grains, mucilages, cardenolides, and alkaloids (Fahn 1979, Konno 2011, Demarco *et al*. 2013, Demarco 2015). Its primary function is defense against herbivory because many of its compounds are toxic. In addition, latex coagulates rapidly, thus sealing plant wounds and inhibiting the entry of pathogens such as bacteria and viruses (Wink 2008, Konno 2011, Bauer *et al.* 2014, Demarco 2015, Dussourd 2017).

Laticifers are present in several families of vascular plants, from ferns to the most derived angiosperm groups, often without phylogenetic relationship, demonstrating a polyphyletic origin (Metcalfe and Chalk 1950, Metcalfe 1967, Lewinsohn 1991, Prado and Demarco 2018). The occurrence of laticifers is considered as a taxonomic and often phylogenetic indicator in several groups.

The currently accepted classification of laticifers was defined by De Bary (1884), who divided them into articulated and non-articulated. Articulated laticifers are

rows of cells that can either keep their end walls intact (not anastomosed) or dissolve them (anastomosed). Non-articulated laticifers are individualized cells. Both types may or may not have ramifications (Esau 1977, Fahn 1979, Mahlberg 1993, Ramos *et al*. 2019).

Although the definition is simple, there are many misinterpretations in the literature attributing the presence of laticifers in families where they do not appear, often confusing them with resin ducts that produce a white secretion (Venning 1948, Mauseth 1978a, b, Wittler and Mauseth 1984).

Another point of divergence in the literature regards the growth of laticifers. Several papers support the theory of intrusive apical growth in non-articulated laticifers (Snyder 1955, Wilson and Mahlberg 1977, Mahlberg 1993) and in some articulated laticifers (Rudall 1994, Canaveze and Machado 2016, Canaveze *et al.* 2019). According to this theory, laticifers would develop in the intercellular spaces of plant tissues (Mahlberg 1993). Although this theory is hard to explain, many authors still support it (Canaveze and Machado 2016, Canaveze *et al.* 2019, Marinho and Teixeira 2018). However, this misconception stems from the observation of supposed acute apexes, often recorded as indications of this type of growth, but which are actually an oblique section to the apical cell of the laticifer due to the sinuous growth of this secretory structure (Demarco et al. 2006, 2013; Demarco and Castro 2008, Gama *et al*. 2017).

The objectives of this paper are to describe laticifers in a structural manner, distinguishing them from other secretory structures and correcting any description errors; to comparatively evaluate the composition of latex in the different families of latescent plants; to identify the type of laticifer in each family based on their ontogenesis; and to evaluate their actual distribution and evolution in vascular plants.

To meet these objectives, this dissertation has been divided into three chapters according to the manner in which it is intended to be published:

Chapter 1: Laticifers and secretory ducts: similarities and differences

Chapter 2: Plant latex and latex-borne defense

Chapter 3: Laticifers in vascular plants

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CHAPTER 1

Laticifers and secretory ducts: similarities and differences

Published: Prado E, Demarco D. (2018). Laticifers and secretory ducts: similarities and differences. In Ecosystem Services and Global Ecology (Hufnagel, L., ed.) IntechOpen: London. 103–123.

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Laticifers and secretory ducts: similarities and differences

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Abstract

During the evolution of terrestrial plants, many protective strategies have emerged, guaranteeing the survival of plants in the most varied environments. Among these strategies, we highlight the chemical defense of plants given by secretory structures, such as laticifers and secretory ducts. These glands are responsible for the production of viscous exudates that can be toxic, deterrent or repellent to herbivores, in addition to acting against microorganisms and sealing wounds. The similarities between latex and resin produced by certain ducts led several researchers to misinterpret their characteristics and generated a great number of divergences in the literature. This work aims to review the similarities and differences between laticifers and ducts and to demonstrate the structure, secretory activity, and chemical composition of the secretion of each one, as well as the evolutionary and ecological aspects that can be associated with the high rate of survival and diversification of the plants that contain laticifers and/or ducts.

Keywords: evolution, latex, resin, tubular secretory systems, protection.

1. Introduction

The huge biological diversity is responsible for relations between different species of plants, animals and microorganisms, with emphasis on the correlation between plants and insects. The interrelationships between these two groups of organisms are already well established in the evolutionary history of both. In addition, they may account for more than 75% of the current biodiversity [1] in both beneficial associations, such as pollination, and in adverse relationships, such as herbivory [2-4].

Herbivory has important implications for the evolutionary processes of the plant community. Its analysis reveals a continuous evolutionary adaptation [5] in which the plants developed physical and chemical defense mechanisms, just as the insects co-evolutionarily improved molecular, physiological and behavioral components in response to these mechanisms [2, 4, 6-10].

Herbivory generates a negative impact on the plant and minimizes its growth, reproduction, and its adaptability to the environment [11, 12]. Therefore, several defensive strategies are observed in different groups of plants that protect them against herbivores and pathogens. These strategies may be 1) physical defenses, like trichomes, calcium oxalate crystals, and sclerenchyma, which provide greater hardness to plant tissue and prevent it from penetration and degradation (13-15], 2) and chemical defenses, through the production of secondary metabolites by secretory cells [3, 6, 16-21]. The secondary metabolites found in the different secretions (or natural products) include a great diversity of alkaloids, terpenoids, cyanogenic glycosides and phenolic compounds that are toxic and play a selective role in relation to the enemies, mainly against herbivory [10, 17, 19, 22, 23], thus enhancing the plant adaptive success in many environments [10, 18, 24].

2. Defensive secretory structures

Secretions are present in all groups of vascular plants and may be composed of a high diversity of secondary and/or primary metabolites [16, 19, 21, 25, 26] and have a well-defined ecological role. Although a single metabolite may predominate within a taxon, especially in the case of some alkaloids [19], when we consider the totality of

compounds produced by plant secretory structures (or glands), they usually vary even within a species due to genotypic variations and abiotic conditions [25].

Different secretions are produced by specialized cells and can be directly released to the environment or stored in the plant in intracellular or intercellular sites [16, 21]. Secretory structures vary enormously in relation to their structural complexity, and may be composed of a single cell (e.g., idioblasts and some laticifers) or many cells, as in the case of more complex structures such as trichomes, colleters, nectaries, osmophores, secretory cavities, and ducts, among others [2, 16, 20, 21, 27-29]. Some of these secreted compounds can be profoundly affected, with their production being increased or reduced when the plant is subjected to some form of stress, such as wounds, infections or variations of climatic or edaphic factors [19, 25, 30].

Among the defensive glands, we highlight the tubular secretory systems that can form an anastomosed and branched network throughout the plant, a similarity that has generated numerous errors of identification between laticifers and resin ducts due to the production of similar secretions [6, 17, 25, 29, 31]. What are the similarities and differences between these two secretory structures?

3. Laticifer and resin duct

Laticifers and ducts can occur as single structures that often anastomose forming an interconnected network through all organs of the plant, whose viscous and mostly terpenic secretion is only released to the outside by the rupture of the secretory system. However, these are the only similarities. Misidentifications are mainly due to the observation of the appearance and color of the secretion in the field, since latex and

resin possess predominantly the same classes of chemical compounds. On the other hand, laticifers and ducts are very different in terms of structure and secretory activity.

3.1. Laticifer

A laticifer is a single cell or a row of specialized cells that contain latex [16] (Figure 1A). When the laticifer is composed of a single cell, it is classified as non-articulated; when it is formed by a row of cells (Figure 1B), it is classified as articulated [32]. Although their classification and morphological variations are very subtle, the identification of the laticifer must be made in the light of an ontogenetic study of the structure, since some articulated laticifers observed in Apocynaceae and Euphorbiaceae can differentiate rapidly next to the promeristem (Figure 1C). In these cases, few cell layers away from the promeristem, the laticifer cells completely dissolve their terminal walls, becoming a continuous tube without border remains between the different cells that compose it (Figure 1D). Thus, this type of articulated laticifer resembles a single cell at maturity, which may or may not be branched (Figure 1E). This has generated numerous divergences in the literature over time, and more detailed studies of the apical portion of the laticifer have tried to unravel its mode of growth among the meristems.

Apparently, the non-articulated laticifer has a more complex development. Several researchers have reported a pre-determined number of laticifer initials present in the embryo, which theoretically develop and branch through the entire body of the plant, regardless of its size [8, 33]. This unlimited elongation would result from an intrusive autonomous growth of the laticifer tip between meristematic cells. This way, this type of laticifer would present cell division without the occurrence of cytokinesis, forming a

long multinucleated, coenocytic tube [9, 33-36] (Figure 1F). Although this type of growth has also been recorded for a few articulated laticifers [35, 37], several studies have demonstrated the impossibility of its occurrence due to the absence of a subcellular apparatus capable of constantly producing cell wall at the laticifer tip [38], besides the lack of records of karyokinesis within laticifers in the main families of latescent plants [7, 39, 40]. Thus, the possible unlimited growth of the laticifer needs to be reviewed, and the record of articulated and non-articulated laticifers in the same genus and even in the same species should be re-evaluated ontogenetically [7, 33, 35, 39-41], since the current data point to the absence of non-articulated laticifers in all the families in which they were described.

Latex is the laticifer's protoplast itself, which has most of the metabolites stored inside a large, central vacuole [7] (Figure 1G, H). This highly heterogeneous content forms a suspension or emulsion of many small particles in a fluid [16], whose typical color is milky white; however, depending on the latex composition, it may be red, orange, yellow, green and even colorless [7, 9, 10, 23, 30, 33, 34, 39, 42].

Although latex is a mixture of many distinct compounds, there is always a predominance of terpenoids in its composition [10, 40, 43] (Figure 2A-C). In general, these terpenoids are triterpenes or tetraterpenes, but rubber tree has up to 45% polyisoprenes (rubber) in its latex composition [30, 43, 44]. In addition, fatty acids, phytosterols, alkaloids, phenolic compounds, proteins, cardenolides, starch grains, among other compounds, have already been identified in the latex of many species [7-10, 30, 39, 42] (Figure 2).

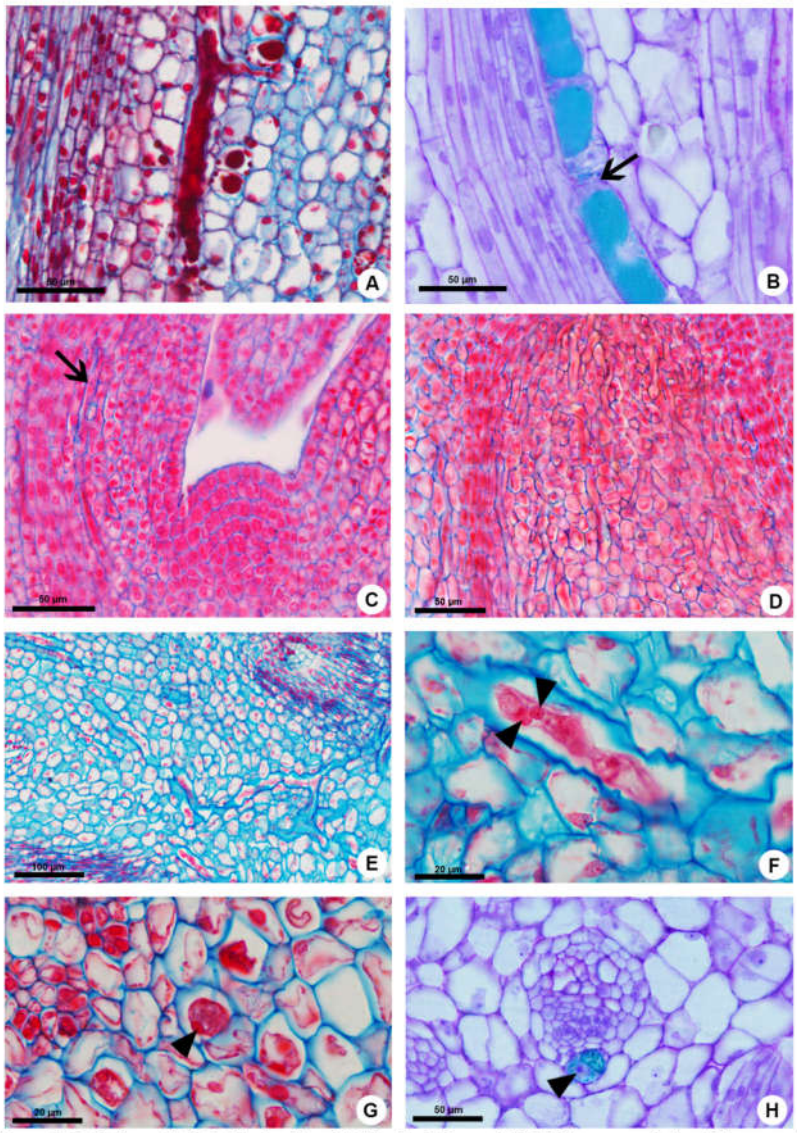

Figure 1. Laticifers. **A, E-G.** *Euphorbia milii* (Euphorbiaceae). **B, H.** *Musa paradisiaca* (Musaceae). **C, D.** *Thevetia peruviana* (Apocynaceae). **A.** Latex within the laticifer. **B.** Articulated laticifer. **C.** Laticifer ontogeny near the promeristem. **D.** Laticifer network. **E.** Branched laticifers. **F.** Multinucleated laticifer. **G, H.** Latex metabolites within the vacuole and peripheral nucleus. Arrow, terminal wall; arrowhead, nucleus.

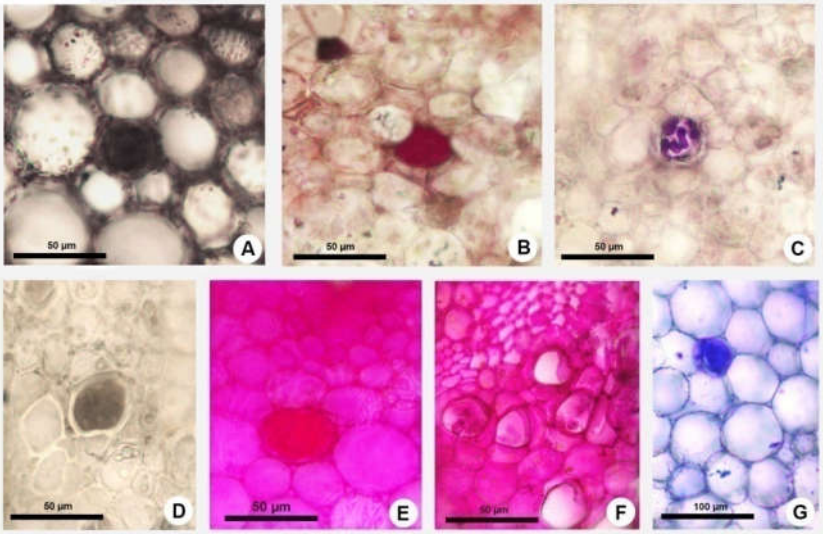

Figure 2. Histochemistry of the laticifers of *Hura crepitans* (Euphorbiaceae). **A.** Detection of lipids with Sudan black B. **B.** Resins identified within the laticifer using NADI reagent. **C.** Globules of essential oils and resins in the latex. NADI reagent. **D.** Detection of phenolic compounds with ferric chloride. **E.** Mucilage identified using ruthenium red. **F.** Polysaccharides within laticifers detected by PAS reaction. **G.** Proteins in the latex identified with coomassie blue.

The function of such compounds is, either individually or synergistically, to protect plants against herbivory and penetration of pathogens; further, they have the ability to seal wounds, since latex polymerizes in contact with the air [6, 7, 10, 23, 25, 30, 33, 39, 40, 45, 46].

The protective function of the latex is reflected in the time of laticifer differentiation, since all the secretory defense structures originate early in the organogenesis. Laticifers are present from the younger portions of the plant and are widely distributed in almost all tissues (Figure 1), but there is a higher frequency of laticifers associated with vascular tissues, especially with the phloem [40, 41] (Figure 1H). This proximity allows a direct transference of the transported nutrients to the laticifer, supplying the intense biosynthetic demand of this cell. This fact becomes even more relevant when it is considered that a single laticiferous cell can produce all the major classes of secondary metabolites [10]. These compounds, which can be extremely toxic, are isolated from the rest of the plant tissues remaining inside the laticifer and will only be released to the environment if there is a rupture of the secretory system [8, 23, 38].

3.2. Resin duct

Ducts are glands formed by a secretory tissue called epithelium that delimits an intercellular space, the lumen, where the secretion is stored (Figure 3A). The ducts are always elongated (Figure 3B) and can remain individualized or anastomose laterally (Figure 3C) forming a complex network of ducts throughout the plant [9, 23, 25]. Although this branched duct system may superficially resemble some types of laticifers, ducts are never composed of a single cell or a single row of cells. Actually, the epithelium of some ducts may have dozens of cell rows lining the lumen.

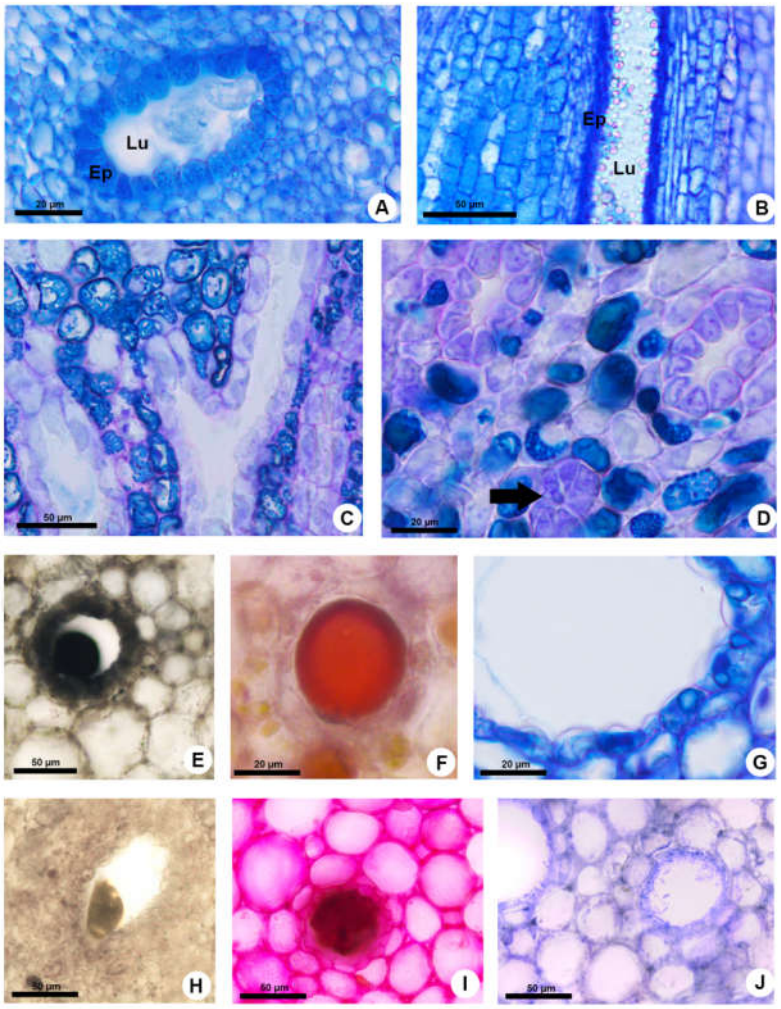

Figure 3. Resin ducts. **A-D.** General view. **A, D-J.** Transverse sections. **B, C.** Longitudinal sections. **A, B, E, H.** *Schinus terebinthifolius* (Anacardiaceae). **C, D, F, I, J.** *Clusia* sp. (Clusiaceae). **G.** *Protium heptaphyllum* (Burseraceae). **E-J.** Histochemistry. **E.** Lipids stained with Sudan black B. **F.** Resins identified using NADI reagent. **G, H.** Phenolic compounds detected by toluidine blue (**G**) and ferric chloride (**H**). **I.** Polysaccharides identified with PAS reaction. **J.** Proteins stained using coomassie blue. Arrow, duct initials rosette; Ep, epithelium; Lu, lumen.

In addition, ducts differ from laticifers in relation to the origin and the mode of secretion storage. The main event in duct morphogenesis is the process of lumen formation. Initially, we observe a set of meristematic cells called rosette (Figure 3D), which may form an intercellular space by means of three processes: 1) schizogeny, in which a space is formed by separation of the rosette initials through an active movement of the cells; 2) lysigeny, in which a space is formed by programmed cell death of one or more central cells of the rosette; 3) schizolysigeny, where the lumen is

initially formed by programmed cell death and then spread apart cells enlarging the intercellular space [9, 23, 25, 34, 47, 48].

After the formation of the lumen and concomitant differentiation of the epithelium, the secretory process is initiated by means of which the produced secretion will be stored extracellularly in the lumen [17, 25, 34, 49] (Figure 3A, B). This secretion's composition varies depending on the group and may be constituted of mucilage, gum or resin.

Despite all the differences between laticifers and secretory ducts, many divergences are found in the literature of some families for which some authors described the secretory structure as ducts, laticifers or latex ducts (= laticiferous canals). This confusion occurs exclusively in relation to the resin ducts, since the resin of some families may be white, especially in species of Anacardiaceae, Burseraceae, Cactaceae, Calophyllaceae and Clusiaceae [42, 47, 50-58].

Although resins are usually associated with the amber coloration, they may also be colorless [52, 59] or white. In the same way that latex varies in color, resins vary in color depending on their composition. By definition, resins are composed of phenolic compounds, terpenoids or a mixture of both [60] but what is observed in those five families is that the resin is composed of several classes of compounds [21, 59], although its constitution is mostly terpenic (Figure 3E, F), such as the resin of the gymnosperms and almost all angiosperms [60]. This fact led some authors to propose mixed terms, such as gum-resin to indicate the heterogeneity of the secretion. However, this term is not comprehensive enough, as this resin may have other compounds, such as phenolic compounds, alcohols, aldehydes, esters, gums, mucilage, proteins, and alkaloids [16, 21, 23, 25, 48, 59, 61, 62] (Figure 3G-J).

The high chemical complexity of some resins confers functions similar to those of the latex, acting against herbivory and microorganisms, besides sealing wounds by the polymerization of their compounds when in contact with the air [6, 63]. The secretion is stored in the lumen and does not come into contact with any surrounding tissue. Its release to the environment occurs only by rupture of the secretory system. Ducts have an early formation during plant organogenesis but due to its more complex structure in relation to the laticifers, they are found in mature stage at a little longer distance from the promeristem than laticifers (Figure 4A). Ducts also occur preferentially in the vascular system (Figure 4B) or in the surrounding area (Figure 4C).

In our study, we have analyzed the five families that have disagreements regarding the presence of resin or latex. In Anacardiaceae, Venning [64] reported the presence of ducts in *Schinus* as laticifers with schizogenous origin, and Fahn and Evert [47] attributed the milky white color of *Rhus* resin to the fact that the secretion contains carbohydrates in its constitution.

The tribe Protieae (Burseraceae) is recognized for presenting resinous latex or latex [50-52]. *Mammillaria* is a genus of Cactaceae described as latescent due to the presence of a milky white exudate [42, 53-57] and Mauseth [65] states that the *Mammillaria* laticifers would have evolved independently of all other latescent families, since their mode of formation is completely different. In addition, *Kielmeyera* (Calophyllaceae) and *Clusia* (Clusiaceae) are registered as latescent [42, 66, 67] due to the production of a white to yellowish exudate [58].

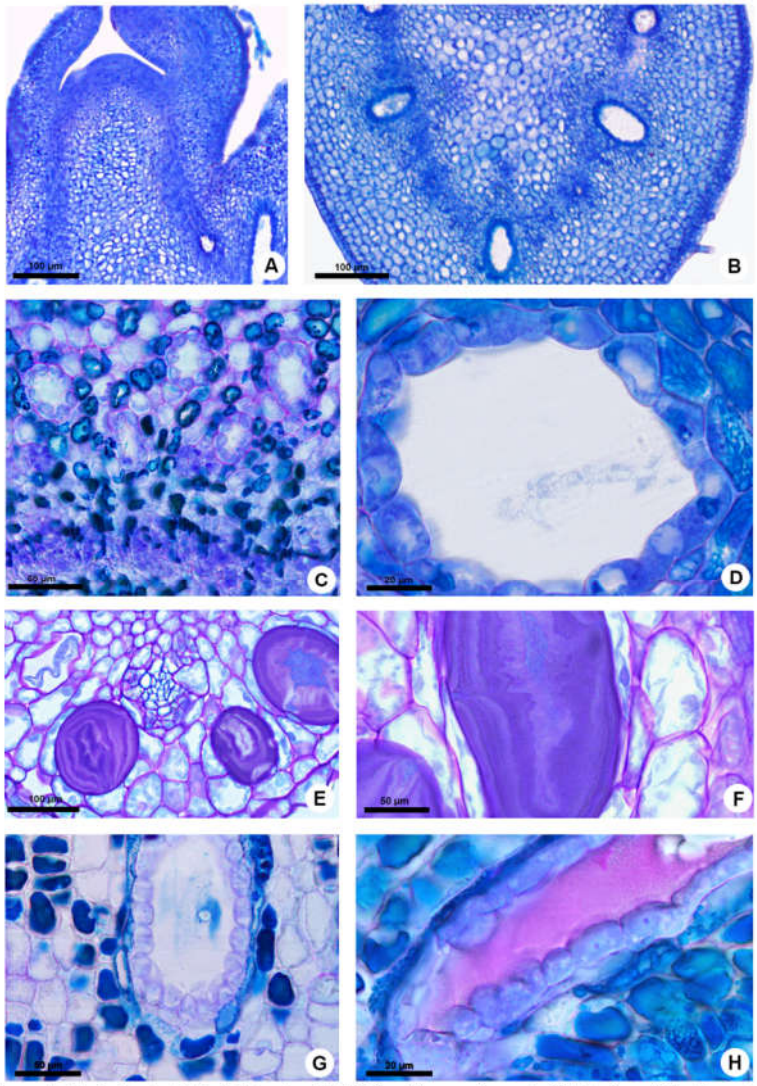

Figure 4. Resin ducts. **A, B.** *Schinus terebinthifolius* (Anacardiaceae). **C.** *Clusia* sp. (Clusiaceae). **D.** *Protium heptaphyllum* (Burseraceae). **E, F.** *Mammillaria* sp. (Cactaceae). **G, H.** *Kielmeyera appariciana* (Calophyllaceae). **A, F, H.** Longitudinal sections. **B-E, G.** Transverse sections.

Our analyses showed that the genera of these five families, in which some authors suggested the presence of latex, actually have resin ducts (Figures 3, 4). The white color of the secretion is due to the high heterogeneity of its composition, which is formed by several types of lipids, mainly terpenoids, phenolic compounds, polysaccharides and proteins (Figure 3E-J).

3.3. Occurrence of laticifers and resin ducts in plant taxa and their distribution according to the environment

The plant ability to produce latex or resin is not related to growth habit and seems to be related to a phylogenetic conserved trait or to a key evolutionary innovation that arose in a particular group, influenced directly or indirectly by the environment in which it lives.

Laticifers occur in about 10% of the angiosperm families, and the resin ducts in other 10% of them. As they usually do not occur in the same groups, both together are found in about 20% of the flowering families (Table 1), being very common defensive secretory structures. Moreover, this number may be underestimated, and laticifers have been identified in several genera of Sapindaceae described as non-latescent due to the inconspicuous latex released when the plant is ruptured [68]. We have noticed that the amount of latex, as well as resin, depends on the gland density in the organ, the degree of anastomosis of the secretory system, climatic and edaphic conditions and even the injuries caused by microorganisms or environmental factors.

According to our updated survey, laticifers are found in Marsileaceae (fern), Gnetaceae (gymnosperm) and 38 families belonging to almost all major lineages of angiosperms. Similarly, resin ducts occur in seven families of gymnosperms, belonging to Ginkgoales and Pinales, and are widespread within angiosperms in which they are present in 40 families (Table 1). Both in terms of absolute and proportional estimates, latescent and resinous families predominate in tropical regions [42, 60] (Table 1). It is estimated that 14% of the tropical species produce latex compared to 6% of the species in temperate regions [42]. In addition, the largest number of resin producing families which have numerous genera that produce copious resins occur in tropical areas [60].

The comparative analysis shows that 17 orders have both laticifers and resin ducts but generally in different families. The occurrence of both secretory structures in the same family was recorded only for Araceae, Salicaceae, Fabaceae, Cannabaceae, Moraceae, Cornaceae and Asteraceae, which are tropical families or have a wide distribution in tropical regions (Table 1).

3.4. Evolution of laticifers and resin ducts and ecological implications

The production of latex or resin is a highly convergent trait that has evolved independently multiple times (Figure 5). Despite the co-occurrence of laticifers and resin ducts being found in only 50% of the angiosperm orders which have these secretory structures (Table 1), it is noticeable the possible presence of laticifers or resin ducts in the ancestor of the same major lineages (Figure 5). This fact may indicate the emergence of an ancestral metabolic capability to synthesize terpenoids, which resulted in similar possibilities to the evolution of laticifers and resin ducts. This hypothesis is strengthened by the correlation between the evolution of resin ducts and a remarkable chemical diversification of terpenoids [70]. However, this issue is not so simple. Many resinous families do not have resin ducts in all their members, and latescent families rarely possess all their representatives with laticifers. Apocynaceae stand up as an exception in which laticifers are ubiquitous [7, 40] but laticifers have apparently evolved multiple times within other families, such as Sapindaceae [68].

Table 1.Occurrence of laticifers and resin ducts in vascular plants according to plant taxa and their distribution. Survey based on Metcalfe [41], Lewinsohn [42], Langenheim [60], Montes [68] and personal observation*.

Groups	Order and families	Laticifer	Resin duct	Distribution
Ferns	Salviniales Marsileaceae	+	-	***Regnellidium***, Southern Brazil and Argentina
Gymnosperms	Ginkgoales Ginkgoaceae	-	+	China
	Pinales Araucariaceae	-	+	Southern S. America, Malesia to Australia and New Zealand
	Cupressaceae	-	+	Northern and southern hemispheres
	Pinaceae	-	+	Northern hemisphere
	Podocarpaceae	-	+	Tropics and subtropics
	Sciadopityaceae	-	+	Japan
	Taxaceae	-	+	Northern hemisphere, scattered in south temperate regions
	Gnetales Gnetaceae	+	-	Tropics
Angiosperms	Nymphaeales Cabombaceae	+	-	World-wide, rather scattered
	Nymphaeaceae	+	-	World-wide
Magnoliids	Piperales Piperaceae	-	+	Tropics
Monocots	Alismatales Alismataceae	+	-	Pantropical, alsotemperate
	Aponogetonaceae	+	-	Old world tropics
	Araceae	+	+	American tropics, W. Indies
	Pandanales Cyclanthaceae	+	-	***Cyclanthus***, Central and tropical South America
	Liliales Liliaceae	+	-	North Temperate
	Asparagales Amaryllidaceae	+	-	World-wide
	Asparagaceae	-	+	World-wide
	Asphodelaceae	-	+	**Xanthorrhoeoideae;** Australia
Commelinids	Arecales Arecaceae	-	+	Indomalesia, esp. W. Malesia
	Zingiberales Musaceae	+	-	Africa, South Asia, Philippines and N. Australia
Eudicots	Ranunculales Berberidaceae	-	+	East Asia, E. North America and South America
	Lardizabalaceae	+	-	South East Asia and Chile
	Papaveraceae	+	-	N. Temperate, S. Africa and South America
	Proteales Nelumbonaceae	+	-	Temperate, E. North America and E. Asia
	Platanaceae	-	+	North Temperate, S.E. Asia
Superrosids	Saxifragales Altingiaceae	-	+	Indomalesia, E. Mediterranean, E Asia, S.E. North America, Central America
	Peridiscaceae	+	-	S.America, tropical W. Africa

	Zygophyllales			
	Zygophyllaceae	-	+	Dry and warm temperate, also tropical
	Celastrales			
	Celastraceae	+	-	World-wide
	Malpighiales			
	Calophyllaceae	-	+	Tropics
	Clusiaceae	-	+	Tropics
	Euphorbiaceae	+	-	Pantropical
	Humiriaceae	-	+	Tropical America, W. Africa
	Malpighiaceae	+	-	Tropicsandsubtropics
Fabids	Salicaceae	+	+	Pantropical, temperate to Arctic
	Fabales			
	Fabaceae	+	+	Tropics
	Rosales			
	Cannabaceae	+	+	Central Asia, N. temperate zone
	Moraceae	+	+	Tropical to warm temperate
	Rhamnaceae	-	+	N. hemisphere to Brazil, S. Africa
	Rosaceae	-	+	Temperate zones and tropical mountains
	Urticaceae	+	-	World-wide, esp. tropical
	Fagales			
	Betulaceae	-	+	North Temperate to Andes and S.E. Asia
	Myrtales			
	Myrtaceae	+	-	Worldwide, esp. tropical-warm temperate
	Sapindales			
	Anacardiaceae	-	+	Tropical, alsotemperate
	Burseraceae	-	+	Tropics
	Rutaceae	-	+	Largely tropical
	Sapindaceae	+	-	Tropics and subtropics, Australia
	Simaroubaceae	-	+	Largely tropical; a few temperate
Malvids	Malvales			
	Bixaceae	+	-	Pantropical
	Cistaceae	-	+	Mediterranean region, N. Africa, N. America, S. South America
	Dipterocarpaceae	-	+	Tropical, esp. Malesia
	Thymelaeaceae	-	+	World-wide, tropical Africa and Australia
	Brassicales			
	Caricaceae	+	-	Tropical America and Africa
	Gyrostemonaceae	-	+	Australia, Tasmania
	Santalales			
	Loranthaceae	+	-	Tropics
	Olacaceae	+	-	Pantropical
Superasterids	Caryophyllales			
	Cactaceae	-	+	*Mammilaria*, America
	Plumbaginaceae	-	+	Tropical, warmregions
	Cornales			
Asterids	Cornaceae	+	+	N. temperate zone, S. America, Indomalesia
	Nyssaceae	+	-	East Asia, Indo-Malesia and E. North America

	Ericales			
	Sapotaceae	+	-	Pantropical
	Styracaceae	-	+	Warm N. temperate to tropical
Campanulids	Aquifoliales			
	Aquifoliaceae	+	-	World-wide
	Cardiopteridaceae	+	-	Tropics
	Asterales			
	Asteraceae	+	+	World-wide
	Campanulaceae	+	-	World-wide
	Goodeniaceae	-		Australia
	Apiales			
	Apiaceae	-	+	World-wide, esp. N. temperate
	Araliaceae	-	+	Largely tropical, fewtemperate
Lamiids	Gentianales			
	Apocynaceae	+	-	Largely tropical to warm temperate
	Rubiaceae	-	+	World-wide, esp. Madagascar and the Andes
	Solanales			
	Convolvulaceae	+	-	World-wide
	Solanaceae	-	+	World-wide, esp. tropical America
	Boraginales			
	Boraginaceae	-	+	Largely north (warm) temperate, some on mountains in the tropics
	Lamiales			
	Gesneriaceae	-	+	**Epithemateae**, tropics
	Scrophulariaceae	-	+	World-wide
	Garryales			
	Eucommiaceae	+	-	Central China
	Icacinales			
	Icacinaceae	+	-	Pantropical

Note. + = present; - = absent. The occurrence of laticifers or ducts in only one infra-familial group was highlighted with the taxon in bold.
*Occurrence not confirmed was not included. Classification *sensu* APG IV [69].

The multiple evolution of these defensive secretory structures may be associated to a sharply increased in insect herbivory during Paleocene–Eocene [1]. In this epoch, angiosperms have become the predominant plant group and coevolved with the insects that fed on these plants and pollinated them [71]. Although the first fossil records of plants with resin ducts were found in pteridosperms from the Carboniferous period of the Paleozoic era [71-74], laticifers were apparently first seen over 250 million years later in the beginning of Cenozoic era [71], when an abrupt global warming seems to be related to an increase of both insect diversity and population density [1].

The emergence of laticifers and resin ducts during evolutionary history of vascular plants represents key innovations that have spurred adaptive radiation in plants. Farrell et al. [6] showed that plants that have laticifers or secretory ducts have more advantages in the environment in which they live in relation to those that do not have them or in which these secretory structures are reduced, promoting a greater diversity in both the reproductive capacity and individual fitness [7, 39]. This can be observed in the higher occurrence of resin and latescent species in tropical regions, where the herbivory rate is higher [40, 42, 60]. If, on the one hand, tropical environments provide better conditions for plant metabolism in terms of photosynthesis and water availability, on the other hand they also favor a greater diversity of phytophagous insects and pathogenic fungi [6, 61, 75].

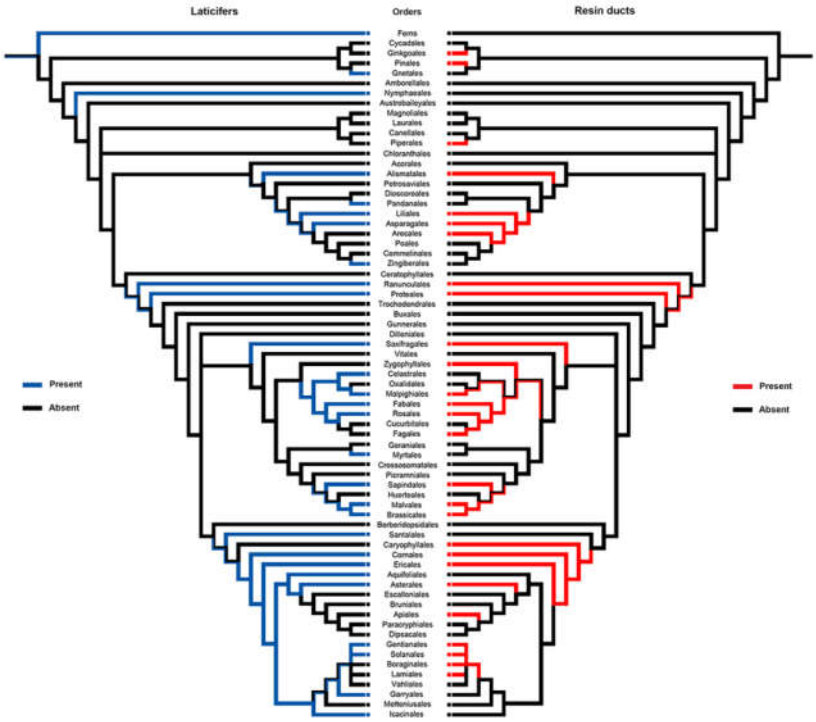

Figure 5. Comparative evolutionary analysis of the distribution of laticifers and resin ducts in vascular plants. All orders containing one latescent or resinous species, at least, were labeled. The data were obtained from the surveys of Metcalfe [41], Lewinsohn [42], Langenheim [60], Montes [68] and personal observation optimized on the current phylogeny [69] using parsimony analysis.

Although specialist insects can feed on some plants that produce latex or resin, generalist ones are highly affected by the properties of these secretions, which are either

toxic or deterrent [2, 5, 23]. Strategies to reduce the intake of toxic plant secretions have appeared in multiple insect lineages, allowing to verify the convergent evolution of similar behaviors in several latescent or resinous plants, regardless of the plant morphology or phylogenetic relationships [3, 5, 6, 17, 23, 25, 26, 30, 76-79].

These specialized insects' ability to avoid the ingestion of toxic compounds involves leaf vein-trecching, vein-cutting, girdling, and leaf clipping strategies, among others, reducing by up to 90% the ingestion of the exudate [17, 23, 30, 79, 80]. It is noteworthy that some specialist insects have developed chemical defenses – such as digestive proteases – against latex compounds [81] and also sequester toxic components from the exudate to reuse them later in their own defense against predators [5, 6, 10, 27, 39].

4. Conclusions

Laticifers and resin ducts have similarities in relation to the secretion, which is mostly terpenic, function as protection against herbivory, present high viscosity and polymerizes in contact with the air, and the resin, at times, is white. However, laticifers and ducts are structurally very distinct and have different origins and mode of secretion storage. It is also important to highlight that, since latex is the own protoplast of the laticifer, when it extrudes, there is not only metabolites in the exudate but also membranes, organelles and nuclei. As the resin is an extracellular secretion, these cellular remnants are not present, and when they are found in its composition, it is due to a completely different process related to a holocrine release of the secretion to the lumen.

Since the secretions are confused only when they are white, it should be noted that, although latex is typically white, and resin is typically amber, both secretions may have different colors and may even be colorless or change their color when in contact with the air. The concept of latex is linked to that of the laticifer and to its complex composition, rather than to its color. Thus, if a white secretion is produced by a duct, it must not be considered latex, and the structure cannot be a laticifer. We propose that the term resin be used in a broad sense for the secretions mainly composed of terpenoids (or phenolics in few cases) which are produced by secretory ducts, regardless of their color, as well as the term latex is used for all secretions produced by laticifers, even when it is not milky white.

The evolutionary analysis of both structures shows that they emerge multiple times in the phylogeny, often in the same order, although they are not usually present in the same plants. Our analyses indicate that the appearance of the terpenoid metabolic route in the ancestral of some major lineages, associated with events of increased herbivory, lead to the emergence of either laticifers or resin ducts in distinct families. In some cases, the presence of both latex and resin within certain families, such as Fabaceae and Asteraceae, certainly conferred greater adaptive success in several environments.

5. Future perspectives

Much remains to be studied about laticifers and ducts. Although their structures have been known for more than a century, and we have clear and objective definitions of them, discrepancies in the descriptions still remain. Divergences about the origin, mode of growth and the lack of information about the chemical composition of latex and resin

of several groups still prevent a series of evolutionary analyses that may clarify the factors that determined the emergence of these structures in different groups, especially considering that both appeared multiple times throughout the evolution of plants.

Acknowledgments

We thank Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES) for financial support and the Laboratory of Plant Anatomy at the Institute of Biosciences of the University of Sao Paulo, where the analyses were performed.

Conflict of Interest

The authors declare no conflict of interest.

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CHAPTER 2

Plant latex and latex-borne defense

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Submitted: Ramos MV, Freitas CDT, Morais FS, Prado E; MedinaMC, Demarco D. 2019. Plant latex and latex-borne defense. In: Latex, Laticifers and their Products. Robert N (ed.). Advances in Botanical Research. Vol. 93. Elsevier: Amsterdam.

1. General Aspects and Definition

Latex has varied composition and properties depending on the group of plants considered due to their multiple emergence during the evolution of vascular plants, but each and every latex has a primary defense function against herbivory (Prado and Demarco 2018). The protection conferred by this secretion ensured the biological success of latescent plants in relation to non-latescent plants in the various biomes (Farrell et al. 1991) and is due to the presence of a wide variety of metabolites in their constitution (Fahn 1979).

Latex is defined as a suspension of small particles dispersed in a liquid with different refractive indices (Fahn 1979), giving it an emulsion appearance (Figure 1A). However, latex is much more than that; it is the product of the laticifer and corresponds to its own protoplast (Demarco *et al.*2006), as all its protoplast is released when the laticifer is broken. This means that the secretion that exits latescent plants is not only composed of primary and secondary metabolites, but also contains plasma membrane, organelles, and the cytoplasmic content, in addition to the nucleus or the various nuclei present in the protoplast (Figure 1B). Therefore, when anatomical cuts are performed without due care for the preservation of the latex inside, only the walls of the laticifers are observed.

Despite the presence of membranes, organelles, nuclei and several components that are not secretory products (e.g., nucleic acids), latex is recognized and studied for the primary and secondary metabolites produced by laticifers (Figure 1C-Q) that are stored basically within their large vacuoles (Fahn 1979). The proportion of these metabolites is variable, which may alter the color of the latex. Thus, not all latex is white, but may also be yellowish, greenish, orange, reddish and even colorless (Mahlberg 1993).

These metabolites act as a chemical barrier to herbivory due to the presence of toxic and dissuasive food components (Dussourd 2017), but also act as a physical barrier because their compounds coagulate fast (Bauer *et al.* 2014), sealing wounds against the entry of pathogens, such as bacteria and viruses (Wink 2008; Konno 2011). In addition, the high viscosity of latex and its rapid coagulation in contact with air can also immobilize insect jaws (Dussourd & Eisner 1987) and is related to the characteristic that joins all types of latex: its predominantly terpenoid composition (Prado and Demarco 2018).

2. Metabolites present in latex

Latex has mainly terpenes (Prado & Demarco 2018), but it is a complex mixture, presenting substances such as rubber, mucilage, phenolic compounds, alkaloids, cardenolides (Konno 2011). Carbohydrates such as starch grains in some Euphorbiaceae. (Demarco et al., 2013), and proteins such as cysteine proteases in Apocynaceae (Freitas et al.2007).

Each class of metabolites acts in the plant's defense (Bennett & Wallsgrove 1994); however, it is worth emphasizing the importance of the synergism of these compounds, where their toxicity can be potentiated when acting together (Wittstock & Gershenzon 2002), as in the case of terpenes associated with phenolic compounds (Wink 2008). In Freitas *et al.* (2007) proteins are shown to be associated with defense functions, since there are satisfactory effects on insects. In *Hevea brasiliensis,* proteins named hevein*,* polymerize the rubber particles, having an important role in sealing wounds (d'Auzac *et al.* 1995).

The various substances that make up the latex can be divided into hydrophilic and lipophilic.

2.1 Hydrophilic Substances

Carbohydrates

Laticifers also secrete carbohydrates, such as sugars found in Compositae latex (Fahn 1979) and polysaccharides or mucilage (Figure 1C-D); however, Groeneveld *et al.* (1982), have shown that the carbohydrates present in the latex are the main source of carbon used by the laticifer in the synthesis of triterpenes in *Euphorbia lathyris*.

The presence of starch grains occurs in the Hippomaneae and Euphorbieae tribes of Euphorbiaceae (Demarco et al., 2013; Figure 1E). However, in Euphorbieae, the grains have varied morphologies and are even used in the interpretation of evolutionary and taxonomic aspects. The rounded and spindle morphology of grains in laticifers are interpreted as being less specialized, while progressive specialization is associated to the larger starch deposition in polarized form, in order to assume an osteoid and eventually lobular morphology, thus leading to an interpretation of the specialization pattern of these taxa (Mahlberg 1975).

Latex production spends great plant energy (Agrawal & Konno 2009); however, according to Biesboer & Mahlberg (1978), the starch grains mobilization is not verified as an energy source.

Proteins

In addition to the secondary metabolites, the proteins identified (Figure 1F) play some defense functions in the latex through proteases, cysteine in *Carica papaya,* and *Ficus*

sp., serine in *Hevea brasiliensis*, *Euphorbia* sp., *Ipomoea* sp., proteases inhibitors, among others (Agrawal & Konno 2009).

Up to 30% of the *Carica papaya* content is formed by proteolytic enzymes (Moussaoui *et al.* 2001), an endopeptidase complex where *papain* is the smallest constituent but with the highest commercial value, because of its use in the food industry (Agrawal & Konno 2009).

Shenoy *et al.* (2009) have found the presence of proteins in strains of *Allium* and exerting antibacterial activity against *Bacillus sublilis*, *Staphylococcus aureus* and *Escherichia coli*.

In *Hura crepitans* (Figure 1F) the crepitin and lectin proteins present in latex grant hemagglutinating properties, besides inhibiting protein synthesis, demonstrating its high cytotoxicity (Barbieri *et al.*1983).

Research conducted by Yan *et al.* (2011) have shown that chitinases from *Ficus carica* have antifungal activity against *Trichoderma viride.*

Other proteins present in latex are protease inhibitors (PIs), with an anti-nutritive function, in which the proteases of herbivores bind and inhibit the digestion of the protein (Agrawal & Konno 2009).

2.2 Lipophilic Substances

Lipids

Latex is composed predominantly of lipids (Figure 1G-M), with terpenes (Figure 1G) being the majority class (Prado & Demarco 2018). Among terpenes, there appears to be a predominance of triterpenes and tetraterpenes (van Die 1955; Rizk 1987). However,

other classes of higher or lower molecular weight terpenes can be found in latex, especially cis-1,4-polyisoprene, popularly known as natural rubber (Mooibroek & Cornish 2000).

Rubber is present in the latex of only a few species; its main function is to increase the latex viscosity and defense functions related to the coagulation properties (Konno 2011). Although it does not occur universally in latescent species, rubber can reach 45% of the latex gross weight in *Hevea brasiliensis* and up to 30% in *Ficus elastica* (Mooibroek & Cornish 2000).

In *Hura crepitans*, diterpenes, called huratoxin, have a structure different from those found in Euphorbioideae (Webster 1986).

On the other hand, in *Euphorbia* spp., the diversity of diterpenes, milliamines, show that there are geographic standards, which are greatly important for phylogenetic and ecological considerations (Webster 1986). Moreover, its toxicity has tumor and inflammatory action in herbivores (Gershenzon & Croteau 1989).

Cardenolides, derived from triterpenoids, have a defense function. Agrawal *et al.* (2012) mention that the higher the cardenolide concentration, the more toxic the plant is due to the high absorption of this nonpolar compound by the mucous membranes of the insects. We may also found fatty acids in some lattices (Figure 1

Phenolic compounds

The phenolic compounds found in latex, such as flavonoids, tannins and diphenols are related to the plant's defense (Rizk 1987, Konno 2011). Tannins linked to proteins inhibit digestive enzymes, thus exerting a negative effect on the growth of herbivores (Yamane *et al.* 2010).

The concentration of phenolic compounds in latex varies depending on the group of plants analyzed, being higher in some plants as *Musa paradisiaca*, *Ipomoea tricolor* (Figure 1N) and *Ficus benjamina*.

Alkaloids

Alkaloids are found in approximately 35 families, mostly angiosperms, where only 14.2% of genera have them (Woolley 2001, Samanani *et al.* 2006). This restricted distribution seems to be related to the type of environment, with a predominance of alkaloids in plants of tropical habitats (Levin 1976). These compounds have high toxicity against herbivores and pathogens (Bennett & Wallgrove 1994), and may be food deterrents (Macías *et al.* 2004).

This class of secondary metabolites greatly varies in terms of molecular structure, as well as ecological and pharmacological activity (Wink 2008; Macías et al. 2004) and, when present in latex, they confer an orange color (Matile *et al.* 1970).

The first study on alkaloids was made from the latex of *Papaver somniferum*, which has approximately 20 types of alkaloids, including *morphine* and *codeine*, potent analgesics used in the pharmaceutical industry (Moussaoui *et al.* 2001).

As seen in some families, such as Amaryllidaceae, Apocynaceae and Asteraceae, latex produced a large amount of alkaloids (Khatoon 2017), which can act synergistically with other alkaloids or compounds (Schardl 2002), thus presenting antimalarial, anesthetic, antibacterial and antiviral properties (Wittstock & Gershenzon 2002).

In *Ipomoea* spp., Convolvulaceae, its alkaloids have antimicrobial and purgative properties granting phytotoxicity (Meira *et al.* 2012), and a study conducted by Bah &

Pereda-Miranda (1997) have found that species of *Ipomoea* are used in plantations to inhibit the growth of invasive plants.

Alkaloids are always among the components found in lesser amounts in latex (Demarco 2015; Figure 1P), being found in extremely small amounts when compared to the other latex components, even in species such as *Papaver somniferum* (Fairbairn & Steele 1981).

When comparing the total composition of latex by histochemical analysis, it can be noted that regardless the group of plant analyzed, its coloration or toxicity, the latex always has all or almost all the main chemical classes of secondary metabolites (Table), showing that, despite the many variations observed in the chemical analysis, in general, all latices are similar and have evolved into a convergent way in the different groups.

3. Exudate trapping

As many of the latex compounds are toxic (Wittstock & Gershenzon 2002, Hagel *et al.* 2008, Agrawal & Konno 2009), the isolation of these substances inside the laticifer can be observed in relation to adjacent tissues, and mature laticifers do not contain plasmodesma (Gama *et al.* 2017).

Although several alkaloids are found in latex, they are not necessarily synthesized by laticifers. In *Catharanthus roseus*, alkaloid precursors are synthesized in specialized parenchyma cells and sent to the laticifers via the apoplast pathway, to finish its biosynthesis (Yoder & Mahlberg 1976).

In *Carica papaya*,it can be noted that the proteinases present in the latex are converted and become highly toxic only after the latex is expelled, being previously stored inactive inside the plant (Azarkan *et al.* 2003).

4. Evolution of latex and ecologic implication

Latex is present in 40 families of vascular plants, often without phylogenetic relation, showing that it has frequently evolved independently (Prado & Demarco 2018).

Latescent families are found mainly among angiosperms, but they are also found in ferns, such as *Regnellidium diphyllum* (Marsileaceae) and gimnospermas *Gnetum gnemon, (*Gnetophyta) (Metcalfe 1967).

The convergent evolution of laticifers is observed from the presence of many similar components of phylogenetically distinct groups (Table). Although the chemical classes of secondary metabolites are the same in the latex of the different lineages of latescent plants, the type of compound may vary among the species of the same family such as in Moraceae, where only some species of *Ficus* have the cysteine protease enzyme, while other species have other types of enzymes (Konno 2011).

In *Asclepias* (Apocynaceae), there is a variation in the presence of cardenolides, with presence in *A. curassavica* and absence in *A. speciosa* and *A. californica* (Konno 2011).

Latex has a plastic nature, showing that latex production may vary according to the environment it is found (Konno 2011). Considering the biotic and abiotic variations, such as drought, light, inter- and intra-specific competition, pathogens and herbivory attack, there will result in the final concentration of compounds in latex (Dicke & van Loon2000). Hao & Wu (2000) have shown that the increase of jasmonic acid in the plant increase latex exudation, as also Agrawal (2011) observed in the production of cardenolides in *Asclepias* sp.

In some groups, such as in Apocynaceae, latex proves to be an ancestral condition, since it is conserved in all species of the family (Metcalfe & Chalk 1950). However, latex is usually present in only a few taxa of each family (Prado & Demarco 2018).

Considering its limited distribution, latex may be an important characteristic for phylogenetic analysis, such as in Malpighiaceae, where latex is a synapomorphy of the Galphimieae tribe, supporting the monophyly (Vega *et al.*2002).

5. Ecologic relations

Even though latex is composed of a large amount of toxic substances, some specialist herbivores present strategies to prevent the plant from defending itself, and some are even capable of sequestering toxic components from the latex and use them in their own defense (Konno 2011, Dussourd 2017).

The occurrence of latex predominantly in tropical environments, where herbivory taxa is higher, highlights that predation as a factor that directs the evolution of this defense system (Prado & Demarco 2018).

By means of behavior strategies such as vein-cutting, vein-trenching and girdling, precise cuts are made in the plant for latex extravasation, reducing in up to 90% the exudate ingestion (Agrawal & Konno 2009, Konno 2011, Dussourd 2017).

When broke, the laticifer releases latex, causing the loss of cell turgor (Buttery & Boatman 1976); consequently, the water from adjacent cells will cross the laticifer's wall, thus contributing to a largest extravasated flow (Pickard 2008). The presence of suberin in the laticifers' walls of Convolvulaceae (Fineran *et al.*1988), prevents the entrance of water in the laticifer and, consequently, the volume of latex expelled when the laticifer é broken is significantly lower (Demarco 2015).

Studies reveal that branched laticifers tend to present herbivore behaviors in *vein-trenching* and *vein-cutting*, as the plant's architecture allows for a higher extravasation of latex (Dussourd & Denno 1991).

These specialized herbivore strategies were described for 11 latescent plant families (Dussourd 2017) and both behaviors have shown to have evolved independently in several lineages of insects and in several environments (Dussourd & Denno 1991). Some specialist herbivores of the Dilophonotini tribe may feed from several latescent species from phylogenetically distant groups, such as Apocynaceae, Caricaceae, Euphorbiaceae and Moraceae (Farrell *et al.* 1991), showing that the behavior to prevent the ingestion of latex is effective according to the structure of the laticifer system and does not depend on the latex composition.

On the other hand, some species have developed physiologic strategies to present the latex toxic action, as observed in the monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Danaidae). The larvae ingest the cardenolides present in latex and still use them in their own defense against predators (Malcolm & Zalucki 1996). By ingesting these cardenolides, which possess the property of inhibiting the potassium sodium pump from cells, they manage to bypass this toxicity through their insensitivity to it. This insensitivity is due to the mutation of a single amino acid at the cardenolide binding site to the plasma membrane (Konno 2011).

Opitz & Muller (2009) have found herbivores with the same sequestration strategy of toxic components for its defense; however, in this case, the compounds were the isoprenoids of *Ipomoea batatas* (Convolvulaceae) and phorbol esters of *Jatropha curus* (Euphorbiaceae).

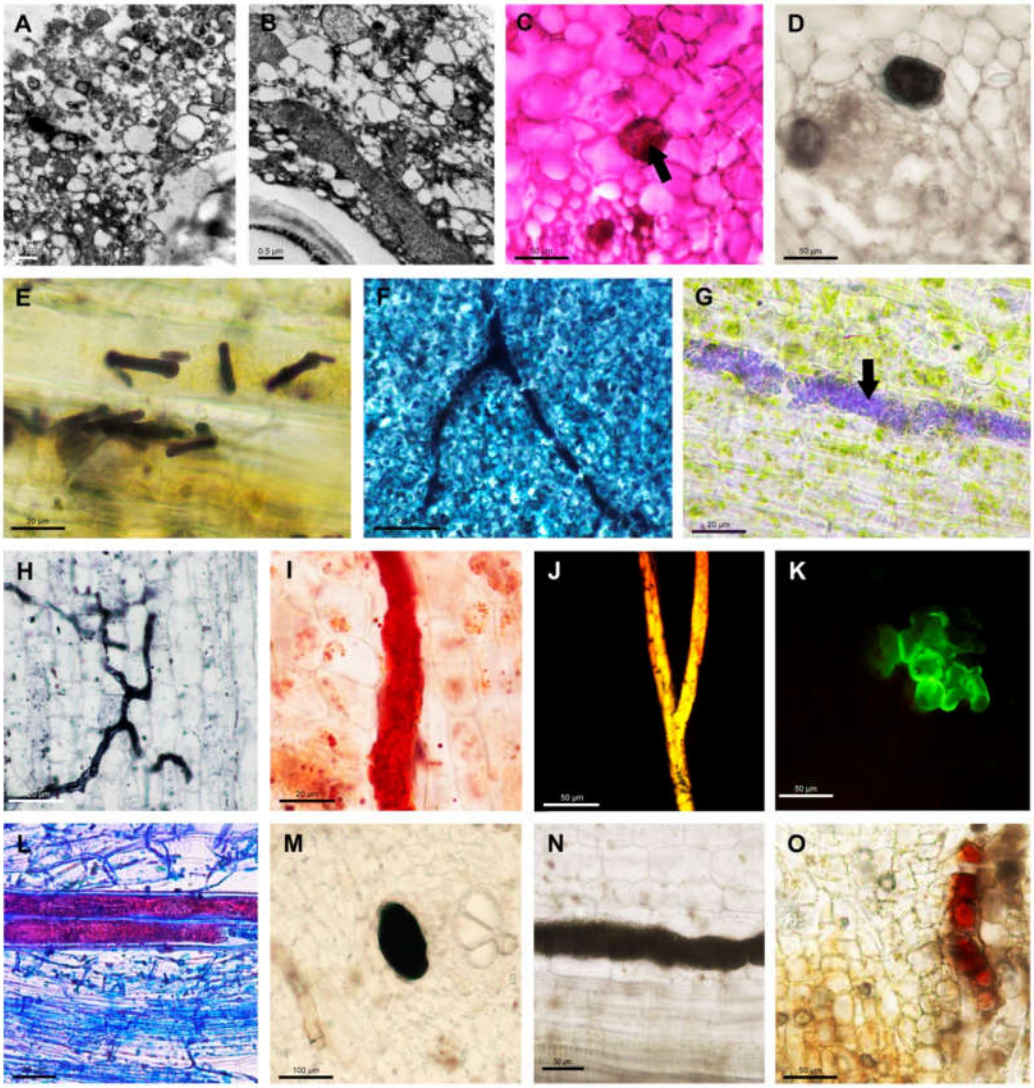

Figure 1. Latex constituents. **A-B.** Electron micrographs of latex in *Asclepias curassavica* L. [tropical milkweed]. **A.** Emulsion aspect of latex containing many small lipophilic particles of different contrast and sizes in a hydrophilic medium. **B.** Presence of organelles within latex. Note an elongated plastid (P) in peripheral position. **C-O.** Latex metabolites identified by histochemical tests. **C.**Carbohydrates (dark pink) detected by PAS reaction in *Allium cepa* L. [onion]. An arrow indicates latex. **D.** Mucilage (black) identified using tannic acid and ferric chloride in *Musa paradisiaca* L. [banana tree]. **E.** Starch grains (black) detected by Lugol's reagent in *Hura crepitans* L. [sandbox tree]. **F.** Proteins (navy blue) stained with aniline blue black in *Hura crepitans*. **G.** Terpenes (purple) identified by NADI reagent in *Urera baccifera* (L.) Gaudich. [scratchbush]. An arrow indicates latex. **H-K.** Lipids observed under bright field (**H-I**) and blue light (**J-K**). **H.** Lipids stained black using Sudan black B. *Hippobroma longiflora* (L.) G. Don [star of Bethlehem]. **I.** Lipids stained red with Sudan IV. *Mandevilla splendens* (Hook.) Woodson [shining mandevilla]. **J.** Yellow fluorescent lipids detected with the use of Nile blue. *Euphorbia milii* Des Moul. [crown of thorns]. **K.** Green fluorescent lipids stained with neutral red. *Musa paradisiaca*. **L.**Neutral lipids (pink) identified using Nile blue in *Euphorbia milii*. **M.** Fatty acids (dark green) detected by copper acetate and rubeanic acid in *Musa paradisiaca*. **N.** Phenolic compounds (dark grey) identified by ferric chloride in *Ipomoea tricolor* Cav. [morning glory]. **O.**Alkaloids (red-brown) detected by Dragendorff's reagent in *Ficus benjamina* L. [weeping fig].

Table 2. Histochemical analysis of latex components in different plant families.

	AMARYLLIDACEAE	MUSACEAE	EUPHORBIACEAE		MORACEAE	URTICACEAE	APOCYNACEAE	CARICACEAE	CONVOLVULACEAE	CAMPANULACEAE
Histochemical test	*Allium cepa* L.	*Musa paradisiaca* L.	*Euphorbia milii* Des Moul.	*Hura crepitans* L.	*Ficusbenjamina* L.	*Urerabaccifera* (L.) Gaudich.	*Mandevillas plendens* (Hook.) Woodson	*Carica papaya* L.	*Ipomoea tricolor* Cav.	*Hippobromalon giflora*(L.) G. Don
Lipids										
Sudanblack B	+	+	+	+	+	+	+	+	+	+
Sudan IV	+	+	+	+	+	+	+	+	+	+
Neutral red	+	+	+	+	+	+	+	+	+	+
Neutral lipids										
Nile blue	+	+	+	+	+	+	+	+	+	+
Terpenoids										
NADI reagent	+	+	+	+	+	+	+	+	+	+
Fattyacids										
Copper acetate and rubeanic acid	+	+	+	+	+	+	+	+	+	+
Phenolic compounds										
Ferric chloride	+	+	+	+	+	+	+	+	+	+
Potassium dichromate	+	+	+	+	+	+	+	+	+	+
Alkaloids										
Dragendorff's reagent	+	+	+	-	+	-	+	-	-	-
Wagner's reagent	+	+	+	-	+	-	+	-	-	-
Carbohydrates										
PAS reaction	+	+	+	+	+	+	+	+	+	+
Mucilage										
Tannic acid and ferric chloride	+	+	+	+	+	+	+	-	+	-
Acidic mucilage										
Ruthenium red	+	+	+	+	+	+	+	-	+	-
Alcian blue	+	+	+	+	+	+	+	-	+	-
Starch										
Lugol's reagent	-	-	+	+	-	-	-	-	-	-
Protein										
Aniline	+	+	+	+	+	+	+	+	+	+

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CHAPTER 3

Laticifers in vascular plants

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Manuscript to be submitted to: Annual Review Plant Biology

Laticifers in vascular plants

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Abstract

Laticifers are specialized latex-secreting cells whose primary function is defense against herbivory and microorganisms. Laticifers are found in several vascular plant families and can be considered as a taxonomic and phylogenetic indicator. However, several misinterpretations occur in the literature, attributing the presence of laticifers in families that do not have them, requiring a review of this structure and its real distribution and evolution. The objectives of the present work are to characterize and distinguish laticifers from other secretory structures, identifying their typology and identifying them in each family based on their ontogenesis. We found that in the 63 families analyzed, only 34 have latex in at least one of their representatives, and many of these families have no phylogenetic relationship with each other, demonstrating that laticifers appeared multiple times in the evolution of vascular plants. We have shown that 18 so-called latescent families actually have resin ducts or secretory idioblasts. We have also verified that all laticifers are articulated and can be classified as anastomosed or non-anastomosed, branched or unbranched, refuting the existence of non-articulated laticifers. Finally, we also refute the intrusive autonomous growth theory because there is no evidence of its occurrence in any of the dozens of families analyzed. This study makes a significant contribution to the study of laticifers and promotes an understanding of the identification, mode of growth, distribution, and evolution in vascular plant families.

Key words: laticifers, latex, distribution, resin ducts, phenolic idioblasts, vascular plants.

Introduction

1. Background and definitions

The presence of a milky secretion that would later be called latex has been observed since ancient Greece and recorded by Greek philosopher Theophrastus. However, it was only after the invention of the microscope in the seventeenth century

that it became possible to observe the structure that produces this secretion (Milanez 1978).

One of the earliest records of laticifers was made by Malpighi (1675), who described latex as "proper juice" contained in vessels called *vasa propria* (Milanez 1978, Mahlberg 1993). Grew supplemented the information in 1682, noting that the compound is not found in all plants.

The term laticifer was coined by Schultz (1839), who observed the Brownian motion of latex particles and described their ability to coagulate when removed from the plant (Milanez 1978, Mahlberg 1993).

Many other researchers have made important contributions to the study of laticifers, such as Schmalhausen (1877 *apud* Mahlberg 1993), who compared them with fungal hyphae and visualized H-shaped laticiferous tubes; Schaffstein (1932 *apud* Mahlberg 1993), who observed laticifers located near the promeristem; and, more recently, Milanez (1978) and Mahlberg (1993), who devoted most of their time to the study of laticifers of different plant groups.

A laticifer is defined as a specialized cell or a row of latex-containing cells (Fahn 1979); the most accepted classification of the structure was made by Bary (1884), who divided laticifers into articulated and non-articulated (Ramos *et al.* 2019).

According to this classification, non-articulated laticifers are composed of individualized cells and may be branched or unbranched (Fahn 1979). Theoretically, they are formed in predetermined numbers in the embryo, denominated laticifer initials and are located in the cotyledonary node (Mahlberg 1961, 1993, Pickard 2008).

Also according to this theory, non-articulated laticifers extend by means of autonomous intrusive apical growth, expanding unlimitedly throughout the plant body through intercellular spaces (Schamalhausen 1877, Mahlberg 1993). This type of

growth would only be possible via two processes: mechanical penetration of the laticifer apex between tissue cells or by the chemical dissolution of the middle lamella by means of pectinase activity. Apparently, the oblique irregular wall at the apex of laticifers would be a clue to this type of growth (Mahlberg 1963, Wilson *et al.* 1976, Serpe *et al.* 2002). In order for a single cell to grow indefinitely through the plant body, it is considered that mitosis would occur without cytokinesis in this type of laticifer, resulting in a coenocytic structure in which the cell wall would not be formed, thus not separating the daughter cells (Mahlberg 1993, Pickard 2008).

Articulated laticifers either consist of a row of cells that can remain individualized and are classified as non-anastomosed articulated laticifers or they can dissolve to form a continuous tubular structure, thus being referred to as anastomosed articulated laticifers (Metcalfe 1967, Fahn 1979, Mahlberg 1993). Such laticifers are formed continuously from the fundamental meristem, the procambium and/or the cambium (Rudall 1986, Demarco *et al.* 2006 2013, Demarco and Castro 2008, Evert 2006).

There are divergences in the literature regarding the typification of laticifers (Demarco *et al*. 2006, 2013, Demarco and Castro 2008), which lead Prado and Demarco (2018) to investigate the existing classification system, since current records point to the absence of non-articulated laticifers in all families in which they were described.

Another occurrence is the mistaken analysis of laticifers with resin ducts and laticifers with secretory idioblasts, causing many descriptive errors in the literature and doubts regarding the actual evolution of the structure.

2. Distribution of latescent plants

Literature data suggest the existence of 40 latescent families with at least one latex-containing representative (Lewinsohn 1991). However, this paper investigates the groups described as latescent in order to reassess the actual distribution of laticifers in families by means of anatomical and ontogenetic analysis, evaluating their typology and how they grow.

Our review found that laticifers are present in 22 orders of vascular plants (Fig 1), and they occur in at least one representative among 34 families. We have also refuted the existence of laticifers in 18 families described as latescent and found that, when a plant has laticifers, they are present in all its organs. Previous descriptions reporting the existence of laticifers in only a few plant organs (Lewinsohn 1991) are the result of the use of inadequate techniques to analyze this secretory structure and the fact that laticifers can be very thin, especially in very small organs or structures (Metcalfe 1967, Lewinsohn 1991). We have observed variations in the diameter of laticifers in all species analyzed in this study. Moreover, in *Sapium* we found laticifers of different diameters making up two independent secretory systems (Demarco *et al.* 2013). Laticifers are absent only in ovules (Demarco *et al.* 2006; Demarco 2017) and, consequently, in seed coats (pers. obs.). Not often, they may also be absent from pollination-related floral well, such as in the *Ficus style* (Marinho and Teixeira 2019).

Below is our review of the distribution of laticifers, their origin, and their classification into the various groups of vascular plants according to the current family circumscription.

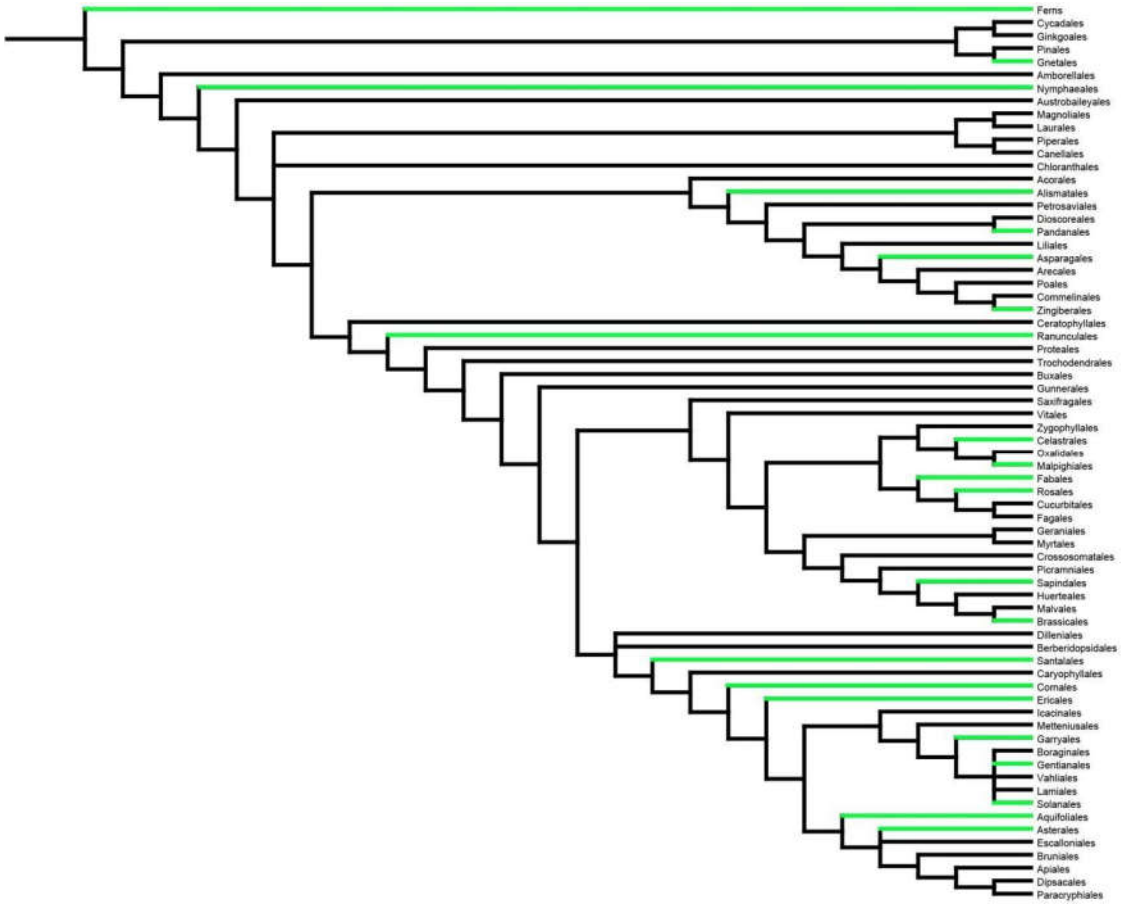


Fig 1: Phylogenetic cladogram of tracheophytes based on Chase *et al.* (2016) indicating the presence of laticifers (green).

Salviniales

The order Salviniales is composed of two families of ferns, Marsileaceae and Salviniaceae (Smith *et al.* 2006); both families are aquatic and are found in tropical and temperate habitats (Nagalingum *et al.* 2008). Laticifers were recorded only in Marsileaceae (Fig 2), and our analysis confirmed their absence in Salviniaceae.

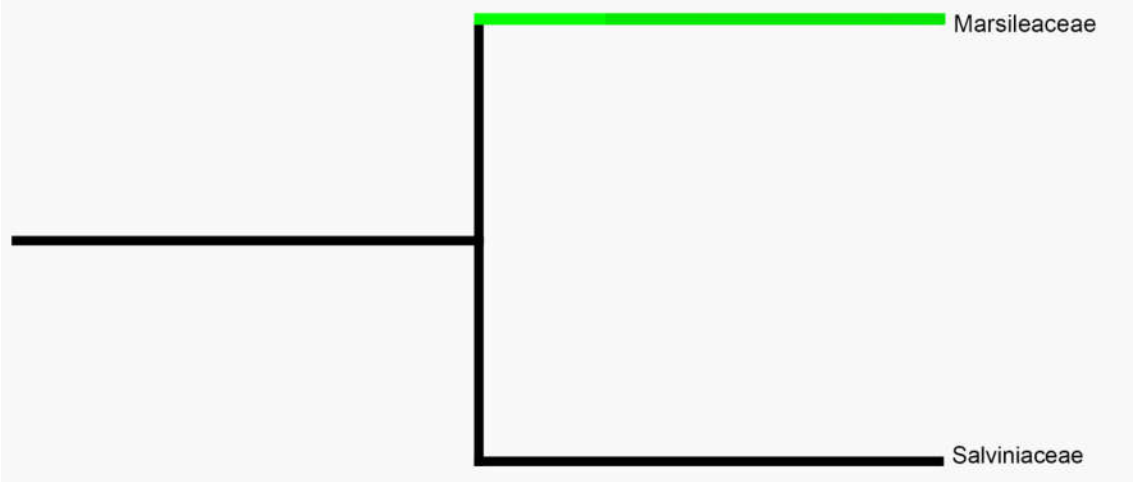


Fig 2: Phylogenetic cladogram of Salviniales based on Smith *et al.* (2006), indicating the presence of laticifers (green) in Marsileaceae.

Marsileaceae

The Marsileaceae is composed of three genera – *Marsilea*, *Pilularia* and *Regnellidium* –, the latter of which is monotypic (Nagalingum *et al.* 2008). Laticifers had been registered only in *Regnellidium*, (Laboriau 1952, Kramer 1990), and there was no record for the other family genera or Salviniaceae.

In our study, we found that *Pilularia* and *Marsilea* have non-anastomosed articulated laticifers (Fig 3 a), measuring about 30 µm in diameter, being a synapomorphy of the family. These genera vary only relation to the location of laticifers. In *Marsilea* laticifers are distributed throughout the cortex (Fig 3 b), while in *Pilularia* they are present in the cortex only in the perivascular space and under the epidermis.

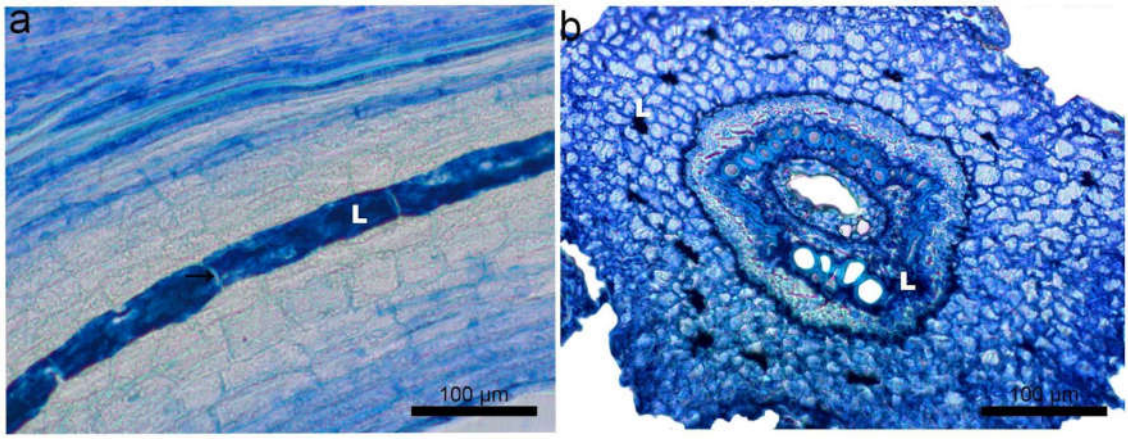


Fig 3: Anastomosed articulated laticifers in Marsileaceae (Salviniales); *Marsilea* sp. a. Longitudinal section indicating terminal wall (arrow) of the laticifer (L); b. Cross section indicating the distribution of laticifers (L).

Gnetales

Gnetales is one of the four orders that make up the "gymnosperms"; it is composed of three families (Doyle 1996), of which only Gnetaceae has laticifers (Fig 4) (Behnke and Herrmann 1978, Tomlinson 2003). This family is different from the others because it is composed of lianescent species, grouped in the genus Gnetum, which occurs in pantropical habitat (Yang *et al.* 2017).

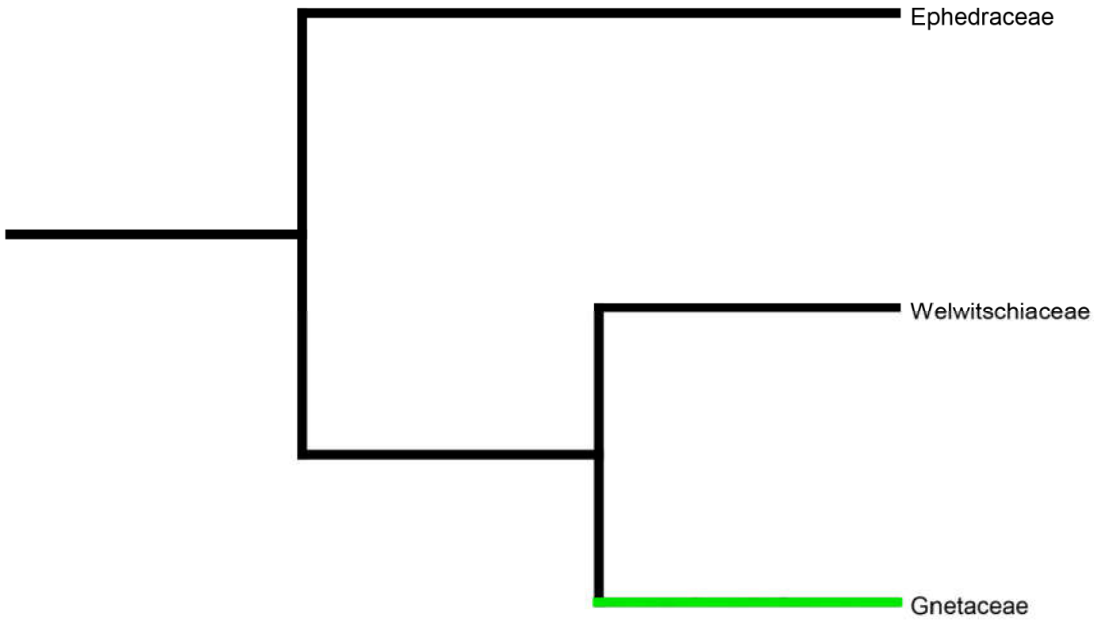


Fig 4: Phylogenetic cladogram of Gnetales based on Doyle (1996) indicating the presence of laticifers (green) in Gnetaceae.

Gnetaceae

Gnetum is similar in its vegetative structure when compared to angiosperms (Hansen *et al*. 1999, Haloi and Barua 2015). Biswas and Johri (1997) found that *Gnetum* shares up to 60% of its characteristics with angiosperms and the presence of laticifers is one of them. Laticifers present in their representatives are easily recognizable by their primary walls and uniformly granular content of lipid nature (Behnke and Herrmann 1978, Tomlinson 2003). Although laticifers of the genus have been classified as anastomosed articulated laticifers (Behnke and Herrmann 1978), in our analyses we found the presence of branched non-anastomosed articulated laticifers (Fig 5a), with a diameter from 20 to 40 µm, and *Gnetum nodiflorum* distributed in the cortex, medulla and vascular system (Fig 5 b).

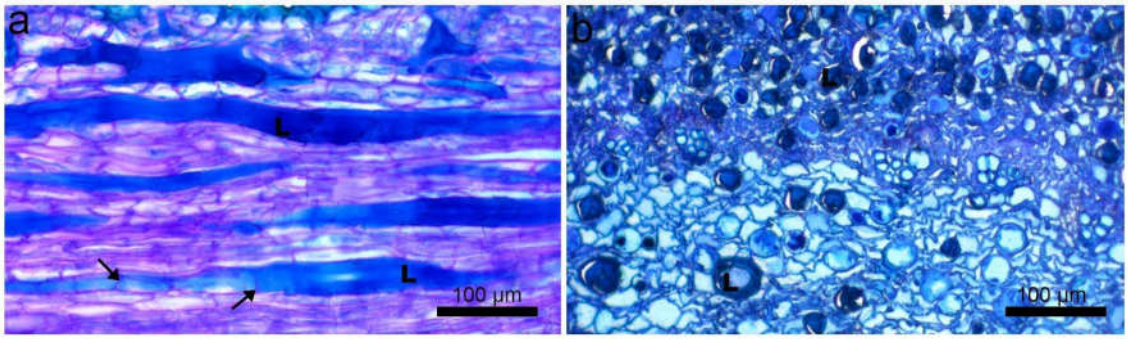


Fig 5: Anastomosed articulated laticifers in Gnetaceae (gnetales); *Gnetum nodiflorum*: a. Longitudinal section indicating terminal wall (arrow) of Laticifer (L); b. Cross section indicating laticifers distribution (L).

Nymphaeales

The Nymphaeales are composed of aquatic herbs of cosmopolitan distribution that correspond to three families: Cabombaceae, Nymphaeaceae and Hydatellaceae

(Borsch *et al.* 2008, Rudall *et al.* 2008). Laticifers appeared only once in the order and were identified in the Nymphaeaceae + Cabombaceae clade (Fig 6) and were absent in the basal family, Hydatellaceae.

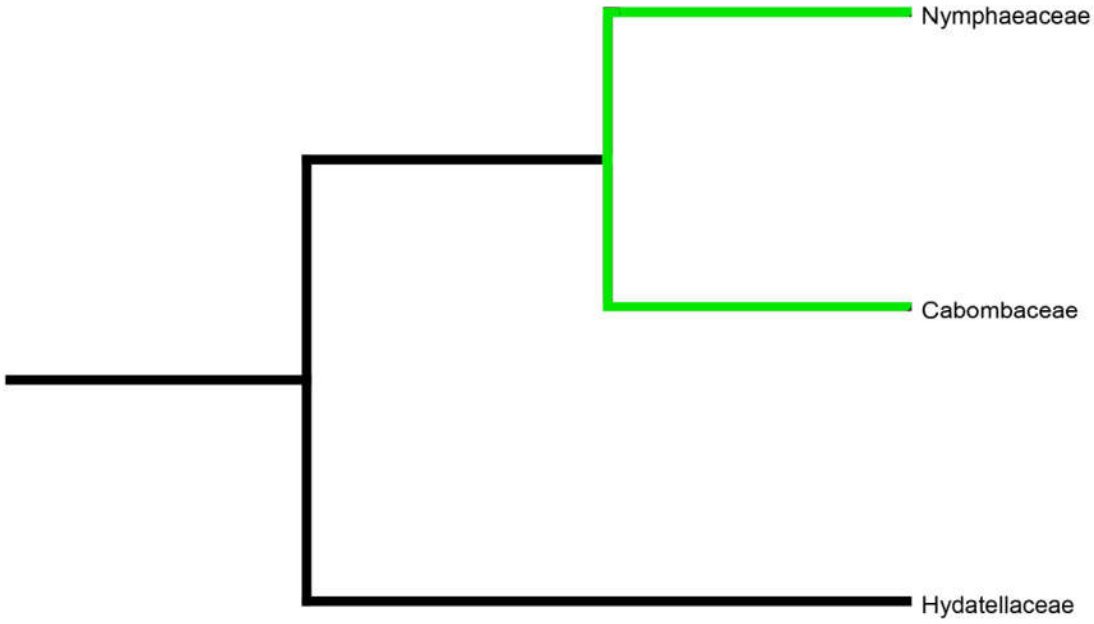


Fig 6: Phylogenetic cladogram of Nymphaeales based on Borsch *et al.* (2008), indicating the presence of laticifers (green) in Nymphaeaceae and Cabombaceae.

Nymphaeaceae

Partially anastomosed articulated laticifers are present in *Nymphaeaceae*; they have already been recorded throughout the plant body of the *Nymphaea* and *Victoria* species (Metcalfe and Chalk 1950, Moseley 1961, Metcalfe 1967, present study), and are usually associated with vascular bundles (Schneider 1976 Fig 7 ab). In the leaf blade of *Nymphaea* sp., we have noticed the presence of laticifers only associated to the larger bundles, while they are indistinctly associated to the various bundles in the cauline portion. Laticifers range in size from 17 µm to 30 µm.

Cabombaceae

In Cabombaceae, a family with two genera (Williamson and Schneider 1993), we confirm the presence of laticifers in *Brasenia*, with a diameter similar to that of *Nymphaea*, which are the articulated type found in the cortex and the phloem (Fig 7 e). However, unlike previous reports made by Metcalfe and Chalk (1950), we refute the occurrence of laticifers in *Cabomba*. Only phenolic idioblasts were found in *Cabomba aquatica* and *C. furcata*. Thus, laticifers are apparently restricted to *Brasenia*.

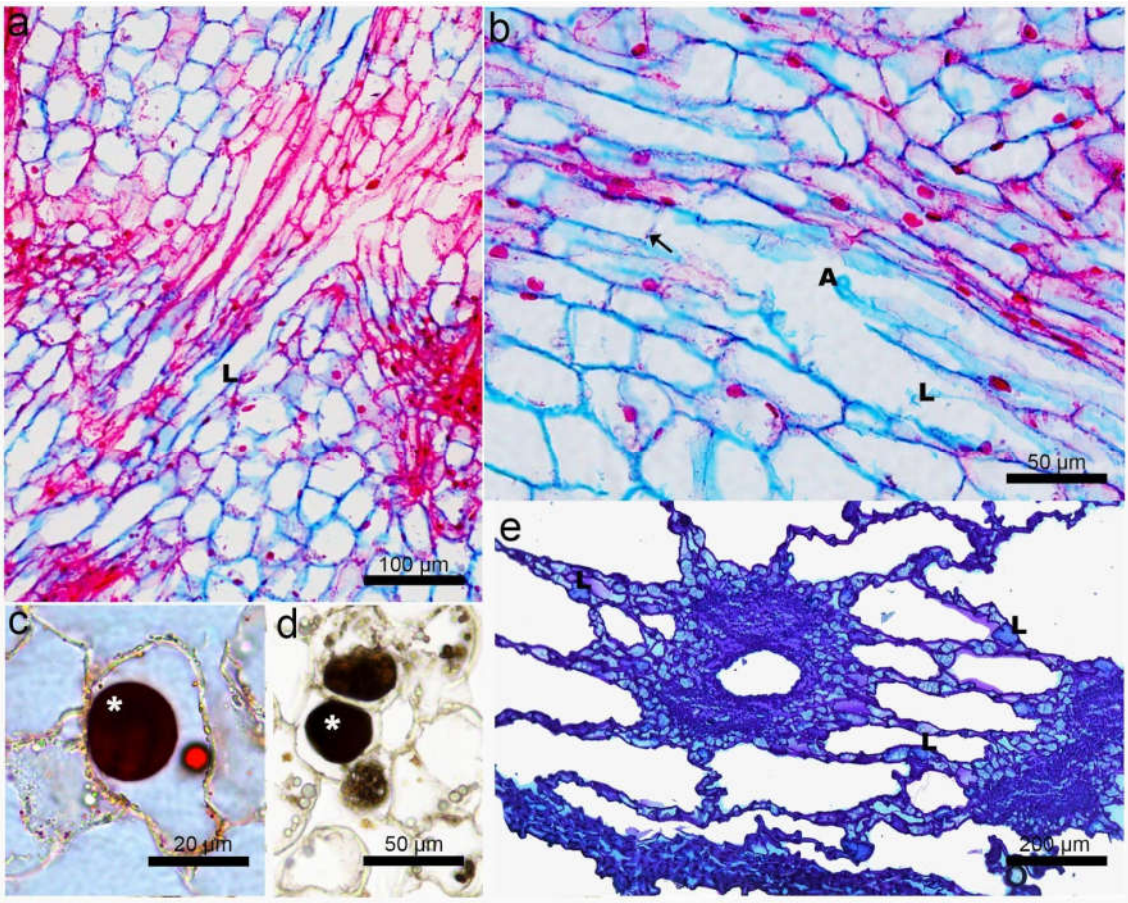


Fig 7: Anastomosed articulated laticifers in Nymphaeales; Nymphaeaceae – *Nymphaea* sp.: a. b. Longitudinal section indicating terminal wall and wall anastomosis of laticifer; cross section: c. Sudan IV test indicating presence of latex lipids; d. Ferric chloride test indicating presence of phenolic compounds in latex; *Cabombaceae – Brasenia* sp.: e. Cross section indicating laticifers distribution; (L – Laticifer; arrow – terminal wall; A – Wall anastomosis; asterisk – Latex).

Alismatales

The Alismatales is composed of 14 families (Ross *et al.* 2016) and previous studies indicate the presence of laticifers in Araceae, Alismataceae, Aponogetonaceae and Juncaginaceae (Fig 8) (Lewinsohn 1991, von Mering and Kadereit 2010). Of these four families, our study refuted the occurrence of latex in Alismataceae. As suggested by Farrell *et al.* (1991), phylogenetic analysis indicates the independent origin of laticifers in the different families of the order.

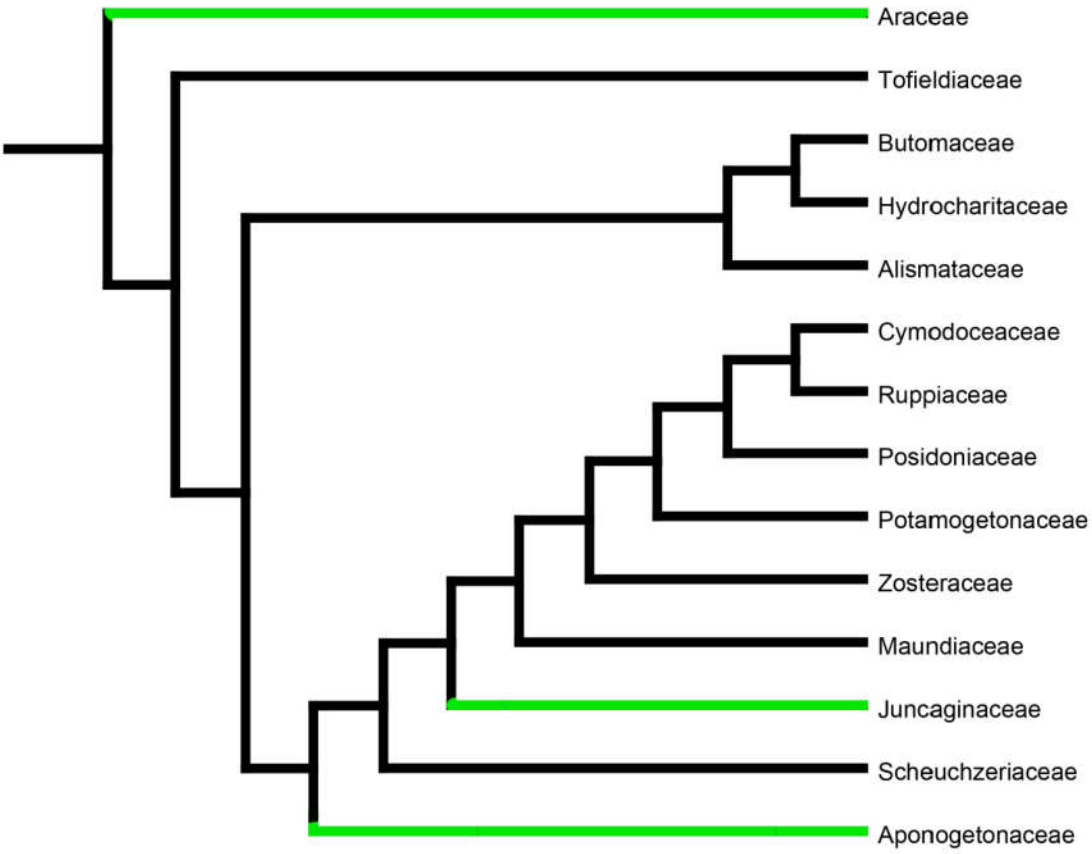


Fig 8: Phylogenetic cladogram of Alismatales based on Rose *et al*. (2016), indicating the presence of laticifers (green) in Araceae, Juncaginaceae and Aponogetonaceae.

Araceae

The Araceae has about 107 genera divided into eight subfamilies; although the family occurs in all continents, they predominate in the tropical habitat (Grayum 1990). Articulated laticifers are present only in the subfamily Aroideae, occurring in the vascular bundles in *Phillodendron*, *Amorphophallus* with diameters from 14 to 28 µm (Fig 9 a-d) (French 1988) and *Dieffenbachia* (Ferreira *et al*. 2006). In our analyses, we observed that *Amorphophallus* has non-anastomosed articulated laticifers in the xylem (Fig a b), as well as phenolic idioblasts. In *Phillodendron*, laticifers are also non-anastomosed articulated (Fig 9 c), but are found in the phloem (Fig 9 d) and also have phenolic idioblasts in their vascular system (Fig 9 d) and resin ducts in the parenchyma.

Juncaginaceae

Juncaginaceae is composed of three genera of cosmopolitan distribution and center of diversity in Australia (von Mering and Kadereit 2010). The circumscription of some genera is undefined. Some works have included *Lilaea* in *Triglochin* due to the sharing of floral characteristics (Lieu 1979, Furness and Banks 2010, Mering and Kadereit 2010, von Mering 2013) and there are reports of laticifers in both *Lilaea* (Thieret 1988) and *Triglochin* (von Mering 2013). However, in our studies, we found the presence of anastomosed articulated laticifers with a diameter ranging from 14 to 28 µm in the vascular system in *Triglochin striatum* (Fig 9 e), but their absence in *Lilaea scilloides,* which has only phenolic idioblasts in its cortex. A similar fact was observed in *Cardiospermum* (Sapindaceae) and the presence of laticifers only in some of the species of the genus corroborated the polyphyly of the genus (Montes 2017), as indicated by the phylogenetic study (Acevedo-Rodríguez *et al*. 2017). Thus, our data

suggest the maintenance of *Lilaea* and *Triglochin* as distinct genera and further studies are needed to re-evaluate the circumscription of both genera. We have also analyzed *Tetroncium*, which has no secretory cells. Thus, Juncaginaceae laticifers appear to be restricted to *Triglochin*.

Aponogetonaceae

Aponogetonaceae is composed of a single genus, *Aponogeton*, with 50 species and tropical and subtropical habitat (Les *et al*. 2005). Anastomosed articulated laticifers (Lewinsohn 1991, Farrell *et al*. 1991, von Mering 2013) were recorded in this family, measuring about 10 µm in diameter and occurring exclusively in the vascular system (Fig 9f). As observed in other families, the species has, besides the laticifers, phenolic idioblasts.

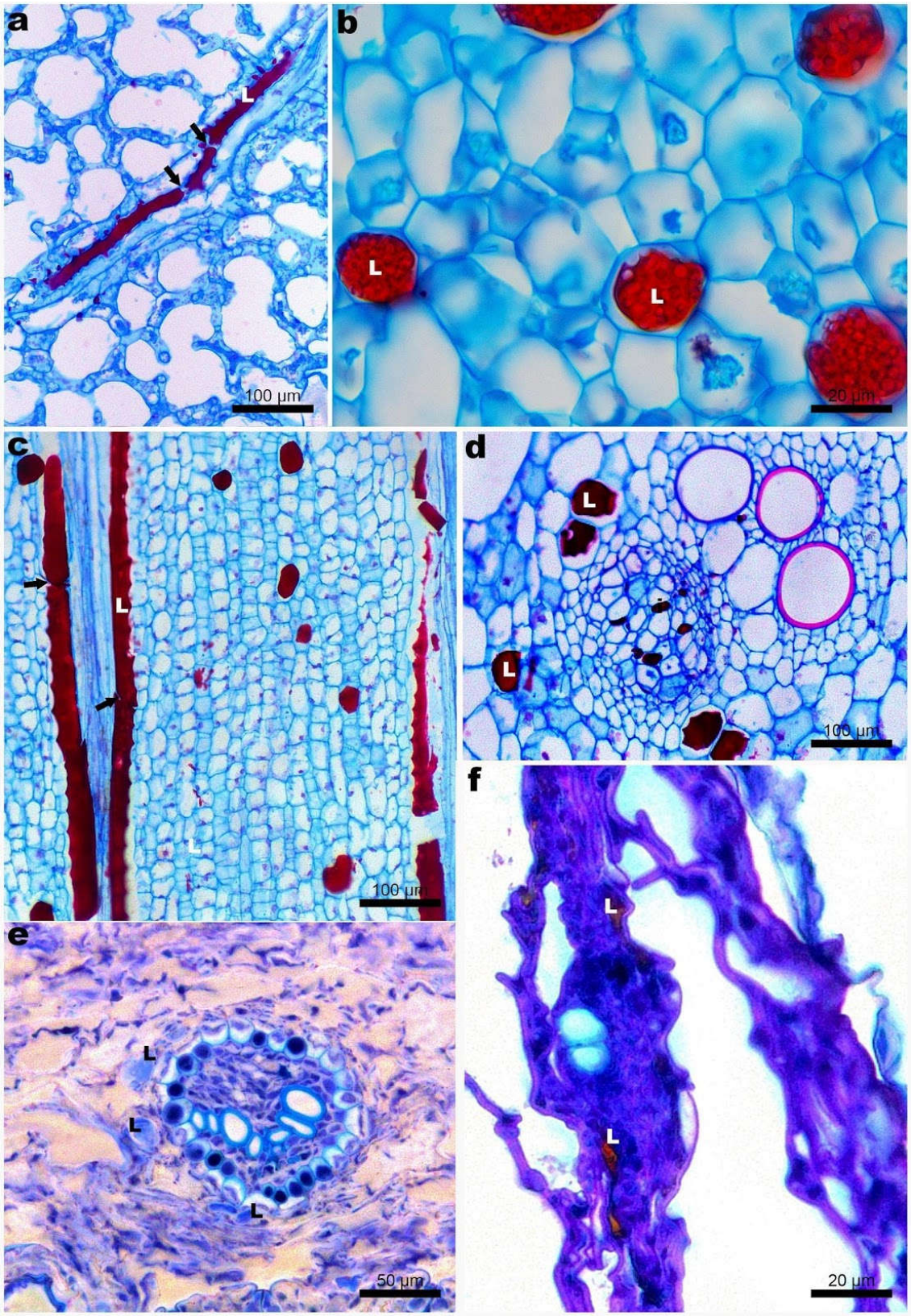


Fig 9: Anastomosed articulated laticifers in Alismatales; Araceae – *Amorphophallus* sp.: a. Longitudinal section indicating terminal wall of laticifer; b. Cross section indicating laticifers distribution; Araceae – *Phillodendron* sp.: c. Longitudinal section indicating terminal wall of laticifer; d. Cross section indicating

laticifers distribution; Juncaginaceae – *Triglochin striatum*: e. Cross section indicating laticifers distribution; Aponogetonaceae – ***Aponogeton elongatus***: f. Longitudinal section indicating laticifers distribution; (L – Laticifer; arrow – terminal wall; i – idioblast).

Pandanales

Pandanales has five families with preference for tropical and subtropical habitats (Rudall and Bateman 2006), with the occurrence of laticifers only in Cyclanthaceae (Fig 10) (Leal 2019, current study).

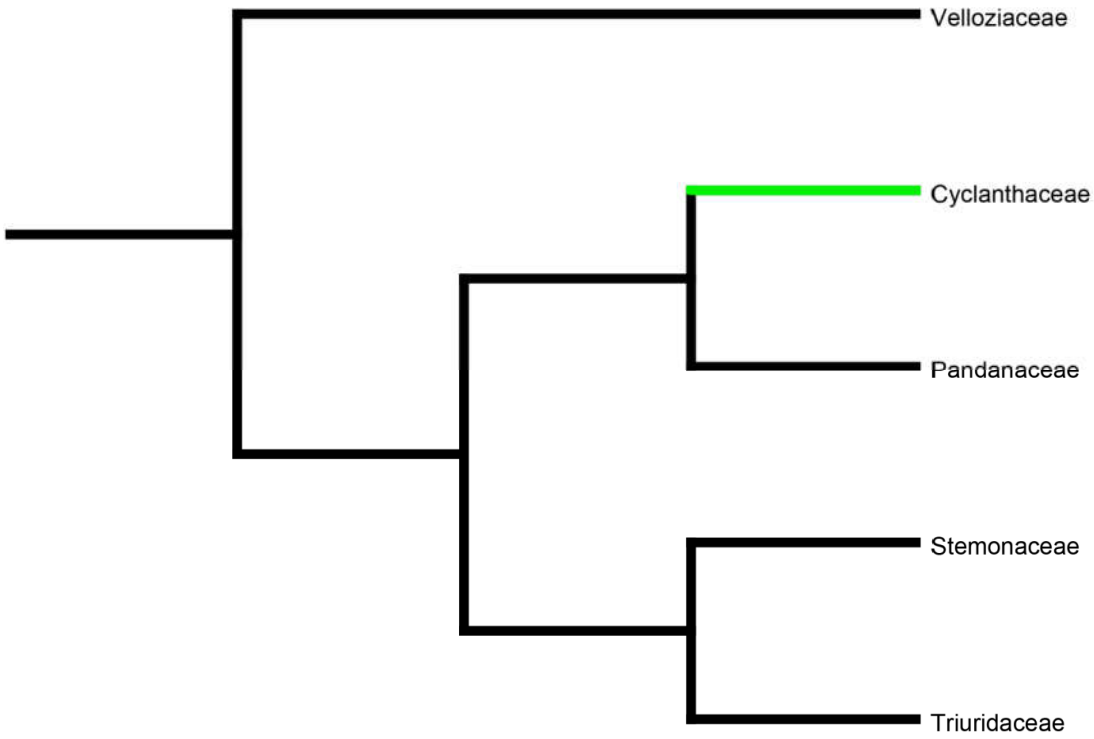


Fig 10: Phylogenetic cladogram of Pandanales based on Rudall Bateman (2006), indicating the presence of laticifers (green) in Cyclanthaceae.

Cyclanthaceae

The laticifers of Cyclanthaceae are present only in the Cyclanthoideae subfamily, which is composed of one single genus, *Cyclanthus*, with two species of tropical habitat in the South and Central America (Leal 2019). Laticifers of this genus

are classified as non-articulated (Wilder and Harris 1982, Harling *et al.* 1998), but our analyses revealed that *Cyclanthus* has anastomosed articulated laticifers (Fig 11 a b), measuring from 30 µm to 43 µm in diameter and with cortical and vascular distribution.

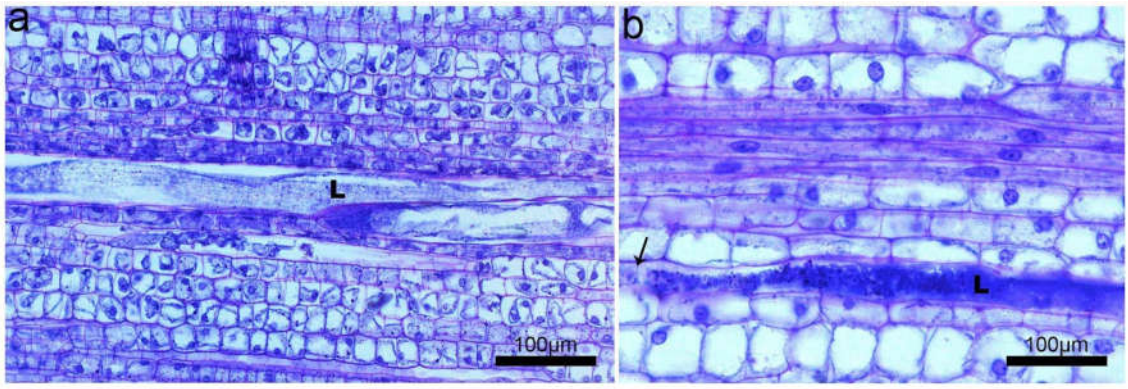


Fig 11: Anastomosed articulated laticifers in Cyclanthaceae (Pandanales) – longitudinal section of *Cyclanthus bipartidus*; a. Indicating unbranched laticifers; b. Indicating remnant of the laticifer's terminal wall; (L –Laticifer; arrow – terminal wall remnant).

Asparagales

The Asparagales has 14 families (Chen *et al.* 2016, Li *et al.* 2019) and 1122 genera, and laticifers were described only in Amaryllidaceae (Fig 12) (Pellicer *et al.* 2017).

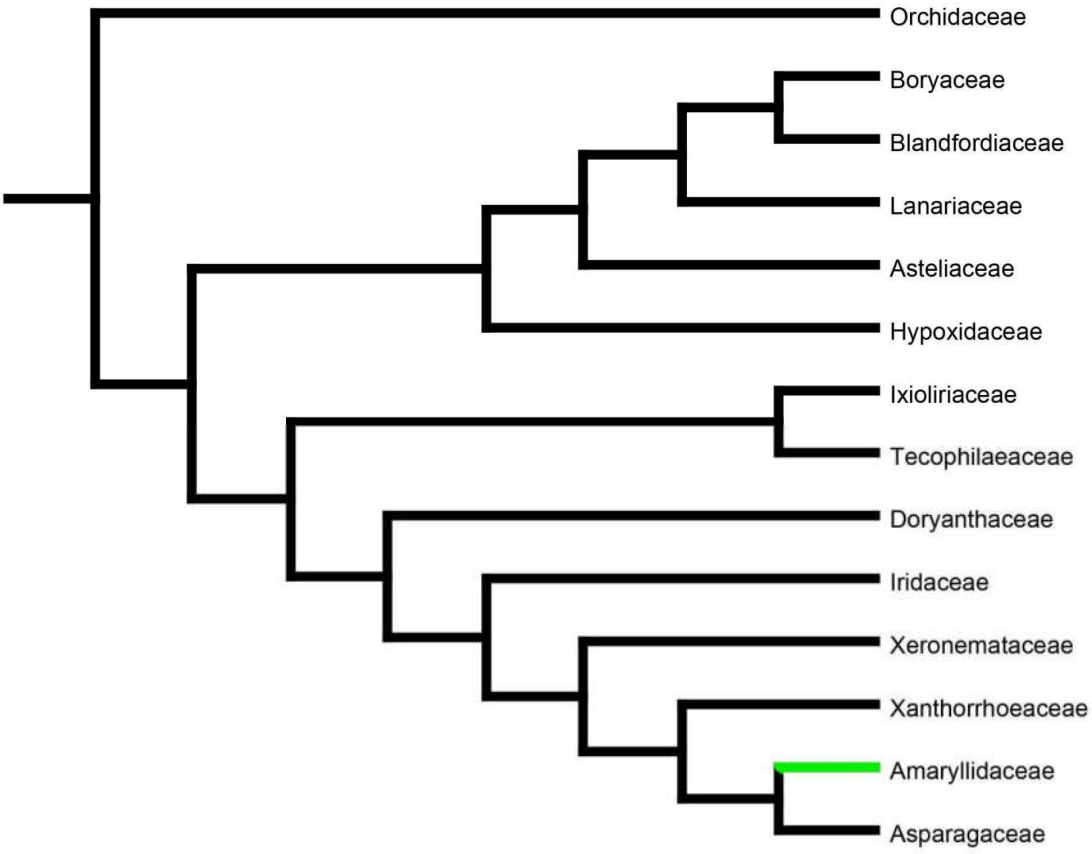


Fig 12: Phylogenetic cladogram of Asparagales based on Li *et al.* (2019), indicating the presence of laticifers (green) in Amaryllidaceae.

Amaryllidaceae

The Amaryllidaceae is composed of 73 genera with cosmopolitan distribution, divided into three subfamilies: Agapanthoideae, Allioideae and Amaryllioideae (Pellicer *et al.* 2017).

Allioideae

There were Laticifers recorded only for the subfamily Allioideae, which is composed of three tribes: Allieae, Gilliesieae and Tulbaghieae (Chase *et al.* 2009, Pellicer *et al.* 2017).

Allieae

The tribe is composed of 14 genera (Pellicer *et al.* 2017), most notably *Allium,* which has seven subgenera and 30 sections (Khorasani *et al.* 2018) and has non-anastomosed articulated laticifers (Fig 13a). Our analysis confirmed the presence of callose on the side (Fig 13b) and terminal walls of the laticifers (Currier 1957, Huang and Sterling 1970) of the *Cepa* section.

The position and structure of laticifers are useful features for the differentiation and delimitation of the subgenera *Allium* and *Amerallium* (Huang and Sterling 1970). In these genera, subepidermal laticifers correspond to the ancestral character, while the intraparenchymal position corresponds to the apomorphic state (Mashayekhi and Columbus 2014). In this study, we observed the laticifers of *A. cepa* and *A. schoenoprasum* distributed in the cortex, especially in the perivascular region, reaching a diameter of up to 35 μm (Fig 13 c).

Gilliesieae

Previous studies differ regarding the presence of laticifers in Gilliesieae. Hegnauer (1963) recorded the occurrence of latex in *Brodiaea* and *Mila*; however, Preston (2013) and Gutiérrez *et al.* (2015) did not observe laticifers in the anatomical analysis of these genera. In this study, we analyzed samples of *Tristagma*, which has only phenolic idioblasts in the cortex, and of *Nothoscordum*, which has no secretory cells.

Agapanthoideae

As in Gilliesieae, Hegnauer (1963) reports laticifers in Agapanthoideae, whose only genus *Agapanthus* is composed of nine species (Kubitzk 1998). However, our analysis of *Agapanthus africanus* did not detect any secretory cells.

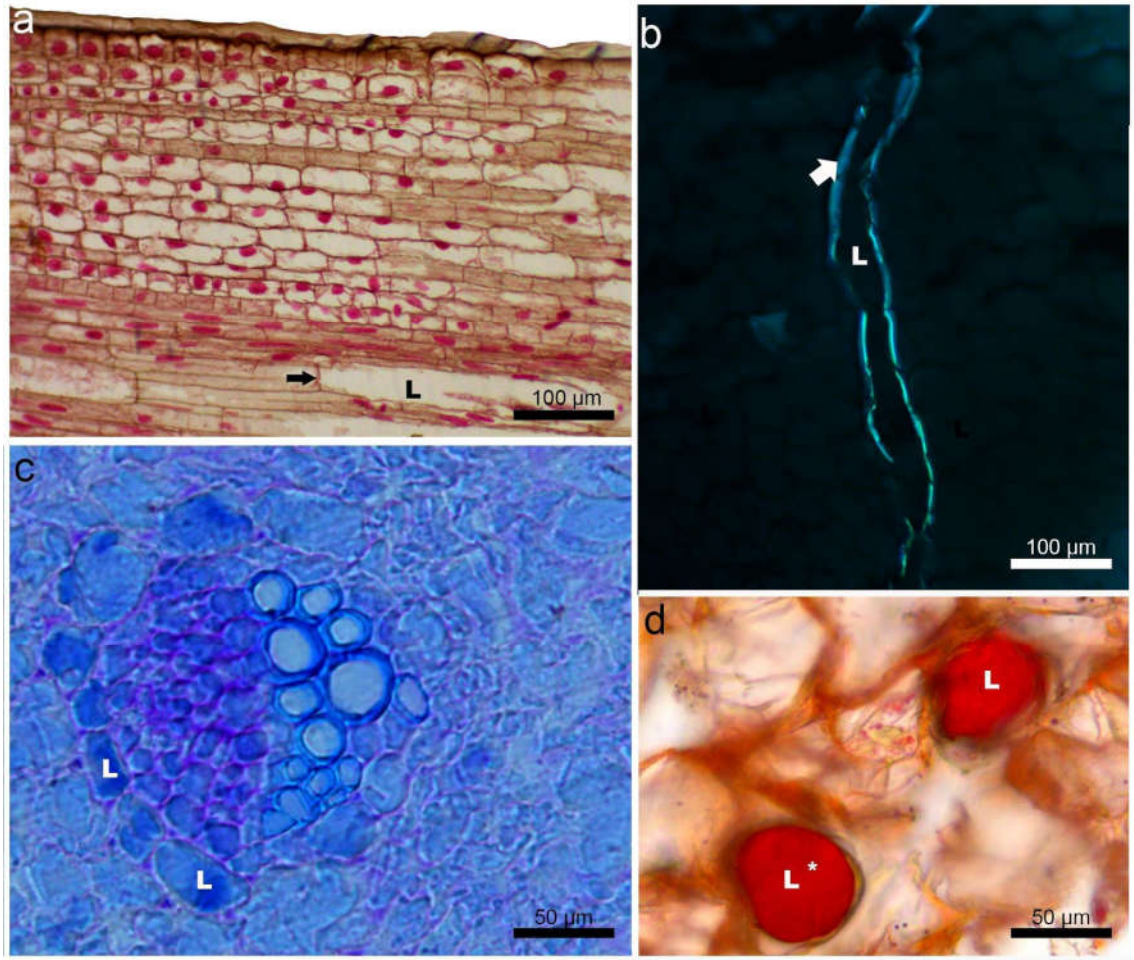


Fig 13: Non-anastomosed articulated laticifers in Amaryllidaceae (Asparagales) – *Allium cepa*: a. Longitudinal section indicating terminal wall of laticifers; b. Blue aniline test in longitudinal section indicating laticiferous wall callose; c. Cross section indicating laticifers distribution; d. Sudan IV cross-sectional test indicating the presence of lipids in the latex; (L – Laticifer; arrow – terminal wall; thick arrow – callose on laticifer wall; asterisk – latex).

Zingiberales

The Zingiberales has eight families (Kress *et al.* 2001, Givnish *et al.* 2018) and laticifers appeared only once in Musaceae (Fig 14) (Fahn 1979, Farrell *et al.* 1991, Lewinsohn 1991, Sumardi and Wulandari 2010).

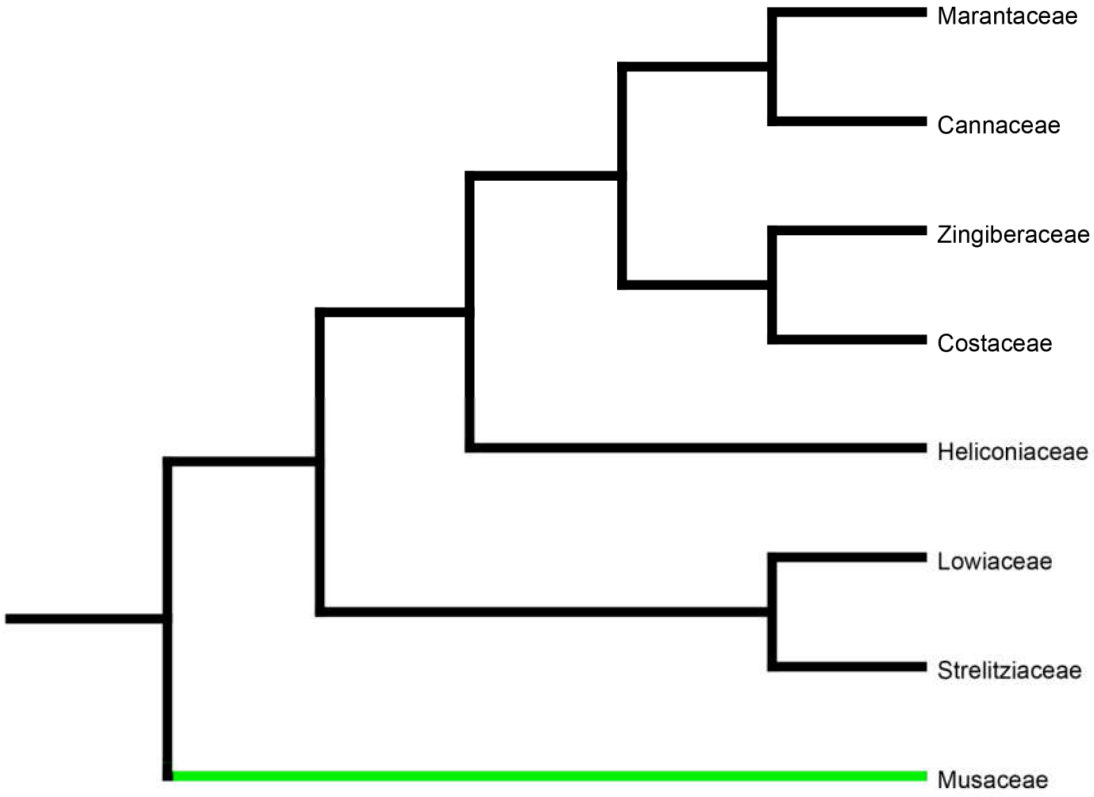


Fig 14: Phylogenetic cladogram of Zingiberales based on Givnish *et al.* (2018), indicating the presence of laticifers (green) in Musaceae.

Musaceae

In the phylogenetic analysis of Zingiberales by Andersson (1998c), the Musaceae presented a basal position due to, among other factors, the presence of laticifers, a synapomorphic characteristic of the family. The family is composed of two genera with 40 native herbaceous species from Africa, the Himalayas, Southeast Asia and Northern Australia (Andersson 1998c).

The laticifers in this family are non-anastomosed articulated (Fig 15 a) and in *Musa paradisiaca* they are distributed throughout the cortex and in the phloem measuring up to 30 µm in diameter (Fig 15 a b), and only one laticifer per vascular bundle was observed in the genus . (Fig 15 a b).

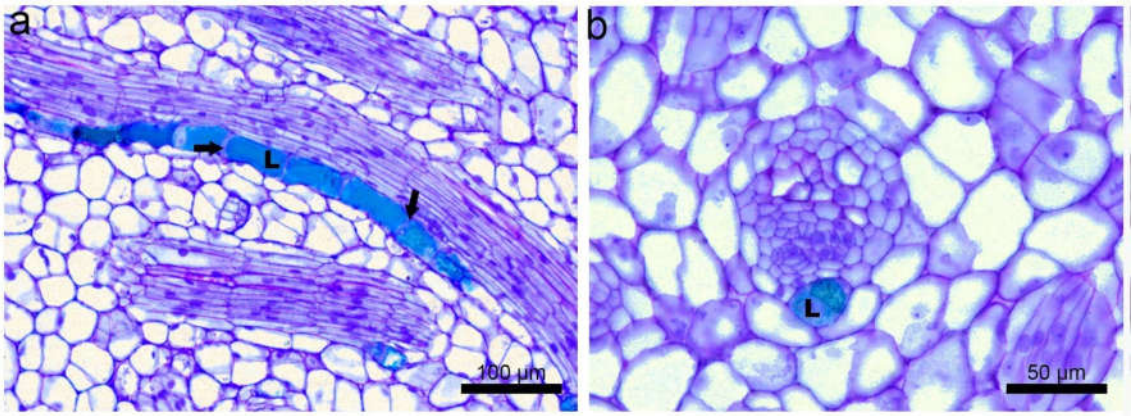


Fig 15: Non-anastomosed articulated laticifers in Musaceae (Zingiberales) – *Musa paradisiaca*: a. Longitudinal section indicating terminal wall in laticifers; b. Cross section indicating laticifers distribution; (L – Laticifer; arrow – terminal wall).

Ranunculales

The Ranunculales has seven families with 199 genera (Wang *et al.* 2009) and the phylogenetic relationship between families is still poorly understood. Papaveraceae is the only one of the order with laticifers (Fig 16) (Hoot *et al.* 2015).

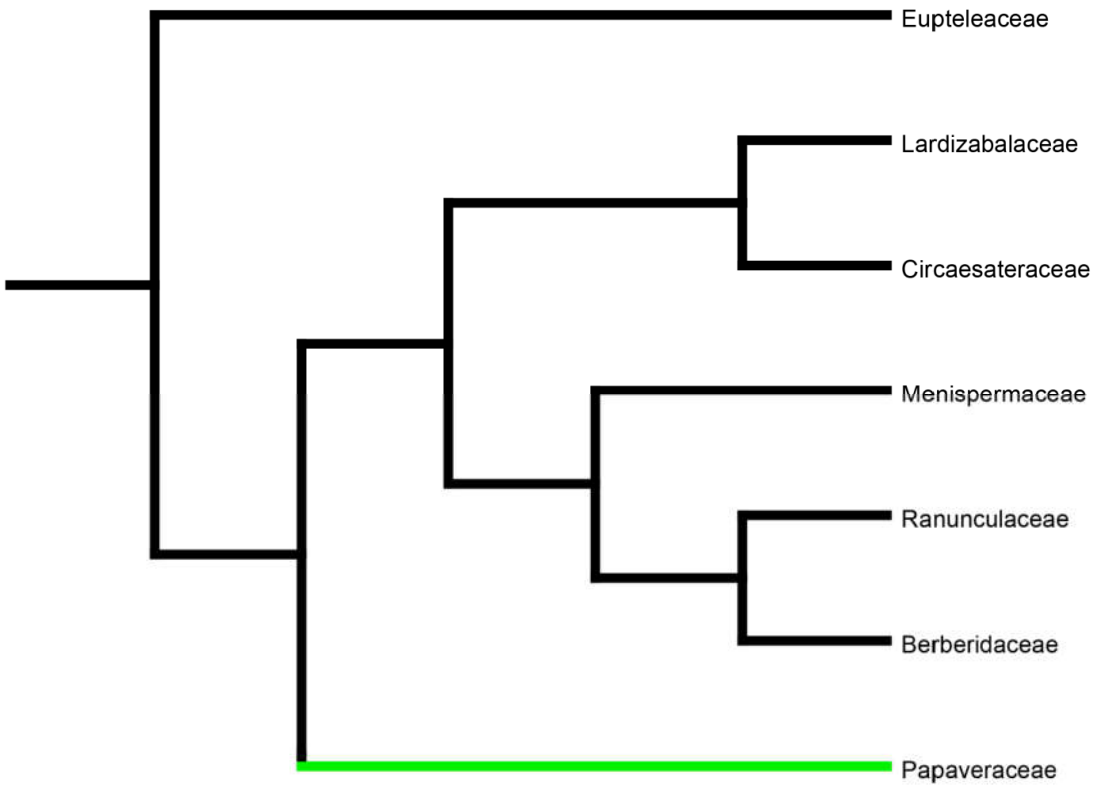


Fig 16: Phylogenetic cladogram of Ranunculales based on Wang *et al*. (2009), indicating the presence of laticifers (green) in Papaveraceae.

Papaveraceae

The Papaveraceae is divided into four subfamilies and only Pteridophylloideae do not have laticifers. In the rest of the family, laticifers are anastomosed articulated (Fig 17 a), having been extensively studied in *Papaver* (Thureson-Klein 1970, Hoot *et al.* 2015) due to the high medicinal value of the alkaloids, morphine, codeine and papaverine present only in latex (Fairbairn and Kapoor 1960, Thureson-Klein 1970). Laticifers have different distribution depending on the genus. In *Papaver*, laticifers anastomose laterally, forming a branched system through the phloem (Fairbairn and Kapoor 1960, Thureson-Klein 1970).

The distribution and origin of laticifers varies in the family, but the diameter found is similar – between 16 and 20 µm. In *Sanguinaria*, laticifers are present in both the cortex and the phloem (Fig 17 b), while in *Chelidonium* (Chelidonieae) laticifers occur only in the vascular system (Fig 17 c), and in *Argemone* (Papavereae), laticifers are composed of short rows of narrow and elongated cells, distributed in the perivascular region (Fig 17 d). This distinct distribution in genera can be used for taxonomic purposes, as observed in *Sapium* (Euphorbiaceae; Demarco *et al.* 2013).

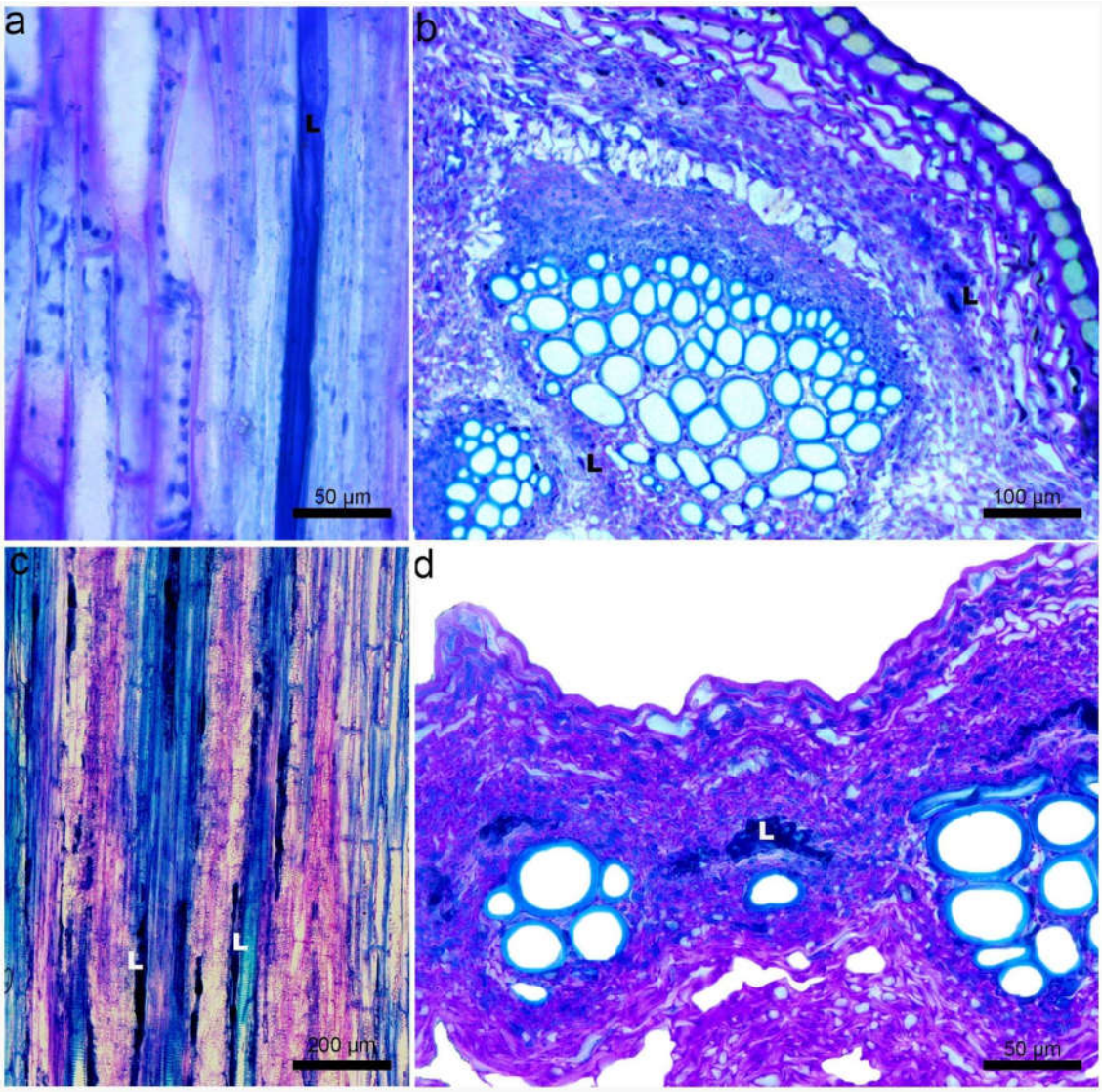


Fig 17: Anastomosed articulated laticifers in *Sanguinaria Canadensis of* Papaveraceae (Ranunculales)*:* a. Cross section indicating laticifers distribution; b. Longitudinal section indicating laticifer; (L – Laticifer)

Celastrales

Celastrales is composed of two families (Chase *et al.* 2016, Sun *et al.* 2016) with about 102 genera (Chinh *et al.* 2016) distributed in tropical and temperate regions (Simmons *et al.* 2001), where only Celastraceae presents laticiferous species (Fig 18) (Lewinsohn 1991).

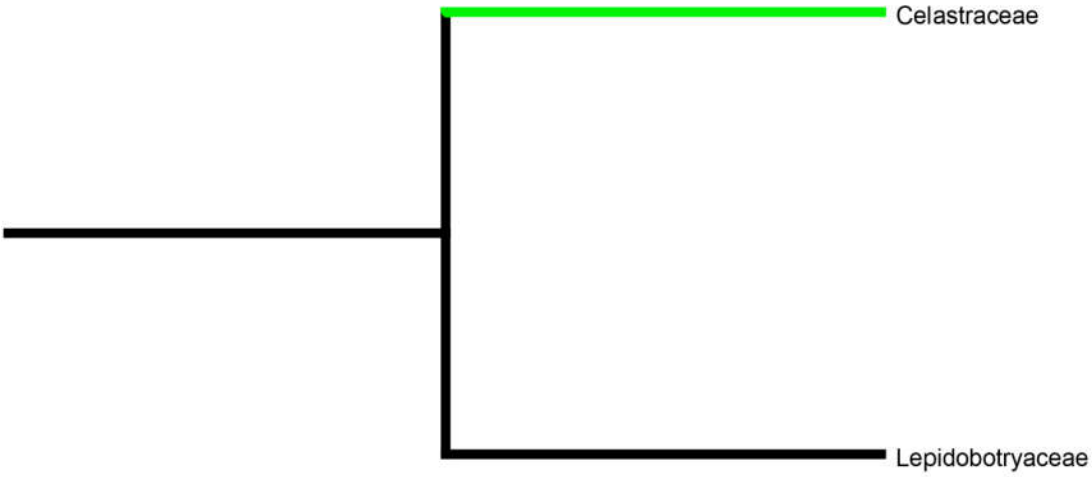


Fig 18: Phylogenetic cladogram of Celastrales based on Chase *et al*. (2016), indicating the presence of laticifers (green) in Celastraceae.

Celastraceae

Celastraceae is composed of herbaceous and liana plants, with predominantly tropical and temperate habitat (Simmons *et al.* 2001). According to the old circumscription, there were no laticifers in the family (Metcalfe and Chalk 1950, Metcalfe 1967). However, with the inclusion of some genera from the old Flacourtiaceae family, which were registered as laticiferous, such as *Salacia*, *Tontelea* and *Hippocratea* (Metcalfe 1967, Hall and Lock 1975, Gomes *et al*. 2005, Gomes *et al.* 2010, Mercadante- Simões 2014), Celastraceae became one of the families with latex. Laticifers have been described as non-articulated (Hall and Lock 1975, Mercadante-

Simões *et al*. 2014), with distribution in the cortex and secondary phloem (Lewinsohn 1991, Gomes *et al*. 2005). However, these data were not corroborated in our study.

Salacia crassifolia and *S. eliptica* do not have laticifers, but only idioblasts. On the other hand, *Tontelea miersii* and T. *micrantha* presented non-anastomosed articulated laticifers (Fig 19 a), with a diameter of 15 µm and distributed in the perivascular region (Fig 19 a b).

Eounymus sp. (subfamily Euonymoideae), was also analyzed for being referred to as a rubber source (Metcalfe 1967, Metcalfe and Chalk 1950), establishing a relationship with latex, since this compound is a product of laticifers (Prado and Demarco 2018). However, our analysis revealed that there are no laticifers in the genus, but only secretory idioblasts.

Hippocratea volubilis (subfamily Hippocrateoideae) was also analyzed and we did not find the presence of secretory cells, but only elongated fibers that are often confused with laticifers (Rudall 1987). Thus, the presence of laticifers was confirmed only in *Tontelea* in Celastraceae.

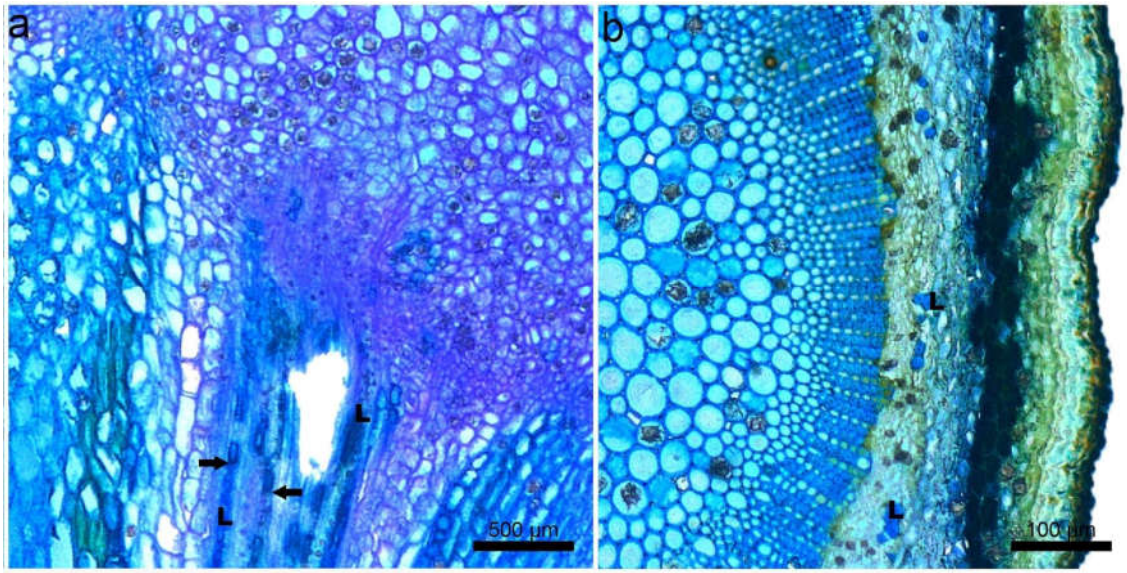


Fig 19: Non-anastomosed articulated laticifers in Celastraceae (Celastrales): *Tontelea micrantha* a. Longitudinal section indicating terminal wall of laticifers; B. Cross section indicating laticifers distribution; (L – Laticifer; arrow – terminal wall).

Malpighiales

Malpighiales is composed of 36 families (Chase *et al*. 2016) with 716 genera, with laticiferous genera in Euphorbiaceae and Malpighiaceae (Fig 20) (Rudall 1987, Lewinsohn 1991, Farrell *et al*. 1991, Mahlberg 1993), with independent emergence.

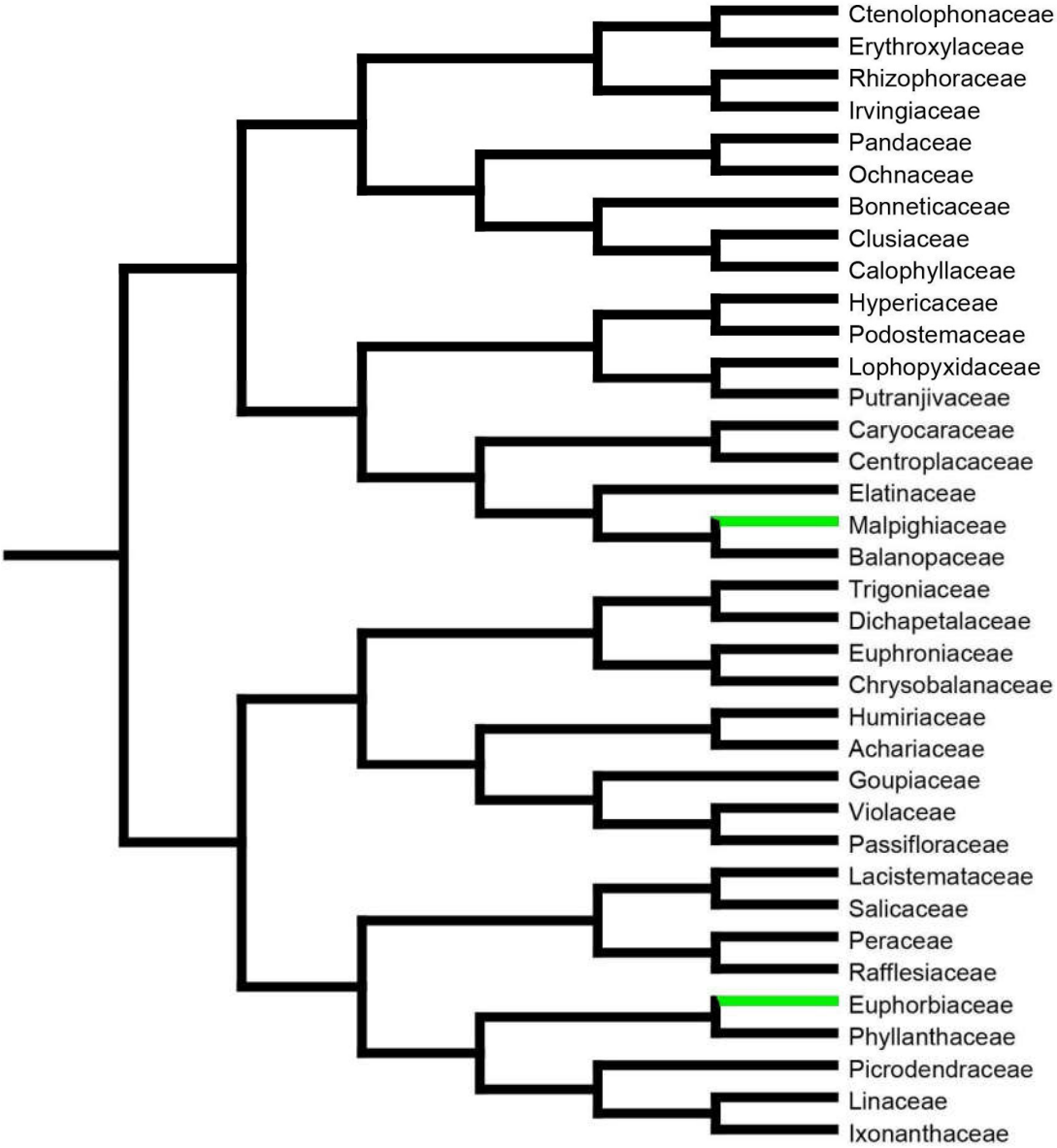


Fig 20: Phylogenetic cladogram of Malpighiales based on Chase *et al*. (2016), indicating the presence of laticifers (green) in Podostemaceae, Malpighiaceae and Euphorbiaceae.

Malpighiaceae

Malpighiaceae is composed of 77 genera with tropical and subtropical habitat (David and Anderson 2010) and laticifers are reported only for the Galphimieae tribe, consisting of four genera: *Galphimia*, *Lophanthera*, *Spachea* and *Verrucularia* (Vega *et al*. 2002, Davis and Anderson 2010); however, Pace *et al.* (2019) have confirmed the presence of laticifers in the secondary phoem and cortex of *Stigmaphyllum* and *Tetrapterys*. Although latex is described for the entire tribe, we have not observed laticifers in *Spachea*, but only phenolic idioblasts. Thus, we have confirmed the presence of laticifers only for *Lophantera*, with wide non-anastomosed articulated laticifers, verifying diameters of up to 45 µm distributed in the cortical and medullary region (Fig 21 a b), and in *Galphimia* and *Verrucularia*. We have also analyzed *Byrsonima*, a genus closely related to Galphimieae; however, the genus has only phenolic idioblasts.

Euphorbiaceae

Euphorbiaceae is composed of 218 genera divided into four subfamilies with pantropical and temperate habitat, where Acalyphoideae, Crotonoideae and Euphorbioideae had representatives registered as laticiferous (Rudall 1987). Articulated and non-articulated laticifers have already been recorded in different genera and the occurrence of both types in the same group was considered an indication of their polyphiletic origin (Metcalfe 1967, Mahlberg *et al*. 1987).

In Acalyphoideae, non-anastomosed articulated laticifers were recorded for *Acalypha*, *Clutia*, *Dalechampia*, *Dicoelia*, *Macaranga*, *Omphalea* and *Pera* (Rudall

1987, 1994, Hayden and Hayden 2000, Krishnamurthy *et al*. 2013). However, anatomical analyses have shown the absence of laticifers in *Acalypha* (present study), *Dalechampia* and *Pera* (Gagliardi *et al*. 2016), which have only secretory idioblasts and further studies are necessary to verify the possible occurrence of laticifers in the other above mentioned genera. According to Rudall (1994), in many genera of Euphorbiaceae, there is the presence of elongated and branched sclereids, which may cause misclassification. According to Metcalfe and Chalk (1950) and Rao and Das (1979), sclereids are common in many genera of Acalyphoideae.

Our study confirmed the presence of laticifers only for the two most derived subfamilies: Crotonoideae and Euphorbioideae, where their diameters range from 15 to 25 µm. In Crotonoideae, laticifers are present in almost all genera and have been considered as non-articulated branches (Rudall 1987, 1994). However, our ontogenetic analysis has shown that *Croton* laticifers are anastomosed articulated, as observed in previous studies by Vitarelli *et al*. (2015) and Gagliardi *et al*. (2016) on *Croton* and *Joannesia* species. Laticifer position may have phylogenetic importance in the subfamily, helping to elucidate genus relations (Mahlberg *et al*. 1987, Vitarelli *et al*. 2015).

Euphorbioideae presents laticifers in all genera (Rudall 1987), where both articulated and non-articulated types were recorded, highlighting those from Hevea recognized as anastomosed articulated and Euphorbia described as non-articulated (Mahlberg *et al*. 1987). However, recent studies show that Euphorbiaceae laticifers are all articulated (Fig 21 c-e) and that laticifers described as non-articulated are actually articulated laticifers whose anastomosis occurs very early between their apical cells (Demarco *et al*. 2013, Ramos *et al*. 2019). In our study, we have analyzed the model species of branched non-articulated laticifer, *Euphorbia milii*, and found that their

laticifers (Fig 21 c) are actually anastomosed articulates (see ontogenesis section). In general, family laticifers are present in the cortex, medulla and phloem (Fig 21 d).

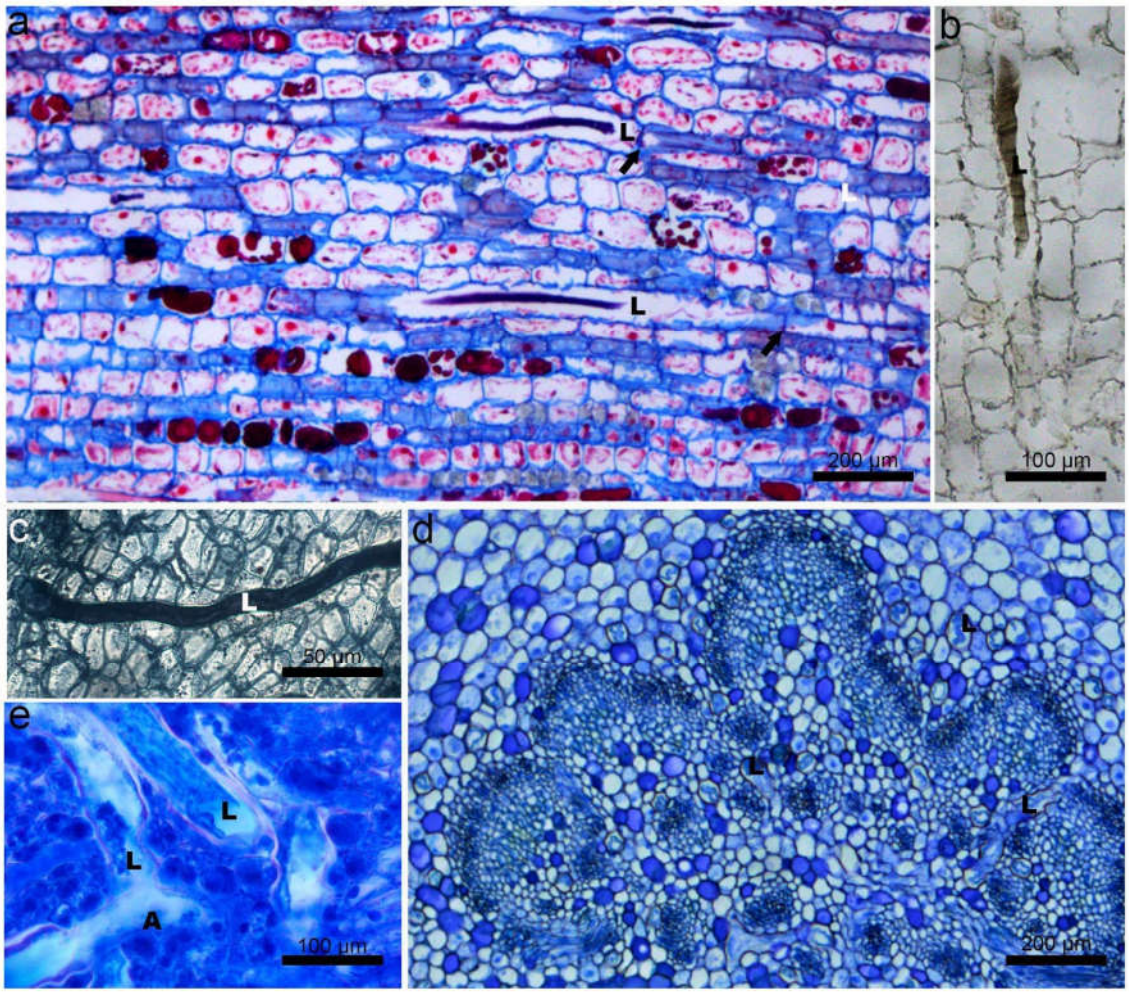


Fig 21: Articulated laticifers in Malpighiales; Malpighiaceae – *Lophantera* sp .: a. Longitudinal section indicating non-anastomosed articulated laticifers; b. Sudan black test – Longitudinal section – indicating latex lipid composition; *Euphorbia milii*: c. Sudan black test – Longitudinal section indicating latex lipid composition; d. Cross section indicating distribution of anastomosed articulated laticifers; Euphorbiaceae – *Hura crepitans*: e. Longitudinal section indicating anastomosed articulated laticifers; (L – Laticifer; arrow – terminal wall; A – Wall anastomosis).

Fabales

Fabales are composed of four families (Banks *et al*. 2008, Sun *et al*. 2016) with about 750 genera (Lewis *et al*. 2005) and there are records of laticifers only in some isolated Fabaceae taxa (Fig 22) (Lewinsohn 1991, Agrawal and Konno 2009).

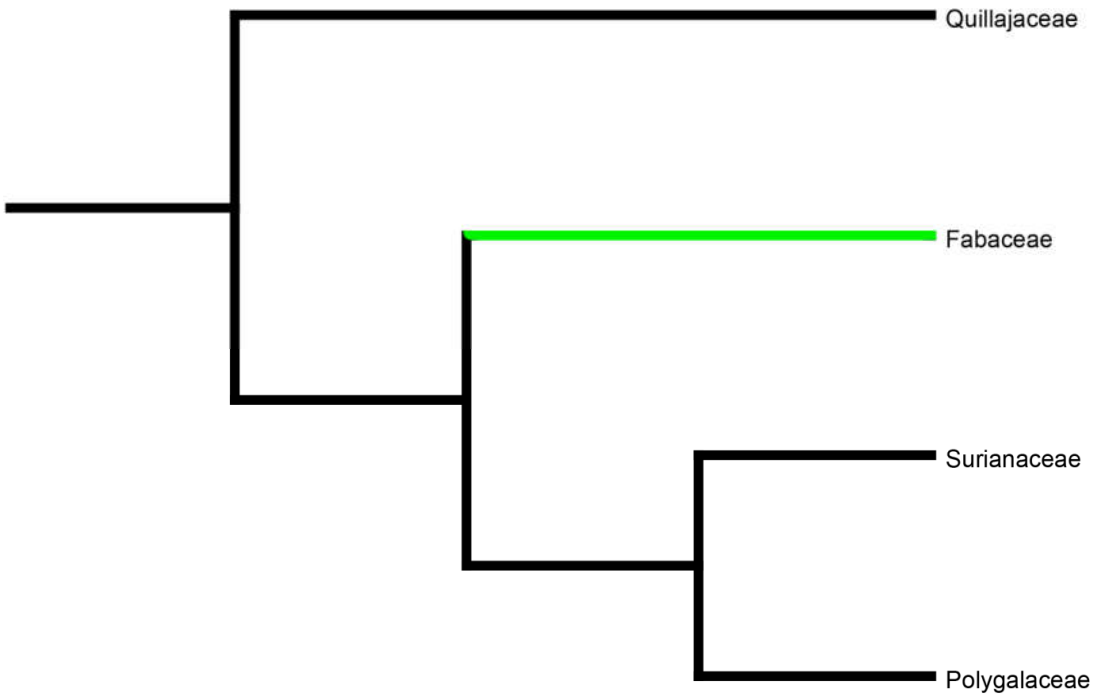


Fig 22: Phylogenetic cladogram of Fabales based on Sun *et al*. (2016), indicating the presence of laticifers (green) in Fabaceae.

Fabaceae

Fabaceae is considered to be the third largest family in number of species and its distribution covers all biomes (Koenen *et al*. 2013), being divided into six subfamilies: Duparquetioideae (comprising one monotypic genus), Cercidoideae (comprising 12 genera), Detarioideae (with 84 genera), Caesalpinioideae (includes in this subfamily the Mimosoid clade, totaling 148 genera) and Papilionoideae (comprising about 503 genera) (Lewis *et al*. 2005, Azani *et al*. 2017).

Papilionoideae

In the Millettieae tribe, laticifers are recorded in some species of Lonchocarpus (Dattolli *et al.* 2011, Silva *et al.* 2012), a neotropical genus formed of about 120 species

(Silva *et al.* 2010); in our analyses of *Lonchocarpus*, we have identified non-anastomosed articulated laticifers (Fig 23 a) with a diameter of 13 µm, distributed in both the fundamental system and the phloem (Fig 23 b), occurring together with phenolic idioblasts. In *Dahlstedtia*, a genus close to *Lonchocarpus* (Mattapha 2017), we also located articulated laticifers occurring in the medulla and the vascular system with diameters of 17 µm (Fig 23 c d). In addition, *Dahlstedtia araripensis* also has secretory idioblasts and oil cavities (Fig 23 c).

In the Phaseoleae tribe, we analyzed *Erythrina speciosa*, which has no laticifers, but only rows of phenolic idioblasts.

Caesalpinioideae

This subfamily has significant floristic importance in the caatinga (Dourado 2013), especially the genus *Mimosa* with about 540 predominantly neotropical habitat species (Marchiori 1996, Koenen *et al.* 2013). In this genus, latex is recorded for *Mimosa laticifera* (Lewinsohn 1991), in which we observed anastomosed articulated laticifers, 14 µm in diameter, present in the phloem and medulla, along with phenolic idioblasts (Fig 23 e).

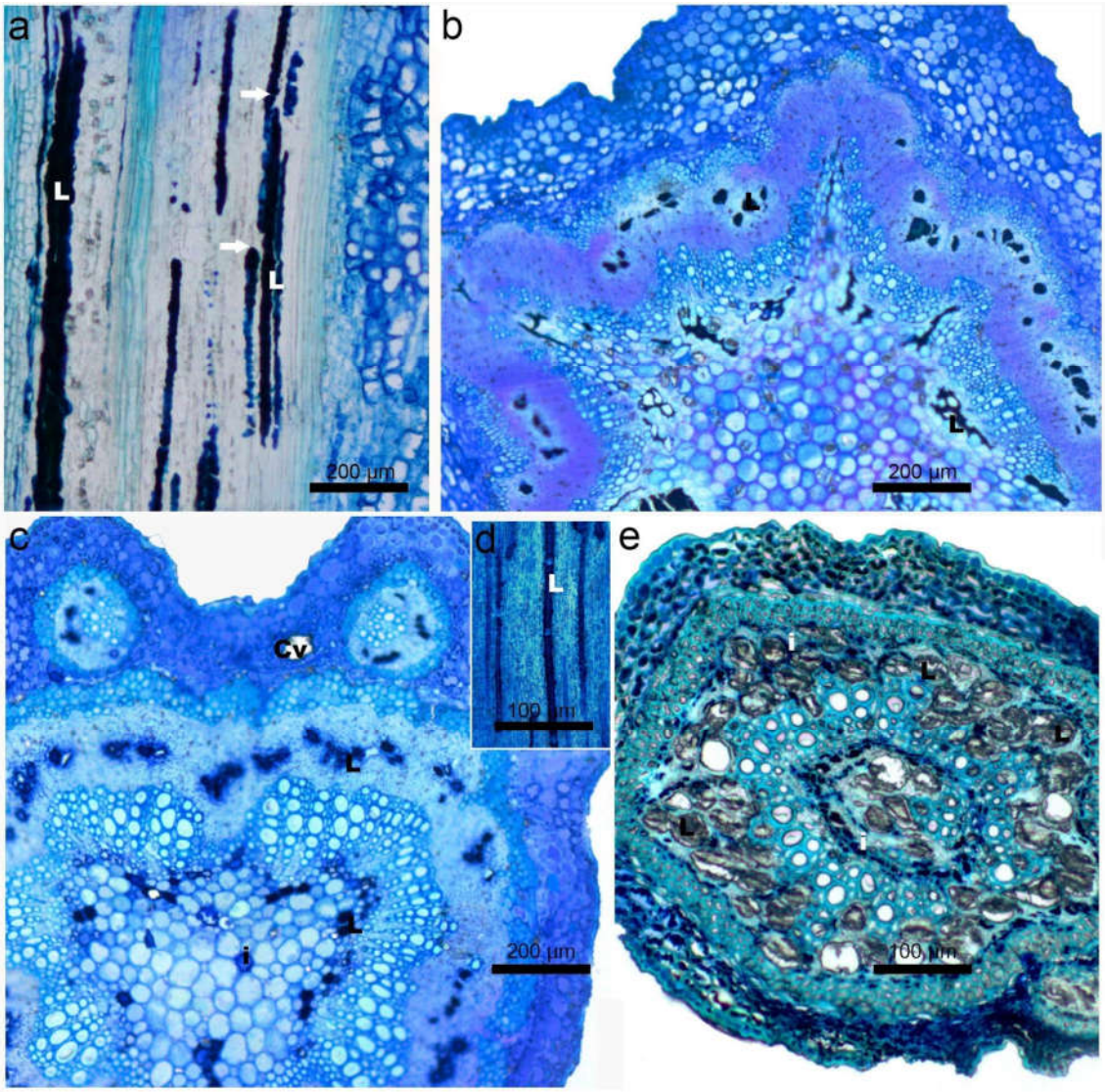


Fig 23: Fabaceae articulated laticifers (Fabales); *Lonchocarpus* sp.: a. Longitudinal section indicating non-anastomosed articulated laticifers; b. Cross section indicating laticifers distribution; *Dahlstedtia araripensis*: c. Cross section indicating distribution of articulated laticifers; d. Longitudinal section indicating articulated laticifers; *Mimosa laticifera*: e. Cross section indicating distribution of anastomosed articulated laticifers. (L – Laticifer; Cv – oil cavity; i – idioblast; arrow – terminal wall of laticifer)

Rosales

Rosales is composed of nine families (Zhang *et al.* 2011) with 261 genera, where laticifers are present in the clade formed of the families Ulmaceae, Cannabaceae, Moraceae and Urticaceae (Fig 24) (Sun *et al.* 2016).

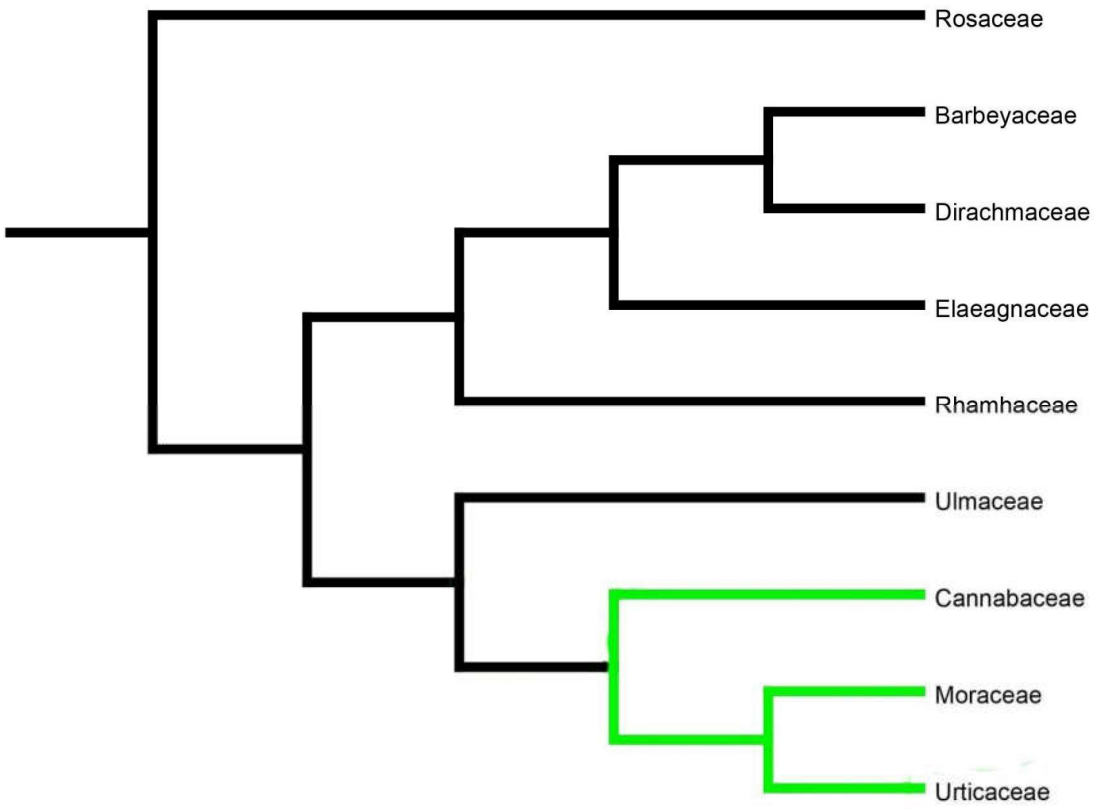


Fig 24: Phylogenetic cladogram of Rosales based on Zhang *et al*. (2011), indicating the presence of laticifers (green) in Cannabaceae, Moraceae and Urticaceae.

Cannabaceae

Cannabaceae is composed of 10 genera with wide geographical distribution (Yang *et al.* 2013). The laticifers in this family have been anatomically analyzed in *Cannabis*, *Humulus* (Hammond and Mahlberg 1994), *Celtis*, *Pteroceltis* and *Trema* (Rudder 2019) and have been described as non-articulated in *Cannabis sativa* and *Humulus lupulus* (Hammond and Mahlberg 1994). However, the analysis of ontogenesis of laticifers of both genera have shown that *Cannabis sativa* has anastomosed articulated laticifers in the phloem (Fig 25 a-c) whose anastomosis is early and occurs very close to the meristem (Fig 25 b). The rapid and total degradation of the walls has resulted in an incorrect classification in previous studies. On the other hand,

Humulus lupulus has non-anastomosed articulated laticifers in the phloem (Fig 25 d). Another difference is the diameter of the genic laticifers, which in Cannabis is wider, from 15 to 24 µm, and in Humulus is narrower, from 10 to 15 µm.

Moraceae

Moraceae has 37 genera widely distributed in the tropical and temperate habitats (Clement and Weiblen 2009). This is one of the major families for which the presence of branched non-articulated laticifers has been described (David 1872, Veedre 1949, Veenendaal and Outer 1990, Mahlberg 1993), occurring in the Moreae, Artocarpeae, Maclureae, Dorstenieae, and Ficeae tribes (Marinho and Teixeira 2019). However, our study has shown that laticifers in this family are branched anastomosed with a diameter ranging from 11 to 28 µm and are distributed throughout the cortex, medulla and vascular system (Fig 25 e f). Again, the rapid dissolution of the terminal walls of the cells that make up the laticifer is the reason for the divergence regarding the typology of the laticifers in this family. Only with accurate ontogenetic studies is it possible to verify the formation of this type of laticifer, as observed in *Ficus* and *Morus*.

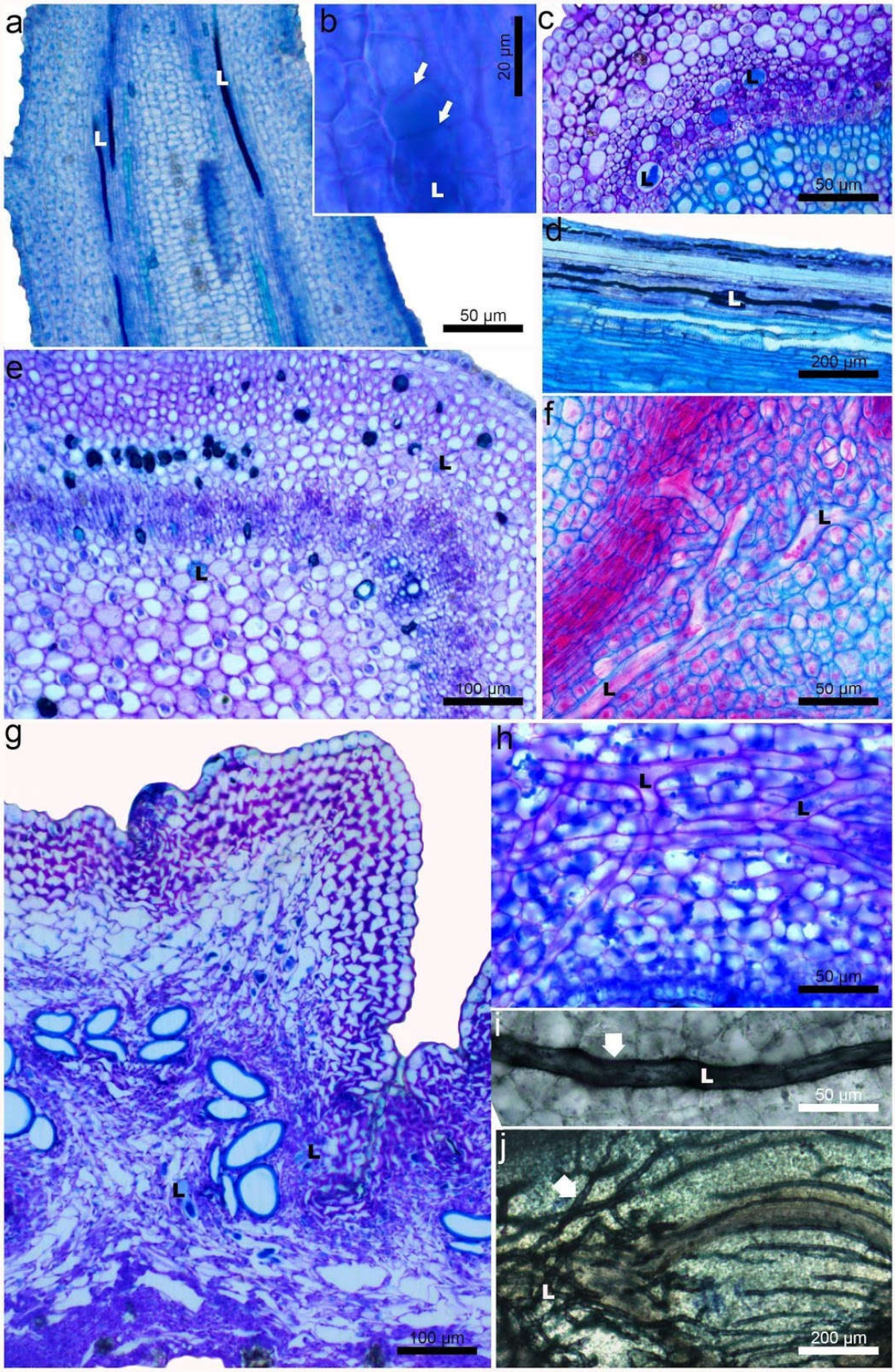
Urticaceae

Urticaceae is composed of 54 genera divided into four tribes with wide geographical distribution, mainly tropical. (Wu *et al.* 2013).

Family laticifers have been studied since the beginning of the last century (Guérin 1905) and Marinho and Teixeira (2019) report the presence of branched articulated laticifers with intrusive growth associated with vascular bundles and the

parenchyma in the Cecropieae tribe, in the genera *Cecropiae* and *Pourouma*. The occurrence of laticifers in Cecropieae still needs to be further investigated, since we observed that *Pourouma guianensis* and *Myrianthus* holstii do not present laticifers, having phenolic idioblasts and mucilaginous cavities, respectively.

Although laticifers appear to be present only in some Cecropieae species, they occur in all Urticeae (Guerin 1923, Gaglioti 2011). In this tribe, laticifers are branched anastomosed with a diameter ranging from 14 to 25 µm and present in the cortex, medulla and phloem (Fig 25 g-h). An unheard of feature for the family is the presence of suberin in the wall of the *Urera baccifera* laticifers (Fig 25 i-j), a fact observed only in Convolvulaceae laticifers and some Sapindaceae genera.


a
L
L
50 µm
b
20 µm
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50 µm
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L
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L
L
50 µm
g
L
L
100 µm
h
L
L
50 µm
i
L
50 µm
j
L
200 µm

Fig 25: Articulated laticifers in Rosales; Cannabaceae – *Cannabis sativa*: a. Longitudinal section indicating distribution of anastomosed articulated laticifers; b. Longitudinal section of the apex indicating presence of terminal walls in laticifer; W. cross section indicating laticifers distribution; Cannabaceae – *Humulus lupulus*: d. Longitudinal section indicating distribution of non-anastomosed articulated laticifers; Moraceae – *Ficus benjamina*: e. Cross section indicating distribution of anastomosed articulated laticifer; Moraceae – *Morus nigra* f. Longitudinal section indicating network of anastomosed articulated laticifers; Urticaceae – *Laportea aestuans*: g. Cross section indicating distribution of anastomosed articulated laticifers; Urticaceae – *Urera baccifera*: h. Longitudinal section indicating network of anastomosed articulated laticifers; i – j: Longitudinal section indicating suberin on the laticifer wall. (L – Laticifer; arrow – terminal wall of laticifer; thick arrow – suberin on wall of laticifer)

Sapindales

Sapindales has nine families (Acevedo-Rodrıguez *et al.* 2011, Acevedo-Rodrıguez *et al.* 2017) and about 479 genera with various secretory structures; however, laticifers are present only in Sapindaceae (Fig 26) (Sun *et al.* 2016, Montes 2017).

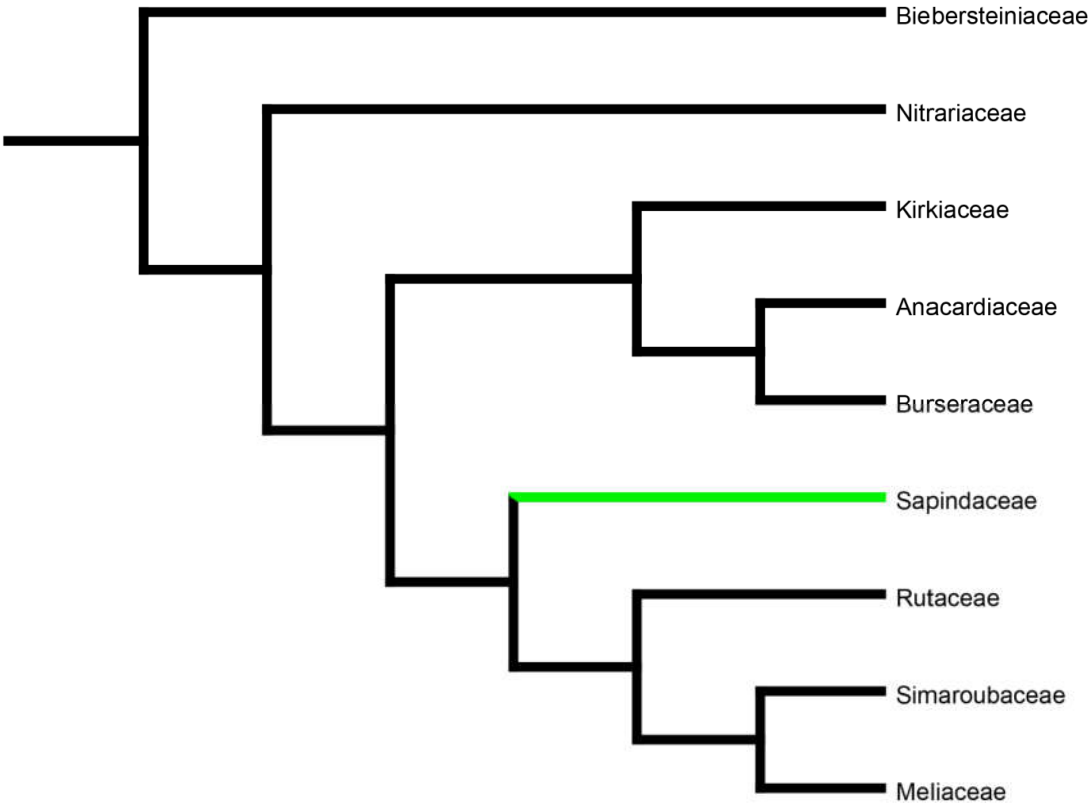

Fig 26: Phylogenetic cladogram of Sapindaceae based on Muellner-Riehl (2016), indicating the presence of laticifers (green) in Sapindaceae.

Sapindaceae

Sapindaceae is composed of 144 genera grouped into four subfamilies (Acevedo-Rodríguez *et al*. 2010, Acevedo-Rodríguez *et al*. 2017). The family has wide geographical distribution and laticifers are found in the subfamilies Hippocastanoideae and some tribes of Sapindoideae. (Montes 2017).

Laticifers have appeared at least six times during the evolution of the family and their presence or absence has taxonomic and phylogenetic importance. In Sapindoideae, laticifers are present in six strains, not always in all genera of the clade, but there is no variation between species of the same genus, except in *Cardiospermum* (Montes 2017), where the presence of laticifers only in some species reinforces the polyphylism of the genus (Acevedo-Rodrígues *et al*. 2017) and helps resolve infratribal relations in Paullinieae (Montes 2017). In Hippocastanoideae, laticifers occur only in Acer and Dipteronia (Montes 2017). Our analyses confirmed their nonexistence in *Billia columbiana* and *Aesculus hippocastanum*, which present only phenolic idioblasts, as well as in *Dodonea viscosa* (Dodonaeoideae).

All laticifers in the family are non-anastomosed articulated, with diameters ranging from 13 to 30 µm in the genera (Fig 27a) (Montes 2017) and their multiple appearance is also reflected in the presence of suberin in the genera *Serjania* and *Paullinia*, or just callose in *Thouinia*, *Diatenopteryx* and *Talisia* (Montes 2017).

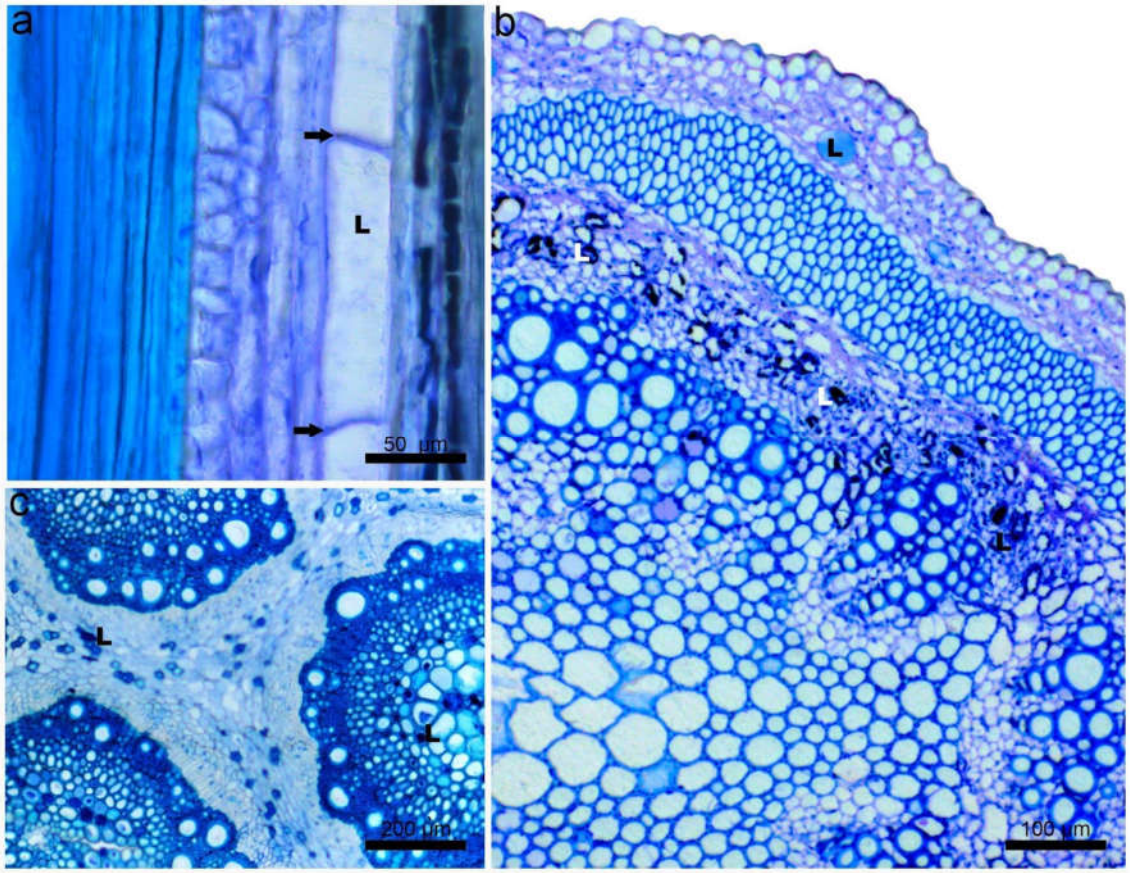

Fig 27: Articulated laticifers in Sapindaceae (Sapindales); *Serjania pinnatifolia:* a. Cross-section indicating distribution of non-anastomosed articulated laticifers; *Lophostigma plumosum:* b. Longitudinal section indicating non-anastomosed articulated laticifers; *Urvillea chacoensis* c. Cross-section indicating distribution of non-anastomosed articulated laticifers (L – Laticifer; arrow indicating terminal wall)

Brassicales

Brassicales is composed of 17 families (Lysak 2018) with about 405 genera and Caricaceae is the only laticiferous family (Fig 28), composed of six neotropical genera that have a distribution center in South America (Kyndt *et al*. 2005).

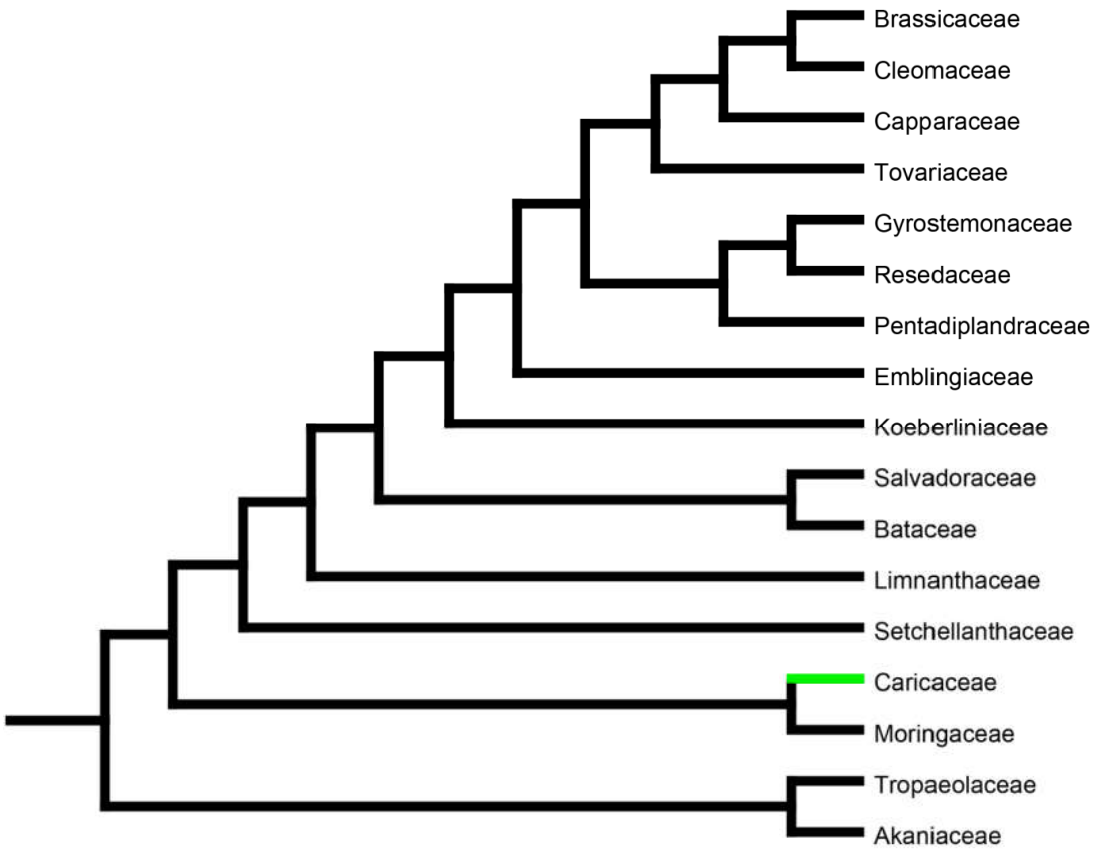

Fig 28: Phylogenetic cladogram of Brassicales based on Lysak *et al*. (2018), indicating the presence of laticifers (green) in Caricaceae.

Caricaceae

The occurrence of latex is widely known in Caricaceae (Miller 1982) due to the presence of proteolytic enzymes in its composition (Metcalfe 1967), which are used in the food and pharmaceutical industry (Agrawal and Konno 2009, Yan *et al*. 2011), and are present in all six of its genera (Miller 1982). Laticifers are of the branched anastomosed articulate type (Metcalfe and Chalk 1950, Lewinsohn 1991, present study) reaching up to 17 µm in diameter and are distributed throughout the fundamental and vascular systems. (Fig 29 a-c).

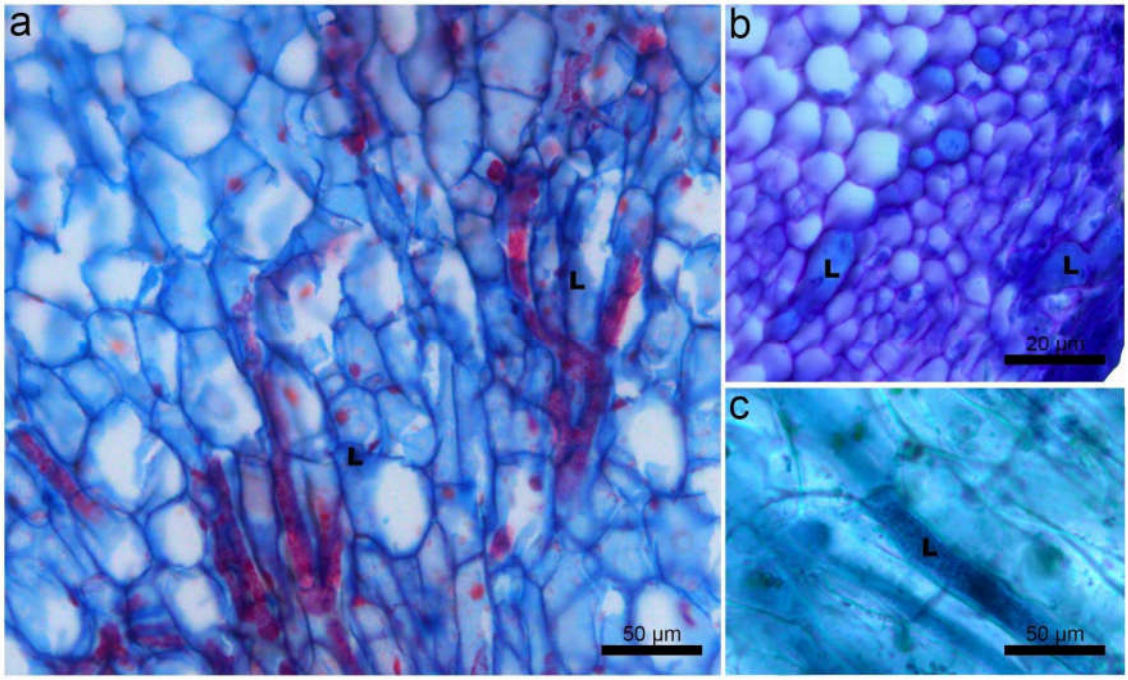

Fig 29: Branched anastomosed articulated laticifers in Caricaceae (Brassicales) – *Carica papaya*: a. Longitudinal section indicating distribution of anastomosed articulated laticifers; b. Cross-section indicating anastomosed articulated laticifers; c. Aniline blue black Test – Longitudinal section indicating latex protein composition. (L – Laticifer)

Santalales

Santalales is composed of 18 families (Nickrent *et al*. 2010) with about 179 genera of wide geographical distribution and many parasitic plant genera (Su *et al*. 2015). The only reports of latex were made for genera that belonged to the Olacaceae (Metcalfe 1967, Lewinsohn 1991) and Loranthaceae (Lewinsohn 1991) families. Currently, genera of Olacaceae have been repositioned according to the most recent phylogenetic analyses (Nickrent *et al*. 2010, Su *et al*. 2015). Our analysis demonstrated the absence of laticifers in genera of both families, according to the current circumscription, but we identified laticifers in Aptandraceae, Coulaceae and Erythropalaceae (Fig 30). According to phylogeny, laticifers have appeared independently at least three times in this order.

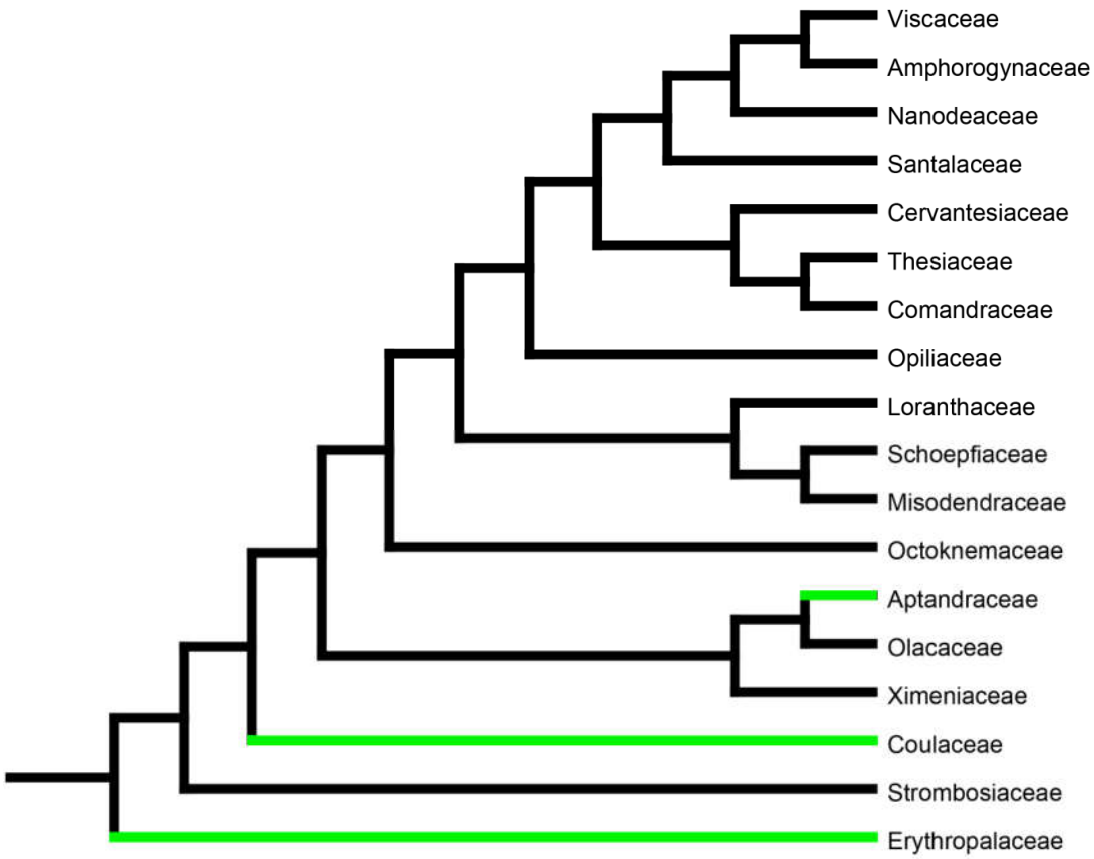

Fig 30: Phylogenetic cladogram of Santalales based on Nickrent *et al*. (2010), indicating the presence of laticifers (green) in Aptandraceae, Coulaceae and Erythropalaceae.

Aptandraceae

Recently raised to the category of family, it is composed of eight genera (Nickrent *et al.* 2010, Su *et al.* 2015). Articulated laticifers are found in the mesophyll of *Aptandra* and *Harmandia* (Baas *et al.* 1982, Malécot 2004).

Coulaceae

Coulaceae is composed of only three genera: *Coula*, *Minquartia* and *Ochanostachys*, with tropical distribution (Su *et al.* 2015). Laticifers and resin cavities

are present in all three genera and are a family synapomorphy (Metcalfe and Chalk 1950, Metcalfe 1967, Baas *et al.* 1982, Sleumer 1984, present study). Laticifers are anastomosed articulated, reach diameters up to 30 µm (Fig 31 a), and are distributed only in the cortex (Fig 31 b).

Erythropalaceae

Laticifers were previously recorded for *Heisteria* (Metcalfe and Chalk 1950, Baas *et al.* 1982, Sleumer 1984), a genus of pantropical occurrence, mainly in tropical America and Africa (Sleumer 1984), which belonged to the Olacaceae family (Malécot 2004) and recently was transferred to Erythropalaceae (Su *et al.* 2015). Laticifers had been classified as non-articulated (Metcalfe 1967, Baas *et al*. 1982), but our analysis revealed that laticifers of the genus are anastomosed articulated measuring up to 40 µm in diameter (Fig 31 c) and with distribution in the cortex, medulla and phloem (Fig d).

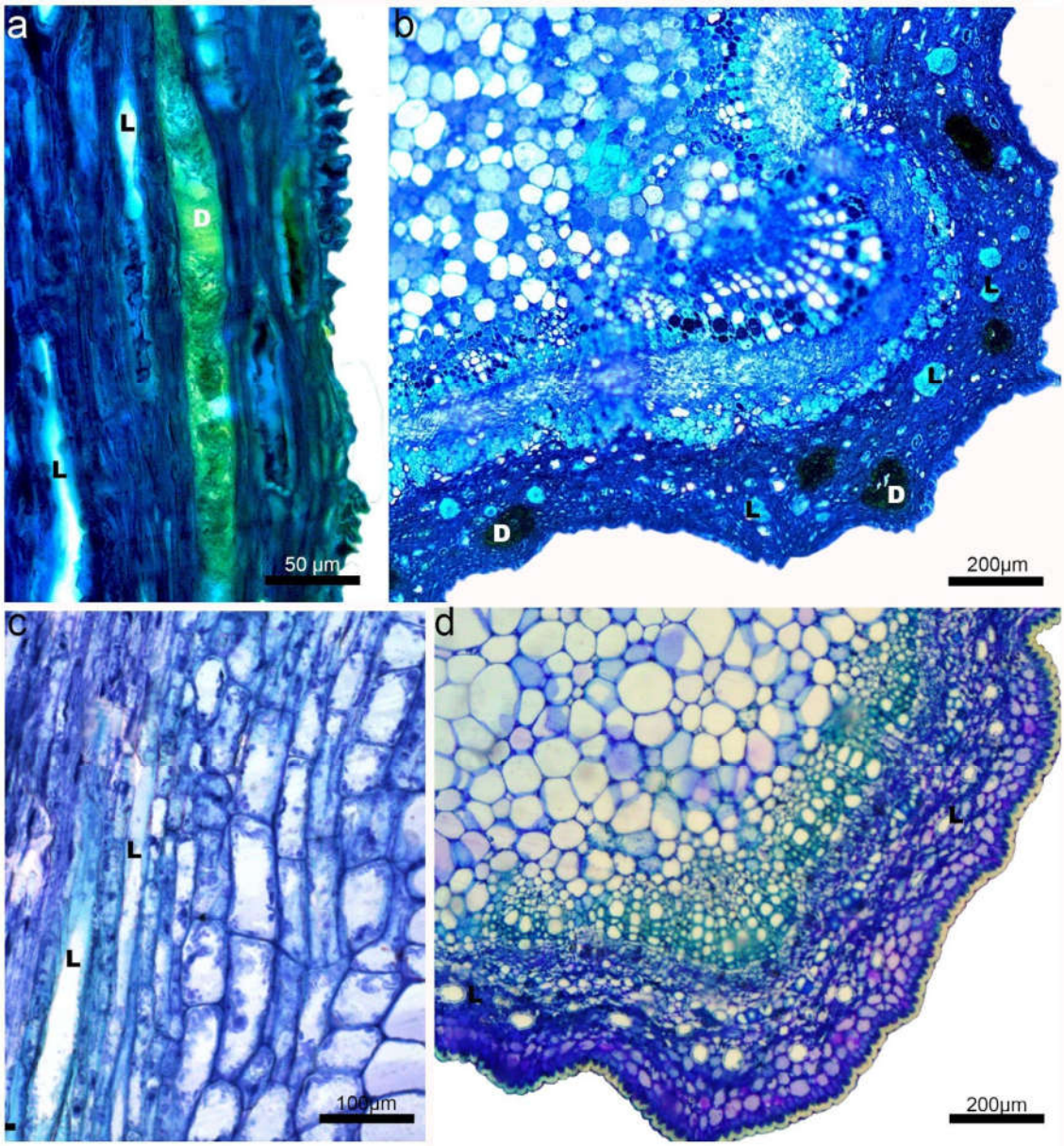

Fig 31: Anastomosed articulated laticifers in Santalales; Coulaceae – *Coula edulis*: a. Cross section indicating laticifers distribution; b. longitudinal section indicating anastomosed articulated laticifer; Erythropalaceae – *Heisteria silvianii*: c. Cross section indicating distribution of anastomosed articulated laticifers; d. longitudinal section indicating anastomosed articulated laticifer; (L – Laticifer).

Cornales

Cornales is composed of ten families with about 42 genera (Fu *et al.* 2017); laticifers were recorded in Alangiaceae (Lewinsohn 1991) and in the genus *Camptotheca* belonging to Nyssaceae (Monacelli *et al.* 2005) (Fig 32). We have also

analyzed representatives of Cornaceae, since the positioning of some genera of Nyssaceae and Cornaceae is questionable (Xiang *et al.* 1998a, Xiang 1999); however, Cornaceae has no laticifers.

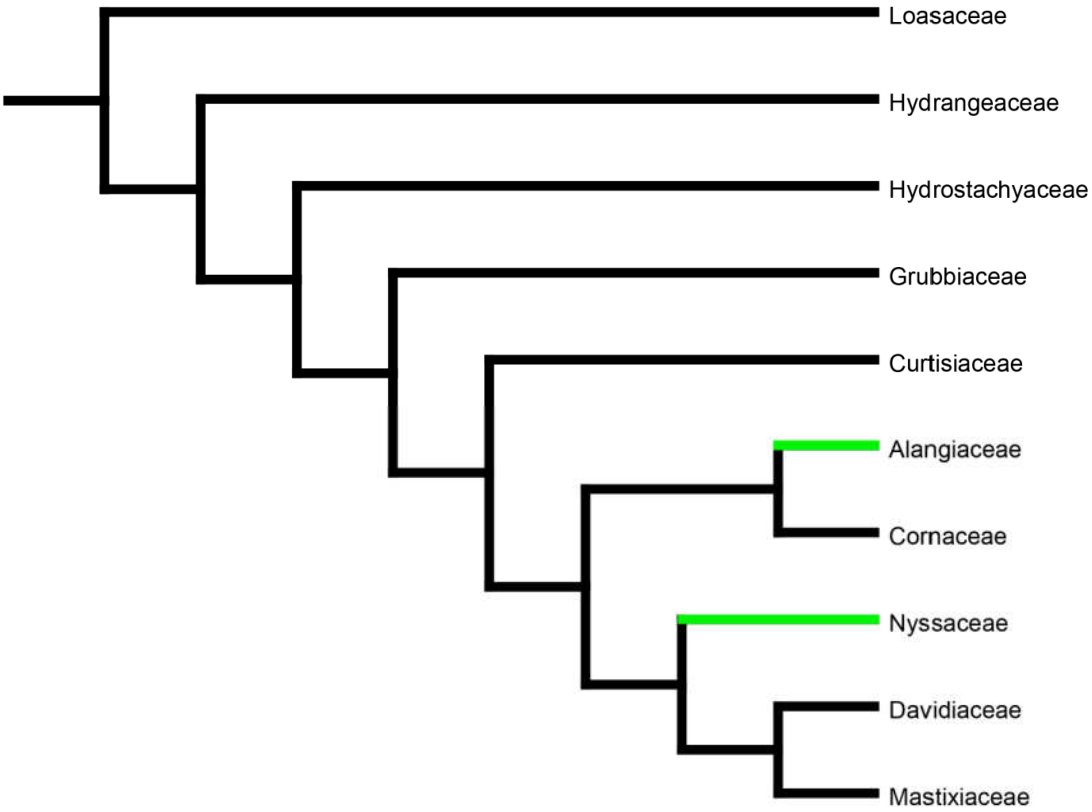

Fig 32: Phylogenetic cladogram of Cornales based on Fu *et al.* (2017), indicating the presence of laticifers (green) in Alangiaceae and Nyssaceae.

Alangiaceae

Alangiaceae is composed of a single genus, with about 24 species, and is distributed in tropical and subtropical regions of East and Southeast Asia (Feng *et al.* 2008). We have found anastomosed articulated laticifers with very thick and extremely wide walls, with a diameter of up to 60 µm (Fig 33 a b) distributed in the perivascular region of the cortex in *Alangium platanifolium*.

Nyssaceae

Nyssaceae is composed of two genera, *Camptotheca* and *Nyssa* (Fu *et al.* 2017) with habitat in East Asia, Indo-Malaysia and North American East (Xiang *et al.* 2008); anastomosed articulated laticifers were found only in *Camptotheca acuminata* (Monacelli *et al*. 2005) (Fig 33 c), with diameters from 16 to 23 µm and distributed in the medulla and perivascular region (Fig 33 d). In *Mastixia* and *Diplopanax*, which were part of the family, resin ducts are observed (Metcalfe and Chalk 1950), but both were transferred to family Mastixiaceae (Fu *et al.* 2017).

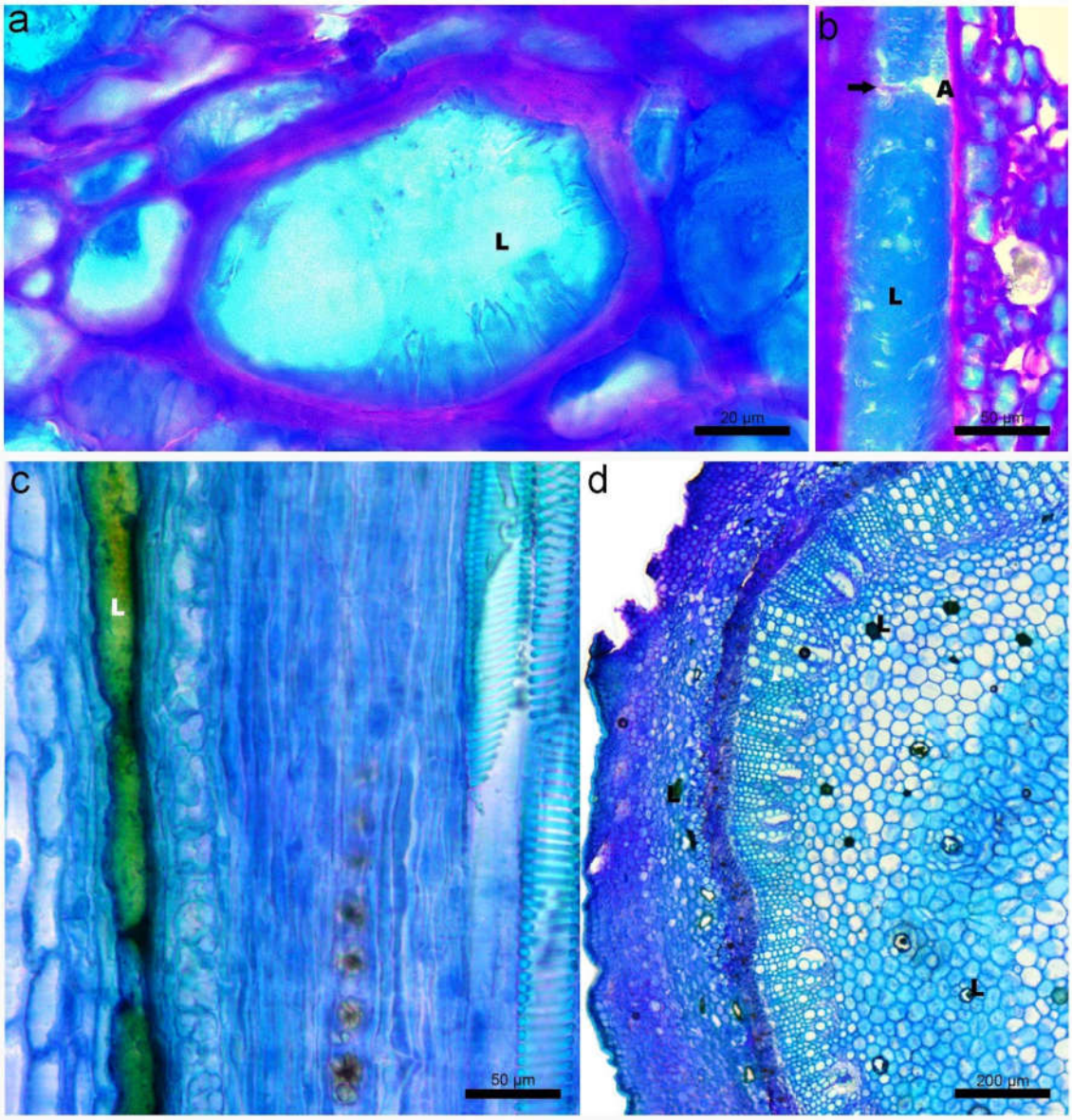

Fig 33: Anastomosed articulated laticifers in carnelians; Alangiaceae – *Alangium platanifolium*: a. Cross-section indicating laticifers; b. longitudinal section indicating laticifer; Nyssaceae – *Campthoteca acuminata*: c. Longitudinal section indicating laticifer; d. Cross section indicating laticifers distribution. (L – Laticifer; A – partial wall anastomosis; arrow – terminal wall remnant).

Ericales

Ericales currently contains 21 families (Chartier *et al.* 2017) with about 346 genera, and laticifers are present only in Sapotaceae (Fig 34) (Lewinsohn 1991).

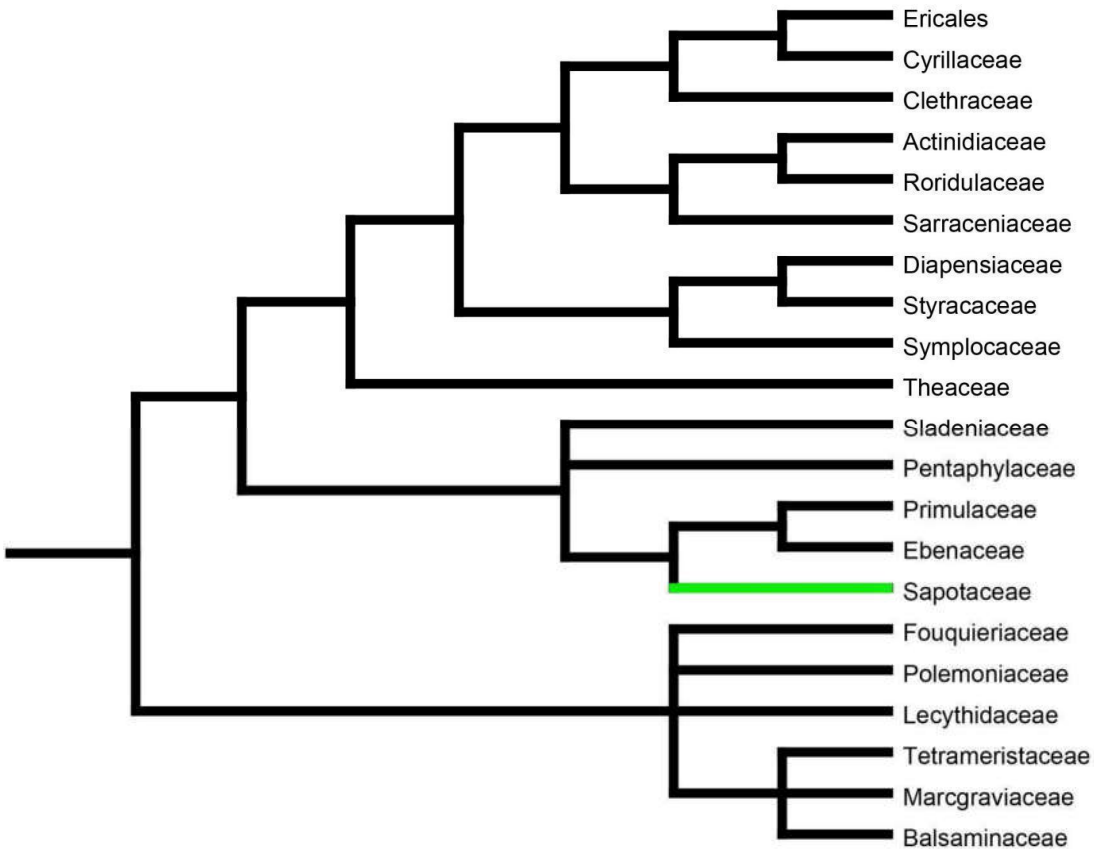

Fig 34: Phylogenetic cladogram of Ericales based on Chartier *et al*. (2017), indicating the presence of laticifers (green) in Sapotaceae.

Sapotaceae

The 53 genera of the family (Govaerts *et al.* 2001) are divided into four subfamilies with pantropical distribution (Rose *et al.* 2018), although the largest species diversity is found in the tropical and subtropical regions of Asia and South America (Swenson and Anderberg 2005). Laticifers are articulated (Metcalfe 1967) and are a family synapomorphy (Solereder 1908, Metcalfe and Chalk 1950). The latex present in the family is not always noticeable (Swenson and Anderberg 2005, Swenson *et al.* 2008) and is very important in economic terms, (Almeida-Jr 2013) such as gutta-percha and chewing gum (Metcalfe 1967).

We observed that the articulated laticifers of *Manilkara* and *Pouteria* have partial anastomosis of the terminal walls and are distributed in the cortical and medullary region (Fig 35 a-d). Laticifers differ greatly in diameter, ranging from 15 to 40 µm. In addition to laticifers, a large amount of phenolic idioblasts are observed in all tissues.

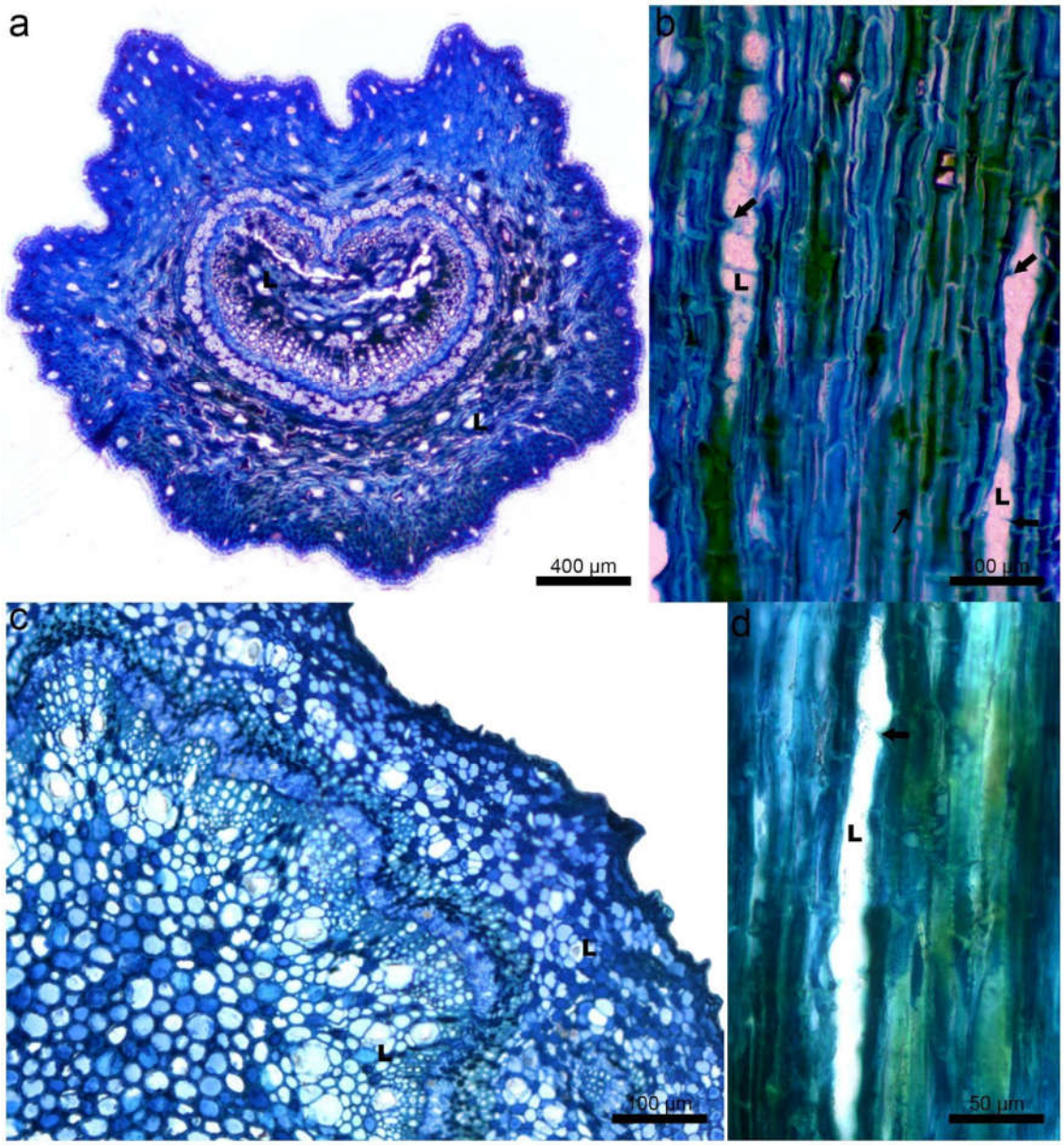

Fig 35: Anastomosed articulated laticifers in Sapotaceae (Ericales) – *Manilkara* sp.: a. Cross-section indicating laticifers; b. longitudinal section indicating anastomosed articulated laticifer; *Caimito Pouteria*: c. Cross section indicating laticifers distribution; d. longitudinal section indicating anastomosed articulated laticifer. (L – Laticifer; arrow – terminal wall remnant)

Garryales

Garryales is composed of only two families, Eucommiaceae and Garryaceae (Stull *et al.* 2015), with subtropical distribution (Lewinsohn 1991). The presence of latex was recorded only in Eucommiaceae (Fig 36) (Metcalfe 1967, Biesboer and Mahlberg 1981, Lewinsohn 1991, Zhang 2016) and our study confirmed its absence in Garryaceae.

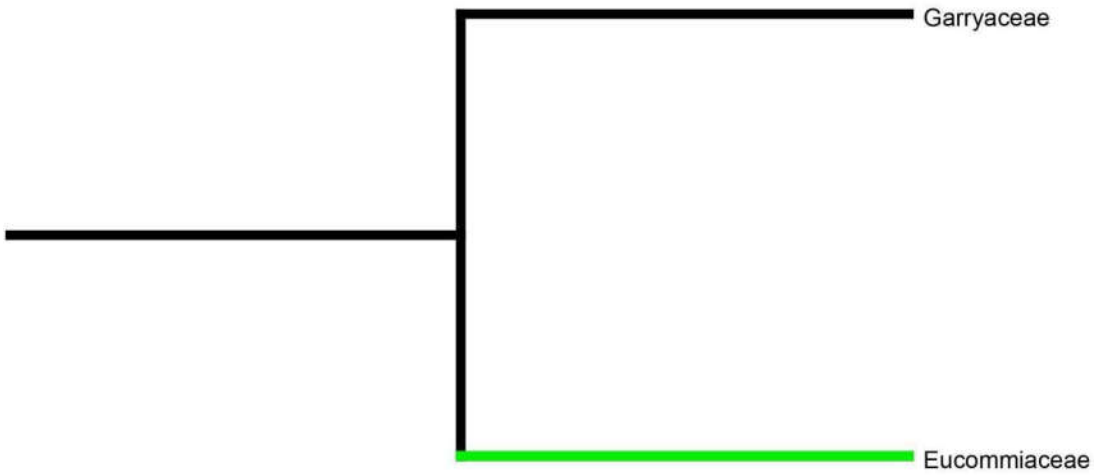

Fig 36: Phylogenetic cladogram of Garryales based on Stull *et al.* (2015), indicating the presence of laticifers (green) in Eucommiaceae.

Eucommiaceae

Eucommiaceae is a monotypic family whose sole representative, *Eucommia ulmoides*, is endemic to China and widely used by the pharmaceutical industry in the manufacture of medicines for hypertension (Zhang 2016). Laticifers present in *E. ulmoides* are non-anastomosed articulated (Fig 37 a-c), have a narrow diameter of up to 7 µm and is distributed in the cortex and phloem (Metcalfe and Chalk 1950, Metcalfe 1967, present study).

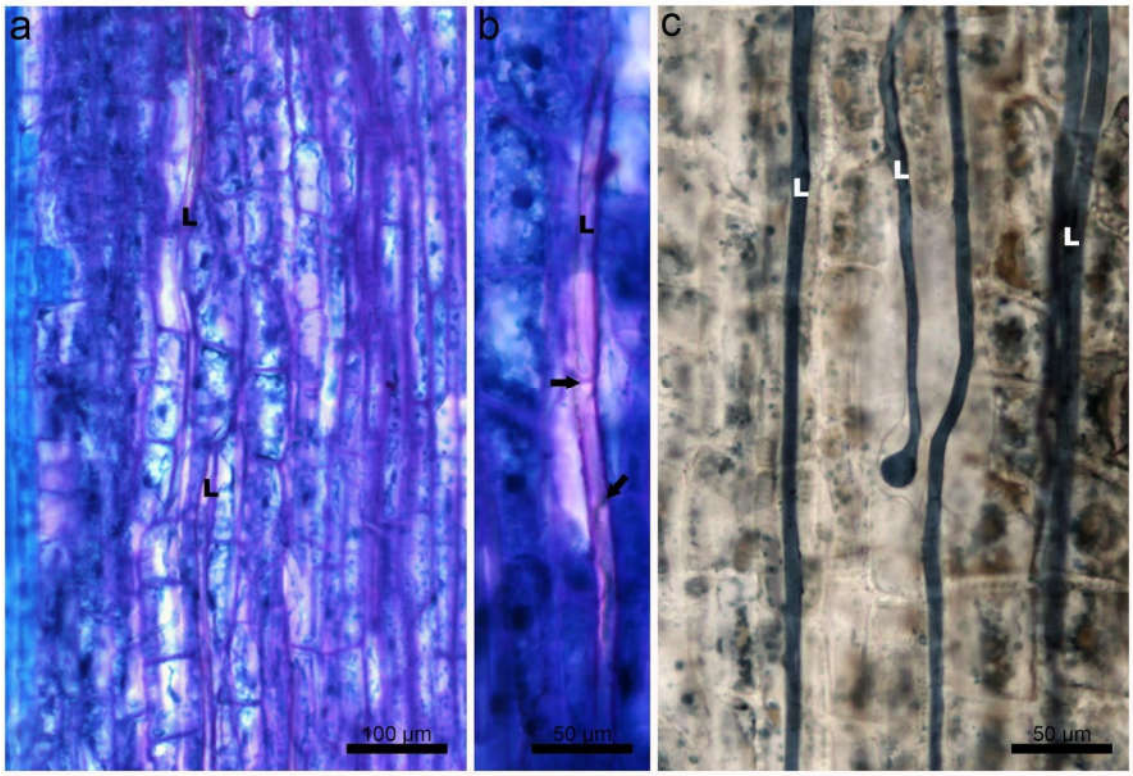

Fig 37: Non-anastomosed articulated laticifers in eucommiaceae (Garryales) – *Eucommia ulmoides*: a. Longitudinal section indicating non-anastomosed articulated laticifers; b. longitudinal section indicating terminal walls of articulated laticifer; c. Sudan black test – Longitudinal section indicating latex lipid composition in laticifers. (L -Laticifer; arrow – terminal wall).

Gentianales

Gentianales has five families with about 1200 genera (Yang *et al.* 2016), and laticifers are only present in Apocynaceae (Demarco *et al.* 2006, Demarco and Castro 2008, Demarco 2015, Gama *et al.* 2017). However, our study has also identified laticifers in Rubiaceae, with at least two independent appearances of this secretory structure in the order (Fig 38).

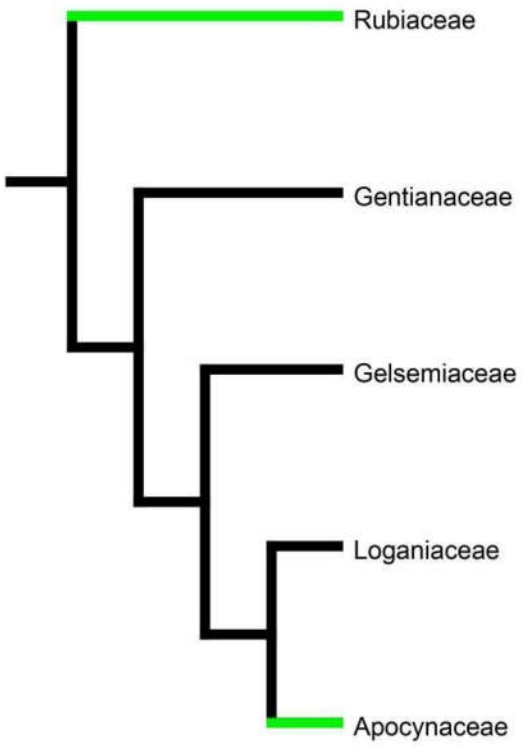

Fig 38: Phabalogen cladogram of Fabales based on Yang *et al.* (2016), indicating the presence of laticifers (green) in Rubiaceae and Apocynaceae.

Rubiaceae

Rubiaceae is composed of about 630 genera with predominantly tropical habitat (Eriksson 1991). Although it is considered as non-laticiferous, a study by Puff *et al.* (1993) reported the presence of laticifers in *Mussaenda* and *Pseudomussaenda*. In this study we identified anastomosed articulated laticifers with diameters from 17 to 25 µm located in the perivascular cortex of *Mussaenda erythrophylla* (Fig 39 a b). Although phenolic idioblasts are abundant (Fig 39 a b), laticifers are distinguished by the production of latex, whose composition is mostly lipid (Fig 39 c) (Prado and Demarco 2018).

Apocynaceae

Apocynaceae is one of the largest families of angiosperms, with 366 genera grouped into five subfamilies (Endress *et al.* 2014) and about 5100 species (Endress 2004; Endress *et al*. 2007) of mostly tropical distribution, all representatives of which produce latex (Lewinsohn 1991, Demarco *et al.* 2006, 2013, Demarco and Castro 2008, Fishbein *et al*. 2018). This is a unique case among laticiferous groups, where all the members of a family with a large number of species and wide distribution produce latex as their main defense strategy. All other families that have laticifers as synapomorphy have few species.

Although laticifers in this family have been classically described as non-articulated, recent ontogenetic analyses have shown that all Apocynaceae laticifers are anastomosed articulated, where terminal wall disintegration is rapid and total, still occurring in the meristematic region (Demarco *et al.* 2006 , Demarco and Castro 2008, Lopes *et al.* 2009, Demarco 2015, Canaveze and Machado 2016, Gama *et al.* 2017, present study). Laticifers range in diameter from 12 to 35 µm and occur in the cortex, medulla, and vascular system, both in the xylem and phloem; transestellar branches can be seen in the family, whose cortical laticifers interconnect with the medullary system (Fig 39 d Demarco *et al.* 2006, Demarco and Castro 2008).

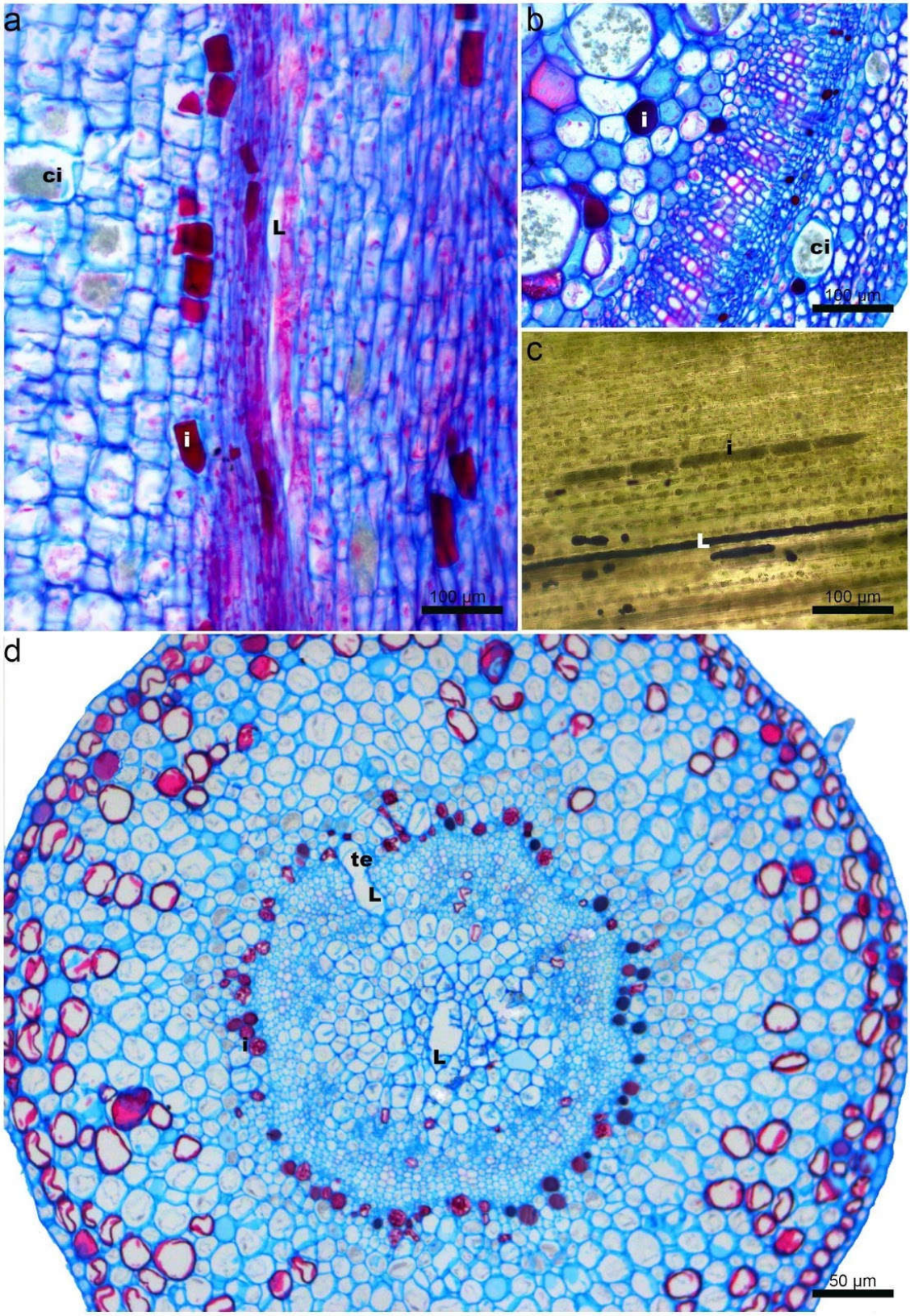

Fig 39: Anastomosed articulated laticifers Gentianales – Rubiaceae: Mussaenda erythrophylla: a. Longitudinal section indicating anastomosed articulated laticifers; b. cross section indicating laticifers distribution; c. Sudan black test – Longitudinal section indicating latex lipid composition in laticifers and

absence in idioblasts. Mandevilla splendes: d. Cross section showing distribution of laticifers; e. transstellar branches (L – Laticifer; arrow – terminal wall; i – idioblast; crystalline ci – idblast; te – transstellar branches).

Solanales

Solanales is composed of five families (Refulio-Rodriguez and Olmstead 2014) with about 165 genera and laticifers are present in family Convolvulaceae family (Fig 40) (Metcalfe 1967, Farrell *et al.* 1991, Lewinsohn 1991, Martins *et al.* 2012).

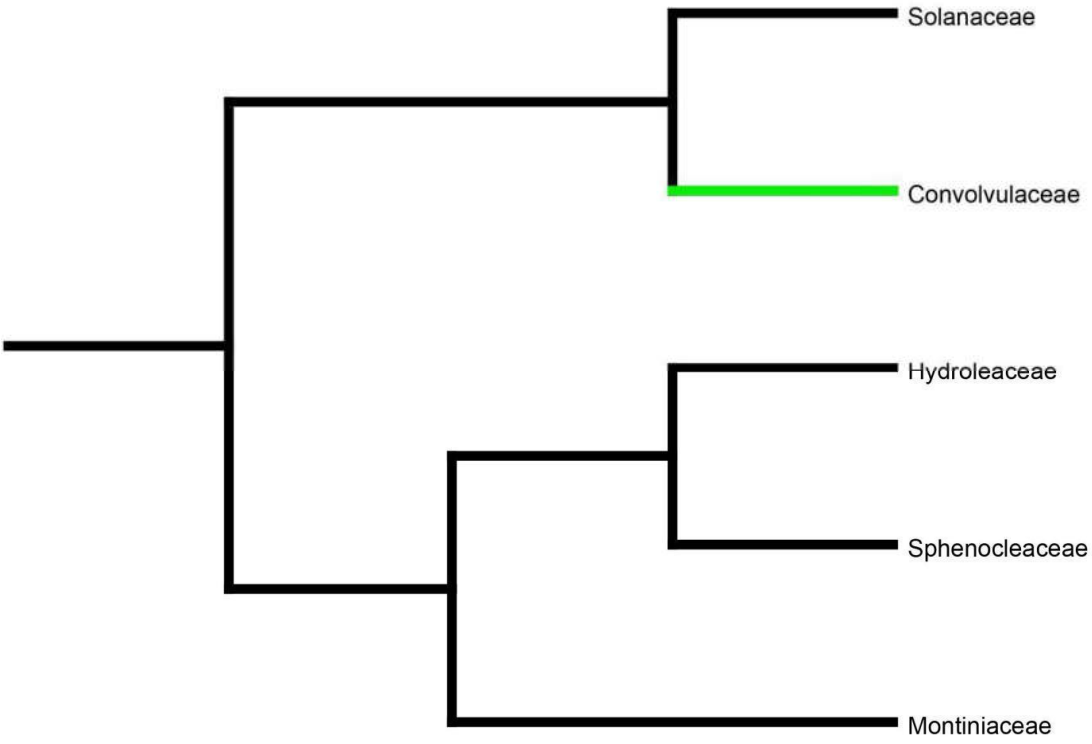

Fig 40: Phylogenetic cladogram of solanales based on Refulio-Rodriguez and Olmstead (2014), indicating the presence of laticifers (green) in Convolvulaceae.

Convolvulaceae

Convolvulaceae consists of 60 genera with cosmopolitan distribution (Stefanović *et al.* 2002). Laticifers are not present in the whole family, they occur only in the most derived subfamily, Convolvuloideae, and have already been described in

tribes Convolvuleae and Ipomoeeae (Fig 41 a-g), being absent in Dichondreae (present study).

Laticifers in this family stand out for having a typical cross-sectional star shape (Fig 41a-b) and the presence of suberin in the wall (Fig 41 e) (Fineran *et al.* 1988, Condon and Fineran 1989). Laticifers are articulated and unbranched forming long rows of cells in the cortex in *Ipomoea tricolor* (Fig 41 c) and only in the phloem of *Calystegia occidentalis* (Fig 41 f) and *Convolvulus crenatifolius* (Fig 41 g). Laticifers present differences in diameter, ranging from 13 to 42 µm in the genera. This family has a unique characteristic among its laticiferous species – its laticifers have partial and late anastomosis, which occurs only in adult organs (Fig 41 d) (Condon and Fineran 1989, present study).

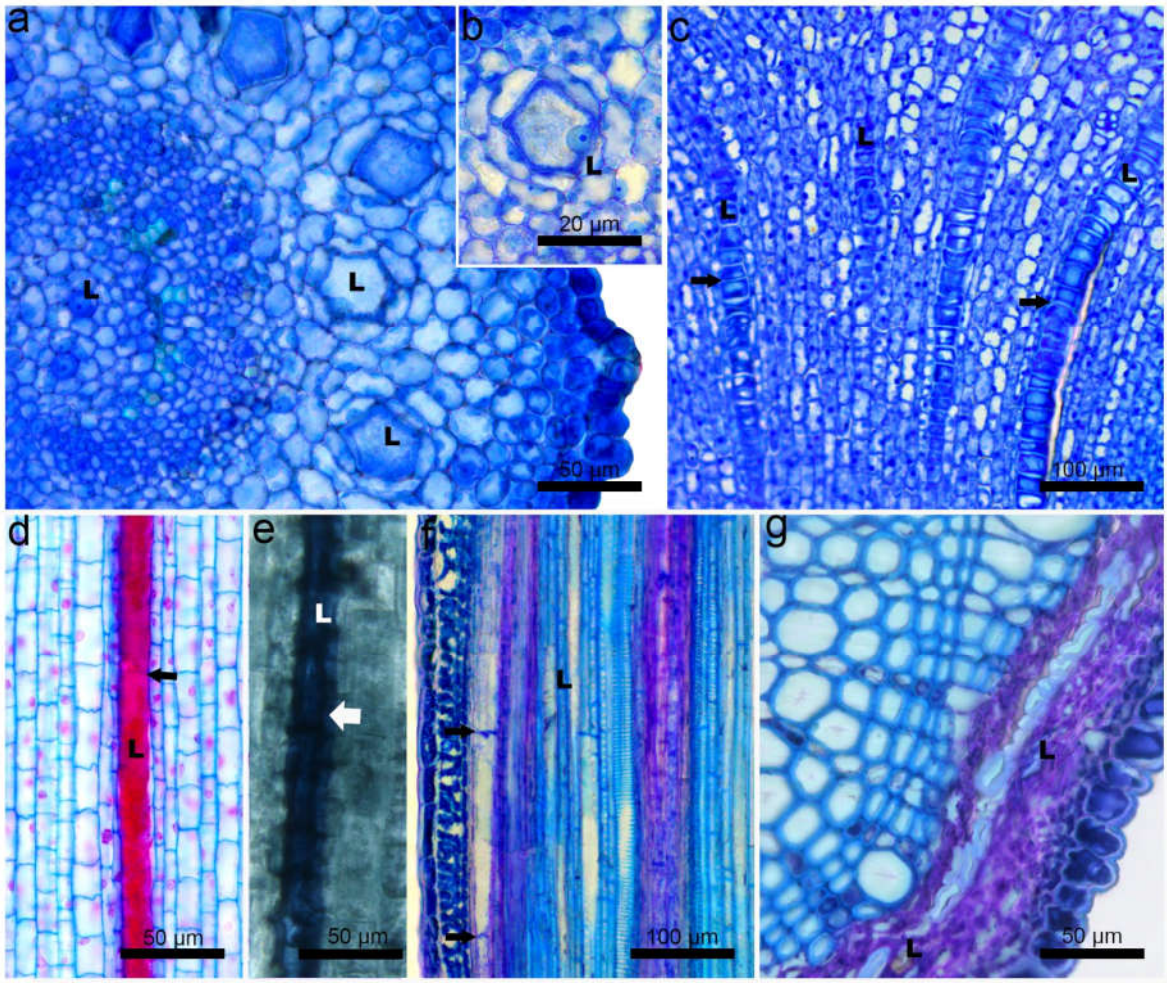

Fig 41: Non-anastomosed articulated laticifers in Convolvulaceae (Solanales); Ipomoea tricolor: a. Cross section indicating laticifers distribution; b. Cross section indicating the star-shape of laticifers; c. Longitudinal section indicating distribution of non-anastomosed articulated laticifers; d. Longitudinal section indicating partial and late anastomosis in adult organs e. Sudan black test – Longitudinal section indicating suberin in the laticifers wall; *Calystegia occidentalis*: f. Longitudinal section indicating non-anastomosed articulated laticifer; *Convolvulus crenatifolius* g. Cross section indicating distribution of non-anastomosed articulated laticifers. (L – Laticifer; arrow – terminal wall of laticifer; thick arrow – suberin).

Aquifoliales

Aquifoliales is composed of five families (Manen *et al.* 2010) with about 21 genera, the vast majority of which are segregated from Icacinaceae (Kårehed 2001, Lens *et al.* 2008, Soltis *et al.* 2011). Laticifers are present only in Cardiopteridaceae (Fig 42) (Metcalfe 1967, Lewinsohn 1991).

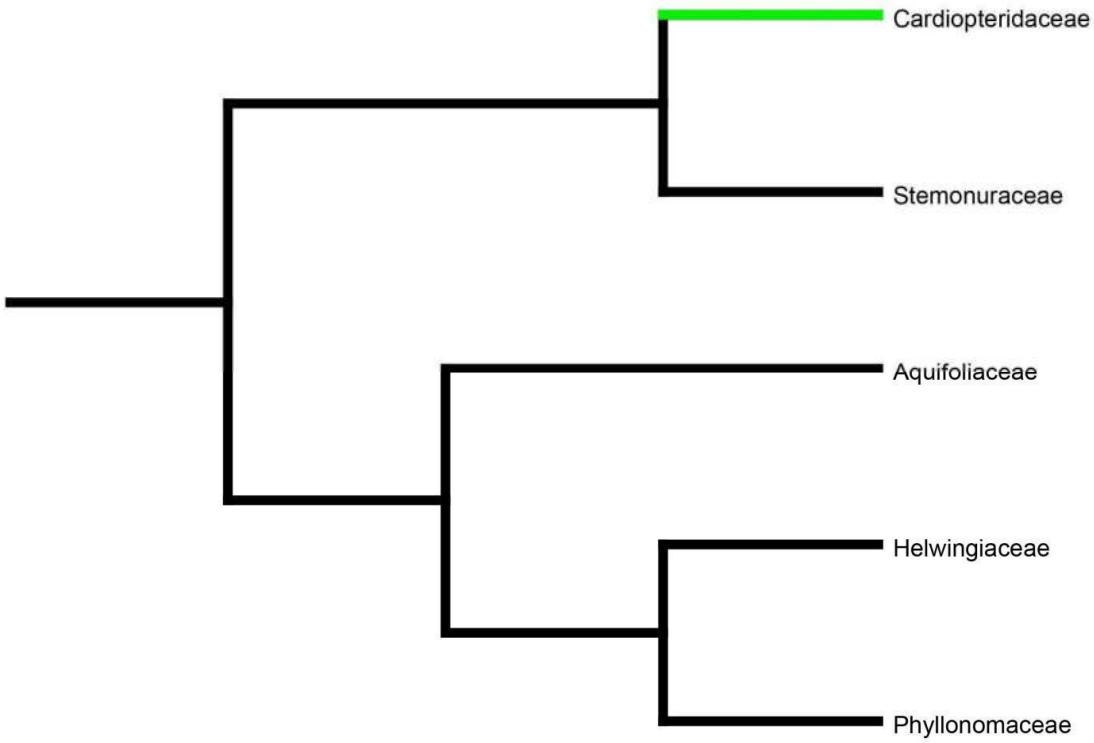

Fig 42: Phylogenetic cladogram of Aquifoliales based on Manen *et al.* (2010), indicating the presence of laticifers (green) in Cardiopteridaceae.

Cardiopteridaceae

Cardiopteridaceae has five morphologically distinct genera, found in the tropical habitat. Apparently, articulated laticifers are present only in Cardiopteris (Thouvenin 1891, Metcalfe and Chalk 1950, Metcalfe 1967, Lewinsohn 1991, Kårehed 2001, Kong *et al.* 2014). We found that *Citronella* has only phenolic idioblasts. Further studies are needed to verify the presence of laticifers in other genera of the family.

Asterales

Asterales is composed of 11 families (Winkworth *et al.* 2008) with about 1743 genera, where the families Roussaceae, Pentaphragmataceae, Stylidiaceae, Phellinaceae, Argophyllaceae, Alseuosmiaceae and Goodniaceae are specifically distributed: Australia, New Guinea, New Zealand, New Caledonia and Solomon islands; Calyceraceae across South America; while Campanulaceae, Menyanthaceae and Asteraceae are cosmopolitan (Lundberg 2009). Laticifers have been recorded for Campanulaceae and Asteraceae (Fig 43) (Savolainen *et al.* 2000, Stytwala *et al.* 2015, Chen *et al.* 2016).

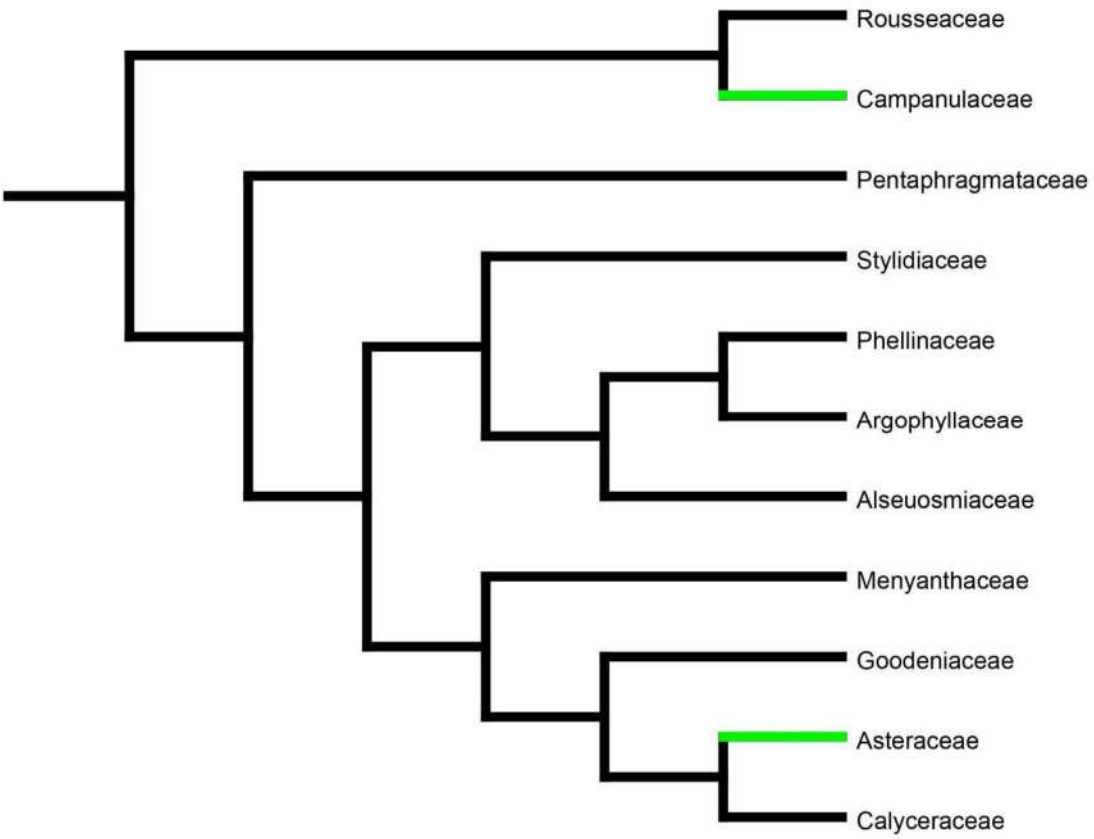

Fig 43: Phylogenetic cladogram of Asterales based on Winkworth *et al.* (2008), indicating the presence of laticifers (green) in Campanulaceae and Asteraceae.

Campanulaceae

Campanulaceae is composed of herbs with cosmopolitan habitat, composed of 84 genera divided into five subfamilies (Lagomarsino *et al.* 2014, Crowl *et al.* 2016), where one of the synapomorphies of the family is the presence of laticifers (Lammers 2007a), which are classified as articulated branched anastomosed (Metcalfe 1967, Lewinsohn 1991, present study).

In *Hippobroma longiflora* (Lobelioideae), laticifers are extremely narrow compared to adjacent parenchymal cells, measuring from 6 to 10 µm, making them difficult to locate (Fig 44 a b). This characteristic is unique and distinguishes laticifers of this family from those of others.

Asteraceae

Asteraceae is composed of about 1620 genera (Lundberg 2009), grouped into 12 subfamilies and has a wide geographical distribution (Panero *et al*. 2014).

According to Carlquist (1976), representatives of this family usually have either laticifers or resin ducts, but not both structures in plants of the same genus.

Vertrees and Mahlberg (1978) described anastomosed articulated laticifers in *Cichorium intybus*, whose origin occurs in both primary tissue – in the fundamental and procambium system –, and in the secondary tissue, in the phloem, and its wall contains callose. We have observed that the occurrence of laticifers and ducts varies depending on gender or subfamily. *Acourtia* (Mutisioideae) has neither laticifers nor resin ducts. On the other hand, *Lactuca* and *Taraxacum* (Cichorioideae) have branched anastomosed articulated laticifers in the cortex and phloem (Fig 44 d) and their diameters range from 14 to 20 µm, while *Centaurea* (Carduoideae) has only resin ducts. Thus, the herbivory protection strategy varies in the family and laticifers may have emerged a few times throughout the evolutionary history of Asteraceae.

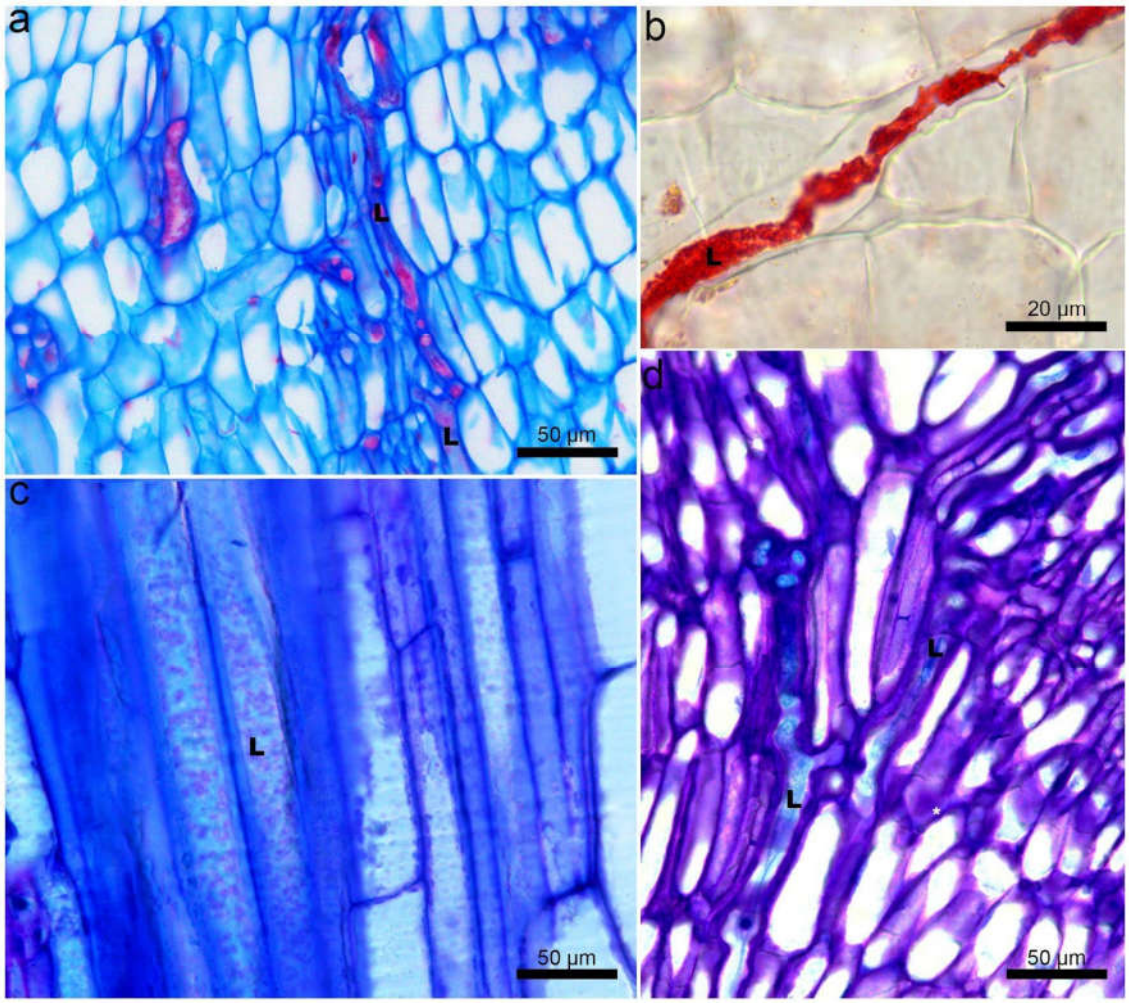

Fig 44: Anastomosed articulated laticifers in Asterales: Campanulaceae: *Hippobroma longiflora*: a. Longitudinal section indicating branched distribution of anastomosed articulated laticifers; b. Sudan Test IV – Longitudinal section indicating latex lipid composition; Asteraceae – *Lactuca indica:* c. Longitudinal section indicating anastomosed articulated laticifers; Asteraceae – *Taraxacum officinale*: d. Longitudinal section indicating anastomosed articulated laticifers. (L – Laticifers).

3. *Non-latescent families*

Below we describe families previously recorded as bearing latex, which was refuted in this work because they do not have laticifers. In addition, we have investigated families of latescent clades to ascertain whether they could have laticifers. The internal secretory structures found are described below (Fig 45 a-k; Fig S1; Fig S2).

Marsileales

Salviniaceae

In Salviniaceae, *Salvinia* (Fig 45 a) and *Azolla* were analyzed and found to have only phenolic idioblasts associated with the vascular system.

Alismatales

Alismataceae

Alismataceae has 15 aquatic genera classified as latescent (Lewinsohn 1991, Pansarin and Amaral 2005, Matias *et al.* 2008). According to Matias *et al.* (2008), the presence of laticifers would be a synapomorphy for Alismataceae; Govindarajalu (1967) reported the presence of non-anastomosed articulated laticifers throughout the plant body in *Sagittaria guayanensis.* We analyzed species of *Sagittaria* (Fig 45 b) and *Limnocharis* and found resin ducts instead of laticifers in the genera analyzed. Thus, the group is resinous rather than latescent.

Liliales

Liliaceae

The description of latex in Liliaceae is not an error, but according to the family's new circumscription, the genera described as latescent (Sterling and Huang

1972) were transferred to Amaryllidaceae (Asparagales) (Fay and Chase 1996, Kim and Kim 2018). Thus, Liliaceae has no latescent representative.

Ranunculales

Lardizabalaceae

Lewinsohn (1991) mentions the presence of laticifers only in the pericarp of *Descainea fargesii* (Lardizabalaceae); however, the species has resin ducts (Zhou and Liu 2011).

There is much controversy in the literature regarding the presence of laticifers and misinterpretations regarding the presence of laticifers due to the similarity of their exudate and often their color with latex. However, both structures differ in relation to their origin, structure, and how they store the secretion (Prado and Demarco 2018). The use of the term "laticiferous duct" (Zhou and Liu 2011) is not correct either. Even if both structures could produce the same chemical classes of metabolites, latex is an intracellular secretion, while duct secretion is extracellular.

Proteales

Nelumbolaceae

Nelumbolaceae consists only of *Nelumbo*, which has two species, *N. nucifera* and *N. lutea,* described as having articulated laticifers (Fig 45 c-d) distributed in the

cortex and vascular system (Williamson and Schneider 1993). Our analysis has shown that *Nelumbo* has only phenolic and non-laticiferous idioblasts.

Saxifragales

Peridiscaceae

There is controversy in the literature regarding the presence of latex in Peridiscaceae. Lewinsohn (1991) cites the genus *Medusandra* (Soltis *et al.* 2007) as being latescent; however, Metcalfe and Chalk (1950) and Metcalfe (1962) refute this by stating that the genus has resin ducts associated with vascular bundles. Our analysis found that *Peridiscus*, a sister genus of *Medusandra* (Breteler *et al.* 2015), has no laticifers either. Thus, there are no records of laticifers in the family.

Malpighiales

Clusiaceae

Clusiaceae consists of about 37 genera that characteristically have resin ducts (Metcalfe and Chalk 1950, Gustafsson *et al.* 2002). However, many studies refer to the species as being latescent (Lewinsohn 1991, Diniz *et al.* 1999, Notis 2004). In a previous study, we corroborated that the family has resin ducts that were mistaken for laticifers because the color of the exudate is eventually milky white (Prado and Demarco 2018).

Calophyllaceae

In the past, *Kielmeyera* (Calophyllaceae) has been recorded as being latescent (Lewinsohn 1991) due to its white-colored exudate. However, as with Clusiaceae, the family has resin ducts only, which are erroneously identified in some studies as laticiferous ducts (Prado and Demarco 2018).

Podostemaceae

Family of aquatic plants found in tropical habitats (Rutishauser 1997), composed of approximately 48 genera divided into three subfamilies – Tristichoideae, Weddellinoideae and Podostemoideae (Ruhfel *et al.* 2016).

Studies by Grubert (1974), Rutishauser and Grubert (1999) report the presence of laticifers in *Mourera fluviatilis* peduncles, which releases a milky white exudate when the plant is sectioned. However, further studies are needed to confirm the structure.

Achariaceae

We have investigated *Hydnocarpus* in this work, which currently belongs to the Achariaceae family, because it was included in the former Flacourtiaceae family, which was dismembered but had latescent representatives. However, our analysis has shown no laticifers in the genus, corroborating its current position in Achariaceae and keeping the family as non-latescent.

Salicaceae

Salicaceae is composed of 54 genera according to the current circumscription, many of which came from the former Flacourtiaceae family, amongst which *Casearia, Laetia* and *Xylosma* (Sun *et al.* 2016). Lewinsohn (1991) mentions that some of the former Flacourtiaceae genera of the family are latescent, such as *Casearia* and *Plagiopteron*. Our study analyzed samples of *Casearia* (= *Laetia*), *Xylosma* and *Salix* and the presence of resin ducts and cavities, but no laticifers, was verified. Thus, we do not confirm the presence of latex in the family.

Phyllanthaceae

Phyllanthaceae consists of 59 genera and has had laticifers recorded by Balaji *et al.* (1996) in *Phyllanthus*. However, we have found no laticifers in the genus, only phenolic idioblasts.

Rosales

Ulmaceae

Ulmaceae is composed of six genera (Sytsma *et al.* 2012, Sun *et al.* 2016) found in temperate and tropical habitats (Sweitzer 1971). The first record of laticifers in the family was made by Leme (2019) in the genera *Ampelocera* and *Zelkova*, considering the occurrence of laticifers throughout the Urticalean clade. However, the data presented is not sufficient to confirm the existence of laticifers in both genera and,

in our study, we have analyzed species of *Ulmus* (Fig 45 e), noting there are no laticifers in the genus, but only phenolic and mucilaginous idioblasts. Apparently, laticifers are absent in the family, but further studies are needed to establish their occurrence in Ulmaceae.

Myrtales

Myrtaceae

Throughout the order Myrtales, laticifers have only been reported for Myrtaceae (Lewinsohn 1991), in the current genera *Angophora*, *Corymbia* and *Eucalyptus gummifera*, all of which belonging to the Myrtoideae subfamily, Eucalypteae tribe.

According to Metcalfe and Chalk (1950), *Eucalyptus* has oil cavities whose epithelium is not very conspicuous, and *Eucalyptus gummifera* would have a secretion that resembles rubber, thus being associated with the occurrence of latex. We have investigated *Eucalyptus gummifera, E. globulus, Corymbia ptychocarpa* and *Angophora costata* (Fig 45 h) and have refuted the occurrence of latex, since all species had only oil cavities, phenolic idioblasts and sclereids, as all other species of the genus did.

Sapindales

Burseraceae

Burseraceae is a family known for having resin ducts (Metcalfe and Chalk 1950), but there is disagreement in the literature regarding the presence of latex in some species due to their milky white secretion, especially in *Protium* (Zapata and Fine 2013, Prado and Demarco 2018), or due to the misinterpreted occurrence of latex in ducts (Lewinsohn 1991, Siani *et al.* 2004, Swanepoel 2011).

Anacardiaceae

As with Burseraceae, descriptions of latex in Anacardiaceae (Lewinsohn 1991) are due to the color of some resins, especially in *Toxicodendron* (Fig 45 g) and *Schinus,* or the misinterpretation of ducts as laticifers (Prado and Demarco 2018). Anacardiaceae, like its sister group Burseraceae, has only resin ducts and phenolic idioblasts as internal secretory defense structures.

Malvales

Bixaceae

The Bixaceae family has four genera (Johnson-Fulton and Watson 2017) found in pantropical habitats (Kirizawa and Abreu 2002), and although Metcalfe and Chalk (1950) have shown that the family has secretory ducts in the cortex and vascular system, they have also been confused for laticifers by some authors (Lewinsohn 1991).

Our analyses of *Bixa orellana* (Fig 45 h) have shown that the species has oil cavities that coalesce, forming elongated ducts in the cortex, medulla and vascular system.

Brassicales

Tropaeolaceae

Tropaeolaceae is a monotypic neotropical family in which former genera *Magallana* and *Trophaeastrum* were synonymized with *Tropaeolum* (Ronse Decraene 2001). We have found that *Tropaeolum pentaphyllum* has only phenolic idioblasts in the phloem, with no laticifers.

Santalales

Strombosiaceae

Strombosiaceae consists of six genera (Sun *et al.* 2015) that were included in tribe Anacoloseae of Olacaceae (Malécot *et al.* 2004). Our analyses of *Tetrastylidium* identified only the presence of phenolic idioblasts.

Ximeniaceae

Ximeniaceae has four genera of parasitic plants (Sleumer 1984, Kuijt and Hansen 2015, Su *et al.* 2015) and was recognized as tribe Ximenieae in Olacaceae (Malécot *et al.* 2004, Su *et al.* 2015). We have analyzed *Ximenia* and determined that secretory cells, including laticifers, were absent.

Olacaceae

Initially, Olacaceae consisted of 28 genera divided into six tribes (Baas *et al.* 1982, Malécot *et al.* 2004), but the vast majority were segregated to form new families in the order. Currently, Olacaceae has only three genera: *Olax, Dulacia* and *Ptychopetalum* (Kuijt and Hansen 2015). *Ptychopetalum* and *Olax* are parasitic plant genera (Sleumer 1984, Su *et al.* 2015), which were sampled in our analysis. Neither genus has laticifers. They have only phenolic idioblasts for internal secretory structures.

Schoepfiaceae

Schoepfiaceae, a former Olacaceae subfamily (Malécot *et al.* 2004), has currently three genera (Kuijt and Hansen 2015). *Schoepfia* is a parasitic plant genus (Baas *et al.* 1982, Sleumer 1984) which, in our analysis, has shown to have only elongated-shape phenolic idioblasts distributed in the perivascular region.

Loranthaceae

This is the largest family in the order, with 77 genera of parasitic plants (Kuijt and Hansen 2015, Liu *et al.* 2018). Lewinsohn (1991) indicated the presence of latex in *Phthirusa* and *Struthanthus*. However, we have only identified the occurrence of phenolic idioblasts in both genera (Fig 45 i).

Cornales

Cornaceae

The family is found in predominantly temperate habitats, distributed in the northern hemisphere, with centers of diversity in eastern Asia, eastern North America, and western North America, consisting mainly of trees and shrubs (Fan and Xiang 2001).

We have analyzed *Cornus racemosa* and found that the species contain only phenolic idioblasts (Fig 45 j).

Caryophyllales

Cactaceae

Cactaceae consists of 127 genera and is recognized for its remarkable adaptation to dry regions (Barthlott and Hunt 1993, Hernández-Hernández *et al.* 2011) due to the presence of mucilage secretory structures (Metcalfe and Chalk 1950, Barthlott and Hunt 1993). However, *Mammillaria* has a milky white secretion, a fact that led Metcalfe and Chalk (1950), Mauseth (1978 a, b), Boke (1980), Wittler and Mauseth (1984), and Barthlott and Hunt (1993) to describe the ducts as articulated laticifers in the medulla and cortex of species of this genus. Mauseth (1978 a, b) further considered that laticifers would have evolved independently from other latescent families due to their entirely different development. However, Prado and Demarco (2018) demonstrated that the genus has resin ducts and the milky white color of the secretion is due to its heterogeneous composition.

Icacinales

Icacinales belongs to Lamiids, comprising two families with 23 genera (Stull *et al.* 2015). Metcalfe (1967) cites laticifers in the family, precisely in *Cardiopteris lobata*; however, molecular studies have redefined the family and many genera were transferred to other families, such as *Eucommia, Discophora, Dendrobangia* and *Cardiopteris* (Kårehed 2001, Lens *et al.,* 2008). Thus, Icacinales has no latescent representatives.

Metteniusales

Metteniusaceae

Metteniusaceae has ten genera (Savolaine *et al.* 2000, Stull *et al.* 2015), amongst which *Dendrobangia*, a genus that once belonged to Icacinaceae and could have laticifers. However, we have found that *Dendrobangia* (Fig 45 k) has very wide and extensive phenolic idioblasts distributed in the cortex and in the medulla, and minor phenolic idioblasts associated with the phloem. Although morphologically distinct, histochemical analysis has shown that their secretion cannot be considered as latex, therefore, they are not laticiferous.

Garryales

Garryaceae

Garryaceae has only two genera: *Garrya* and *Aucuba*, and forms a clade with Eucommiaceae (Soltis *et al.* 2000, Liston 2003, Burge 2011), which is latescent. Stull *et*

al. (2015) state that the presence of latex would be a synapomorphy of the order, however, *Garrya* has resin ducts and we did not detect any secretory cells in *Aucuba*.

Aquifoliales

Stemonuraceae

Stemonuraceae is composed of 12 genera (Potgieter *et al.* 2016) and has been studied because it forms a clade with Cardiopteridaceae, which is latescent. However, we have not identified any internal secretory cells in *Discophora*.

Aquifoliaceae

Aquifoliaceae is formed solely by genus *Ilex*, which was segregated from Icacinaceae (Karehed 2001). We have found that *Ilex* has only phenolic idioblasts in its cortex and vascular system.

Helwingiaceae

Helwingiaceae consists only of genus *Helwingia*, which also derives from Icacinaceae (Karehed 2001, Xiang 2016). Our analyses have not identified the presence of any secretory cells in the genus.

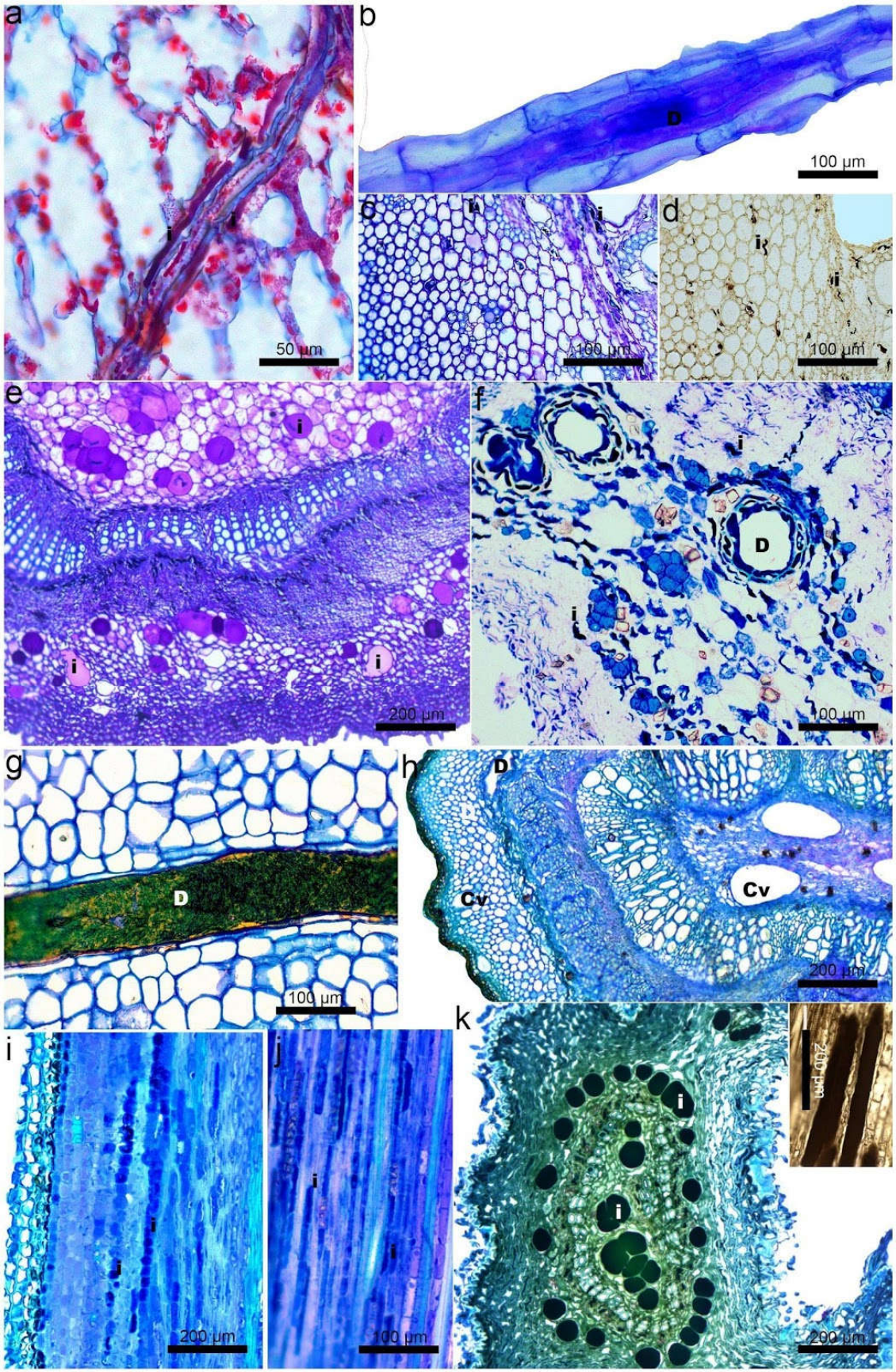
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Fig 45: Non-latescent genera – Salviniaceae (Salviniales) – *Salvinia* sp.: a. Longitudinal section indicating the presence of phenolic idioblasts; Alismataceae (Alismatales) – *Sagittaria montevidensis:* b. Longitudinal section indicating the presence of a resin duct; Nelumbonaceae (Proteales) – *Nelumbo nucifera:* c. Cross section indicating the presence of phenolic idioblasts; d. Ferric chloride test – Cross section indicating the presence of phenolic idioblasts; Ulmaceae (Rosales) – *Ulmus glabra*: e. Longitudinal section indicating the presence of phenolic idioblasts; Myrtaceae (Myrtales) – *Angophora costata*: f. Cross section indicating the presence of resin ducts and phenolic idioblasts; Anacardiaceae (Sapindales) – *Toxicodendron*sp.: g. Longitudinal section indicating the presence of resin ducts; Bixaceae (Malvales) – *Bixa orellana*: h. Cross section indicating the presence of resin cavities; Loranthaceae (Santalales) – *Phthirusa podoptera:* i. Longitudinal section indicating the presence of phenolic idioblasts; Cornaceae (Cornales) – *Cornus racemosa:* j. Longitudinal section indicating the presence of phenolic idioblasts; Metteniusaceae (Metteniusales) – *Dendrobangia boliviana*: k. Cross section indicating the presence of phenolic idioblasts; l. Ferric chloride test – Longitudinal section indicating phenolic composition; (i – Idioblasts; D – Resin duct; Cv – Resin cavity).

4. Distribution in plant body

Latescent plants have laticifers in all their organs and, in most groups, laticifers are present in the fundamental system, in the middle of the parenchyma, with a higher concentration near the vascular system, especially near the phloem. We have also observed that fundamental laticifers usually occur in the cortex and medulla or in the cortex only; medullar-only laticifers are very rare, as seen in *Mimosa* (Fabaceae).

Laticifers can also be vascular, and phloem laticifers are very common. Those that occur in both the phloem and xylem are less frequent, and exclusively xylemic laticifers are extremely rare, as observed in *Amorphophallus* (Araceae).

Regarding secondary laticifers, plants that have vascular laticifers formed from the procambium usually also have secondary laticifers, i.e., formed from the cambium. However, we have observed a small number of cases displaying the formation of secondary vascular laticifers (usually in the phloem) in plants that did not have them in the primary vascular system, such as in Sapindaceae.

5. Ontogeny

The ontogenesis or development of laticifers involves two processes: 1) the formation, which corresponds to the addition (or not) of cells to the laticifer and their corresponding differentiation; and 2) the growth, which corresponds to cell expansion and modifications of format.

Formation

According to the most widely used classification (by Bary 1884), laticifers could be formed in two ways: 1) from a single cell, classified as non-articulated; 2) from the addition of cells forming a row, classified as articulated.

Subtypes were established to explain the diversity of laticiferous systems observed in plants. Non-articulated laticifers could be branched or unbranched, and articulated laticifers could be anastomosed (with total or partial dissolution of the end walls) or non-anastomosed (Ramos et al, 2019). Although the classification is simple, our study has identified that laticifers can be formed in a wide variety of manenrs, since anastomosed articulated laticifers can lose their end walls completely, forming continuous laticifers (*sensu* Milanez 1978), and having exactly the same morphology of laticifers described as non-articulated. Thus, the only safe way to classify laticifers is by means of ontogenetic analysis. However, this analysis is not simple, since in families such as Apocynaceae, Euphorbiaceae and Moraceae the addition of cells to the laticifer is rapidly followed by the dissolution of their end walls, and few cells with intact walls being found close to the apex of the laticifers in these families (Demarco *et al*. 2006, 2013; Demarco and Castro 2008; Gama *et al.* 2017).

Recent studies have shown that the misidentification of the type of laticifer arises from the fact that the end walls of laticifers from families such as Apocynaceae,

Euphorbiaceae and Moraceae, among others, are dissolved very early during cell differentiation, while still among the very meristems from which they originated. These misconceptions are widely observed in Apocynaceae (Metcalfe 1967, Inamdar *et al.* 1988, Mahlberg 1993) and Euphorbiaceae (Rudall 1989, 1994, Wiedenhoeft *et al.* 2009, Vitarelli *et al.* 2015), among other families such as Eucommiaceae, Cannabaceae, Moraceae and Urticaceae (Biesboer and Mahlberg 1981, Furr and Mahlberg 1981, Van Veenendaal and Den Outer 1990).

Divergences in the literature have raised doubts about the real distribution of the different types of laticifers.

In our studies, we have analyzed 119 species (Table S1) from 63 families of vascular plants (Table 1) and have found that they all have anastomosed or non-anastomosed, branched or unbranched articulated laticifers (Fig 46 a-e). We have paid special attention to the analysis of families recorded as having laticifers of the latter type, including species used as models for their description. However, ontogenetic analysis has shown that all species have articulated laticifers with early anastomosis of their separating walls (Fig 11 a b, Fig 17 a, Fig 21 c-e, Fig 25 a-c e-j, Fig 31 a c, Fig 39 a c d). Just as the early union of cells led many authors to classify laticifers erroneously for decades, the late anastomosis observed in Convolvulaceae (Fig 41 d) has also given rise to several misconceptions.

Although today we have verified the absence of non-articulated laticifers, many articles have been published in the past and can still be found in the recent literature about this type of laticifer and its unusual growth for a plant cell. In search of an explanation of how a single cell could grow indefinitely through the body of a plant, a number of theories have been devised by several authors (see historical synthesis in Mahlberg 1993). The central theory found in many works is that the non-articulated

laticifer would not be a unicellular structure, but rather a cenocytic structure derived from a predetermined number of laticifer initials present in the embryo, which would undergo mitosis without cytokinesis. (Mahlberg 1993). However, in all species where laticifers have been previously classified as non-articulated we have verified that they are constantly produced in the plant's meristematic regions. Furthermore, we have demonstrated that they are all formed by cell addition (Fig 46 a-b), with subsequent dissolution of the end walls and fusion of protoplasts, resulting in a syncytial structure with as many nuclei as the cells that formed them (present study; Demarco *et al.* 2006, 2013, Demarco and Castro 2008; Gama *et al.* 2017).

Just as anastomosis occurs among the cells of the laticifer row, laticifers of some plant groups anastomose laterally with other laticifers forming branches, the interconnection of which can form a unique and sinuous system throughout the plant body, as noted in Apocynaceae, Euphorbiaceae, Moraceae and Urticaceae (Fig 39 d, 46 c-e) (Demarco *et al.* 2006, 2013, Demarco and Castro 2008). When there are no branches, laticifers remain in continuous rows as seen in Cannabaceae (Fig 25 a, Leme 2018) and Convolvulaceae (Fig 41 c, Fig 46 b, Metcalfe and Chalk 1950, Fineran *et al*. 1988, Condon and Fineran 1989).

Another variation observed in laticifers regards their length. Laticifers can be extremely long and branched, forming an anastomosed network that connects all laticifers in the organ or even in the whole plant, but can also be extremely short with only two cells. These short laticifers are usually unbranched, non-anastomosed and although they do not form a pattern in any family, some genera are characterized by having laticifers that are only two to ten cells long, as seen in *Phillodendron* (Araceae) (Fig 9 c) and *Musa* (Musaceae) (Fig 46 a), or with countless cells as observed in *Ipomoea* (Convolvulaceae) (Fig 41 c; Fig 46 b).

Order	Family	Subfamily/ Tribe	Genus	Laticifer	Reference
Salviniales	Marsileaceae	-	Whole family	+	Present work (*Marsilea, Pilularia*); Laboriau 1952, Kramer 1990 (*Regnellidium*)
	Salviniaceae	-	Whole family	-	Present work (*Azolla; Salvinia*)
Gnetales	Gnetaceae	-	*Gnetum*	+	Present work
Nymphaeales	Nymphaeaceae	Nymphaeoideae	*Nymphaea*	+	Present work
	Cabombaceae	-	*Cabomba*	-	Present work
			Brasenia	+	
Alismatales	Araceae	Aroideae	*Amorphophallus*	+	Present work
			Phillodendron	+	
	Alismataceae	*Sagittaria* clade	*Sagittaria*	-	Present work
			Limnocharis	-	
	Aponogetonaceae	-	*Aponogeton*	+	Present work
	Juncaginaceae	-	*Triglochin*	+	Present work
			Tetroncium	-	
			Lilaea	-	
Pandanales	Cyclanthaceae	Cyclanthoideae	*Cyclanthus*	+	Present work
Asparagales	Amaryllidaceae	Alloideae – Alliae	*Allium*	+	Present work
		Alloideae – Gilliesieae	*Tristagma*	-	Present work
			Nothoscordum	-	
		Agaphanthoideae	*Agaphanthus*	-	Present work
Zingiberales	Musaceae	-	Whole family	+	Present work
Ranunculales	Lardizabalaceae	Lardizabaloideae	*Decaisnea fargesii*	-	Zhou and Liu 2011
	Papaveraceae	Papaveroideae-Papavereae	Whole tribe	+	Present work (*Argemone*); Hoot *et al.* 2015
		Papaveroideae – Chelidonieae	Whole tribe	+	Present work (*Chelidonium, Sanguinaria*); Hoot *et al.* 2015

		Pavaveroideae-Fumarioideae	Whole tribe	+	Hoot *et al*. 2015
Proteales	Nelumbolaceae	-	*Nelumbo*	-	Present work
Saxifragales	Peridiscaceae	-	*Medusandra*	-	Metcalfe and Chalk (1950); Metcalfe 1962
			Peridiscus	-	Present work
Celastrales	Celastraceae	Euonymoideae	*Euonymus*	-	Present work
		Salacioideae	*Salacia*	-	Present work
			Tontelea	+	
			Cheiloclinium	+	Gomes and Lombardi 2010
		Hippocrateaoideae	*Hippocratea*	-	Present work
Malpighiales	Clusiaceae	Clusieae	*Clusia*	-	Present work
	Calophyllaceae	Calophylleae	*Kielmeyera*	-	Present work
	Podostemaceae	Podostemoideae	*Mourera*	-*	Grubert (1974); Rutishauser and Grubert (1999) – references reports the opposite
	Elatinaceae	-	-	-*	Pace *et al.* 2019 - references reports the opposite
	Malpighiaceae	Byrsonimoideae	*Byrsonyma*	-	Present work
		Malpighioideae-*Galphimia* Clade	*Spachea*	-	Present work (*Spachea, Lophanthera*) Vega *et al.* 2002 (*Verrucularia, Galphimia*)
			Lophanthera	+	
			Verrucularia	+	
			Galphimia	+	
		Malpighioideae-Tetrapteroids Clade	*Tetrapterys*	+	Pace *et al.* 2019
		Malpighioideae-*Stigmaphyllon* Clade	*Stigmaphyllon*	+	Pace *et al.* 2019
	Achariaceae	Erythrospermeae - Pangieae	*Hydnocarpus*	-	Present work

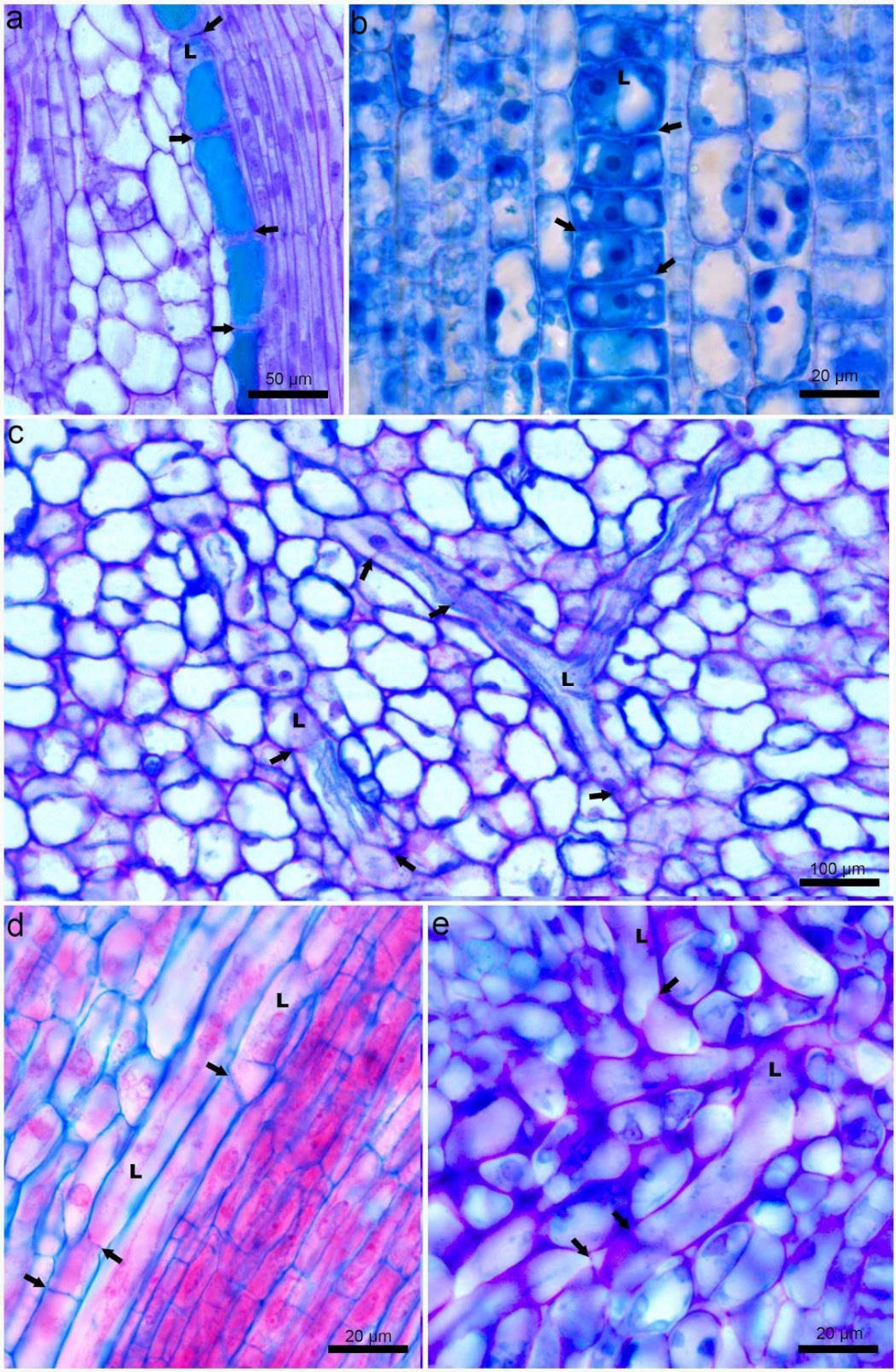
	Salicaceae	Salicoideae – Saliceae	*Salix*	-	Present work
			Xylosma	-	
		Samydoideae	*Caseria*	-	Present work
	Euphorbiaceae	Acalyphoideae	*Acalypha*	-	Present work
		Crotonoideae	Whole subfamily	+	Present work (*Croton*); Rudall 1987
		Euphorbioideae	Whole subfamily	+	Present work (*Euphorbia, Hura*); Rudall 1987
		Phyllanthoideae-Phyllantheae	Whole subfamily	-	Present work (*Phyllanthus*); Gama *et al.*2016; Cardoso-Gustavson *et al.* 2011)
Fabales	Fabaceae	Caesalpinioideae-Mimosid Clade	*Mimosa*	+	Present work
		Papilionoideae – Millettieae	*Lonchocarpus;*	+	Present work
			Dahlstedia	+	
		Papilionoideae – Phaeseoleae	*Erythrina*	-	Present work
Rosales	Ulmaceae	-	*Ulmus*	-	Present work
			Ampelocera	-	Leme 2019 – reference reports the opposite
	Cannabaceae	-	Whole family	+	Present work (*Cannabis, Humulus*) Leme 2019
	Moraceae	Moreae Ficeae Artocarpeae Maclureae Dorstenieae	Whole family	+	Present work (*Ficus, Morus*); Marinho and Teixeira 2019
	Urticaceae	Cecropieae	Cecropia	+	Marinho and Teixeira 2019
			Pourouma	-	Present work
			Myrianthus	-	

		Urticeae	Whole tribe	+	Present work (*Laportea, Urera*); Guérin 1905; Gaglioti 2011
Myrtales	Myrtaceae	-	Whole family	-	Present work (Myrtoideae – Eucalypteae- *Angophora, Corymbia, Eucalyptus*)
Sapindales	Burseraceae	-	Whole family	-	Present work (*Protium*); Metcalfe and Chalk 1950
	Anacardiaceae	-	Whole family	-	Present work (*Toxicodendron, Schinus*); Metcalfe and Chalk 1950
	Sapindaceae	Dodonaeoideae	*Dodoneae*	-	Present work
		Hippocastanoideae – Hippocastaneae	*Billia*	-	Present work
			Aesculus	-	
		Sapindoideae - Paullinieae	Whole tribe (except for some *Cardiospermum* sp.)	+	Present work (*Urvillea, Serjania, Lophostigma*); Montes 2017
Malvales	Bixaceae	-	Whole family	-	Present work (*Bixa*); Metcalfe and Chalk 1950
Brassicales	Tropaeolaceae	-	*Tropaeolum*	-	Present work
	Caricaceae	-	Whole family	+	Present work (*Carica*); Miller 1982
Santalales	Erythropalaceae	-	*Heisteria*	+	Present work
	Strombosiaceae	-	*Tetrastylidium*	-	Present work
	Coulaceae	-	*Coula*	+	Present work
			Minquartia	+	
	Ximeniaceae	-	*Ximenia*	-	Present work
	Aptandraceae	-	*Aptandra*	+	Baas *et al.* 1982

			Harmania	+	
	Olacaceae	-	*Ptychopetalum*	-	Present work
			Olax	-	
	Schoepfiaceae	-	*Schoepfia*	-	Present work
	Loranthaceae	-	*Struthanthus*	-	Present work
			Phthirusa	-	
Caryophyllales	Cactaceae	-	*Mammillaria*	-	Present work
Cornales	Cornaceae	-	*Cornus*	-	Present work
	Alangiaceae	-	*Alangium*	+	Present work
	Nyssaceae	Nistoid Clade	*Camptotheca*	+	Present work
Ericales	Sapotaceae	Sapotoideae	*Manilkara*	+	Present work
		Chrysophylloideae	*Pouteria*	+	Present work
Metteniusales	Metteniusaceae	-	*Dendrobangia*	-	Present work
Garryales	Eucommiaceae	-	*Eucommia*	+	Present work
	Garryaceae	-	Whole family	-	Present work (*Garrya, Aucuba*)
Gentianales	Rubiaceae	Cinchonoideae - Mussaendeae	*Mussaenda*	+	Present work
	Apocynaceae	-	Whole family	+	Present work (*Mandevilla*); Demarco iet al. 2006, 2013; Demarco and Castro 2008
Solanales	Convolvulaceae	Convolvuloideae – Ipomoeeae	Whole tribe	+	Present work (*Ipomoea*)
		Convolvuloideae – Convolvuleae	Whole tribe	+	Present work (*Convolvulus, Calystegia*)
		Dichondreae	*Dichondra*	-	Present work
Aquifoliales	Cardiopteridaceae	-	*Cardiopteris*	+	Thouvenin 1891

			Citronella	-	Present work
	Stemonuraceae	-	*Discophora*	-	Present work
	Aquifoliaceae	-	*Ilex*	-	Present work
	Helwingiaceae	-	*Helwingia*	-	Present work
Asterales	Campanulaceae	Lobelioideae	Whole family	+	Present work (*Hippobroma*); Carlquist 1969
	Asteraceae	Mutisioideae	*Acourtia*	-	Present work
		Carduoideae	*Centaurea*	-	Present work
		Cichorioideae	*Lactuca*	+	Olson *et al.* 1969
			Cichorium	+	Vertrees and Mahlberg 1978
			Taraxacum	+	Vertrees and Mahlberg 1978
		Asteroideae	*Parthenium*	+	
			Chrysothamnus	+	Metcalfe 1967
			Hymenoxys	+	

Table 1: Description of latescent orders and families; (plus – presence of laticifers; minus – absence of laticifers; asterisk with minus (- *) – our analysis from the works described).


a
L
50 µm
b
L
20 µm
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20 µm

Fig 46: Ontogenesis of laticifers from shoot apical meristems. a-b. Unbranched, non-anastomosed laticifers. a. *Musa paradisiaca*; b. *Ipomea tricolor*. c-e. Branched anastomosed laticifers. c. *Euphorbia milii*; d. *Morus nigra*; e. *Urera baccifera*. (**L** – Laticifer; arrow – end wall).

Our study has shown that all laticifers are composed of rows of cells and disproves the existence of non-articulated laticifers. This classification, widely used throughout the 130-year old history of the study of laticifers, cannot be sustained in light of current accurate ontogenetic analyses. In view of the present result, we propose abandoning the differentiation between articulated and non-articulated laticifers and classifying laticifers solely as anastomosed or non-anastomosed, branched or unbranched. Although we cannot exclude the hypothesis that a unicellular laticifer might exist in a plant that has not been analyzed yet, if it exists, it will be the size and shape of an idioblast.

Growth

Another topic of divergence in the study of laticifers is the way in which they grow through the plant. Initially, non-articulated laticifers derived from early embryonic laticifers were described as having intrusive autonomous growth (Metcalfe 1967, Mahlberg 1993, Rudall 1994). This description would explain why the structure would grow through intercellular spaces like a fungus hyphae and, when there was no intercellular space, the laticifer apex would produce enzymes to digest the middle lamella and penetrate between cells (Mahlberg 1993 and references therein). This theory was later expanded to include some articulated laticifers, such as in *Hevea brasiliensis* (Rudall 1987) and Apocynaceae (Canaveze and Machado 2016, Canaveze *et al.* 2019), Moraceae and Urticaceae (Marinho and Teixeira 2019). This theory is mainly due to the observation of supposed acute apices of some laticifers (Fig 47 a) and the presence of pectinase activity in the apical region of laticifers (Wilson *et al.* 1976, Allen and Nessler

1984). However, careful analysis of serial sections shows that all laticiferous apexes have straight end walls, as do the other cells in the meristem from which they originated. The observation of supposed acute apexes is due to the sinuous path of most laticifers. Thus, in regions where the laticifer changes its growth direction the cell is obliquely sectioned and appears to have an acute apex, but in fact, the laticifer apex is in another plane or layer of cells (Fig 47 a-d) (Demarco *et al.* 2006, Demarco and Castro 2008).

Pectinase activity has been previously identified in the laticifer wall in its apical portions, as well as in its vacuole, and is associated with loosening of the cell wall, increasing its plasticity and degradation of the middle lamella, facilitating the intrusive growth of the laticifer among the other cells (Wilson *et al.* 1976, Allen and Nessler 1984). However, this enzymatic activity is related to the degradation of the end walls of anastomosed laticifers, as observed in *Papaver* (Nessler and Mahlberg 1981), and its presence in the vacuole is due to the wall digestion product that is translocated to the vacuole (Giordani 1980).

Immunocytochemical studies have identified the presence of wall compounds specific to laticifers, or that occur in different proportions in laticifers relative to other plant cells, and relate these differences to the occurrence of intrusive growth (Serpe e*t al.* 2001, 2002, 2004, Canaveze and Machado 2016, Canaveze *et al.* 2019). These works actually show that laticifers have walls with particular characteristics, but have no relation with possible intrusive growth. Previous studies have shown that laticifer walls have inherent characteristics related to cell expansion (Demarco *et al*. 2006), but this does not imply intrusive growth. Other substances may be present in the laticifer wall, such as suberin in the laticifers of Convolvulaceae (Fineran *et al.* 1988) and some Sapindaceae and Urticaceae genera (present study, Montes 2017), callose in some

Sapindaceae genera (Montes 2017) and in some species of *Allium* (present study, Currier 1957).

Intrusive autonomous growth would only be possible by means of a specific subcellular organization of the laticifer apical cell capable of continuously producing walls in a polarized manner, as well as enzymes to digest the middle lamella of adjacent cells. However, this cellular apparatus is not found at the apex of laticifers (Gama *et al.* 2017). In addition, laticifers are always strongly adhered to adjacent cells by the middle lamella, without any intercellular space, and the independent growth of surrounding cells is not possible (Demarco 2015).

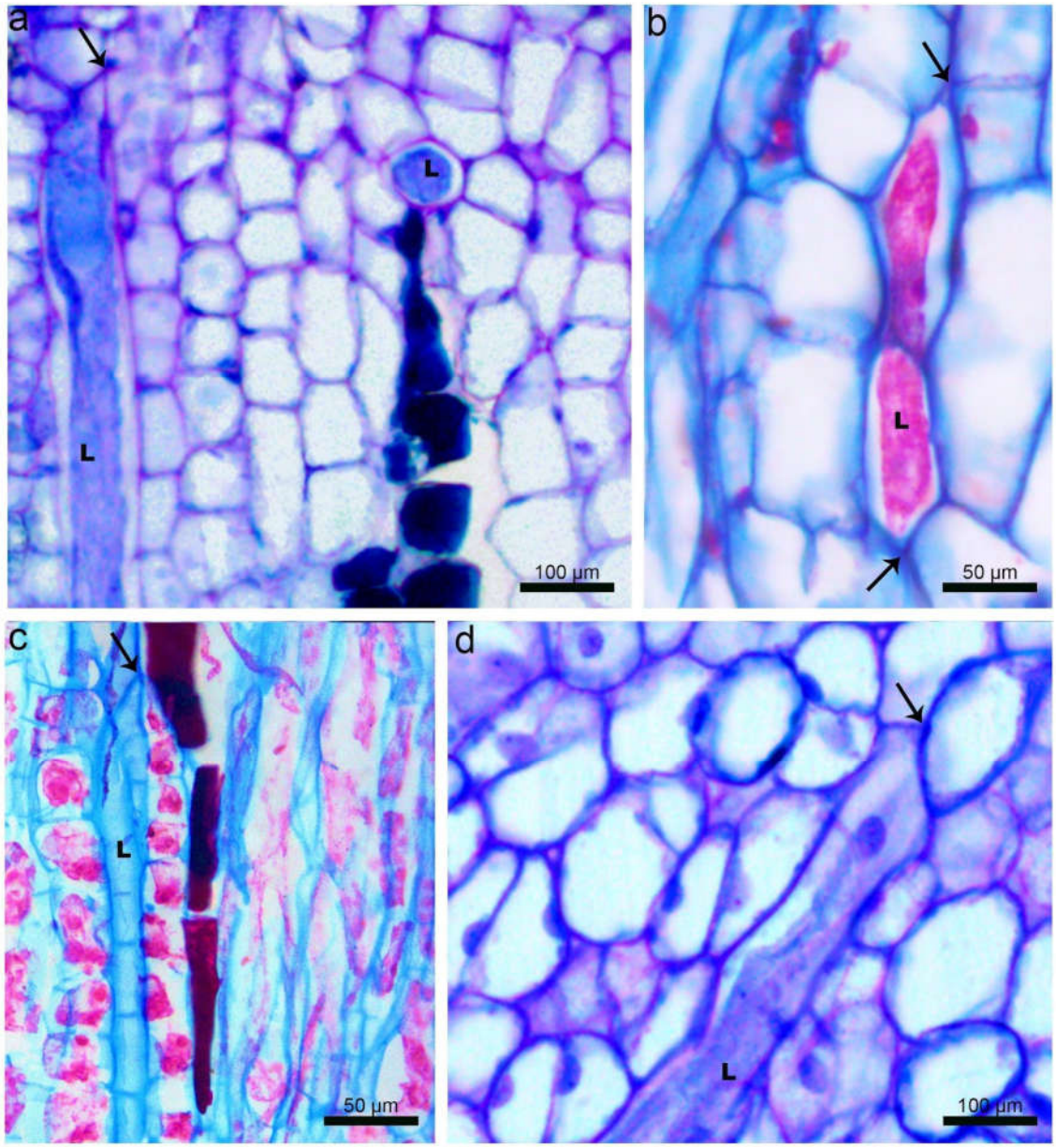

Fig 47: Apex of laticifers from different families. Laticifers formed in different directions in a. *Ipomoea tricolor*; b. *Carica papaya*; c. *Mandevilla splendens*; d. *Euphorbia milii*; (L – Laticifer; thin arrow – supposed acute apex of laticifer).

After analyzing the laticifers of all vascular plant groups, we found no evidence of intrusive autonomous growth. However, histological studies have come to differing conclusions regarding the observation of the same results, therefore, more specific studies involving enzymatic and molecular analysis are needed to verify whether intrusive autonomous growth actually occurs in some laticifers.

6. *Latex and its functions*

The primary function of latex is to defend the plant from herbivory (Prado and Demarco 2018) due to the high toxicity of its compounds (Konno 2011, Dussourd 2017). In addition to acting as a chemical barrier against herbivores, latex also acts as a physical barrier when it coagulates, rapidly sealing wounds and preventing the entry and proliferation of pathogens such as bacteria and viruses (Wink 2008, Konno 2011, Bauer *et al.* 2014, Demarco 2015, Dussourd 2017).

Latex is the product of laticifers and corresponds to its entire protoplast (Demarco 2015, Chapter 2), because when latex overflows, not only does it release primary and secondary metabolites, but also plasma membrane, organelles, and nuclei (Chapter 2). Laticifers produce the largest diversity of metabolites among all secretory structures. Latex has mostly terpenes in its composition (Prado and Demarco 2018; Chapter 2), with a predominance of triterpenes, tetraterpenes or polyisoprenes (rubber), depending on the species analyzed. Latex also has all other chemical classes of compounds in less quantity, forming an emulsion and giving a heterogeneous appearance to this secretion. Among the metabolites, we should highlight carbohydrates (including mucilage and starch grains in some Euphorbiaceae (Biesboer and Mahlberg 1981) and Cannabaceae (Leme 2018) phenolic compounds, alkaloids, fatty acids, cardenolides, and others (Chapter 2, 1979 Fahn 1979 Konno 2011, Demarco *et al.* 2013, Demarco 2015).

Latex will have different properties and composition depending on the group of plants analyzed (Prado and Demarco 2018), and each class of metabolites will act to defend the plant (Bennett and Wallsgrove 1994). However, it is important to emphasize

the synergy of these compounds, demonstrating their boosted toxicity (Wittstock and Gershenzon 2002), as in the case of terpenes associated with phenolic compounds (Wink 2008).

If compared, the composition of latices from different plant groups contains all or almost every major chemical classes of secondary metabolites. Despite variations in metabolite amounts, such compositions are generally similar, and their evolution in different groups is shown to be homoplasic (Chapter 2).

7. Resin Ducts and Idioblasts

There is much divergence in the literature regarding the definition of secretory tissues; however, our study clarifies the main issues involving laticifers, resin ducts and secretory idioblasts.

Secretory structure classification is based on anatomical and histochemical analyses that establish the structure's origin, its storage mode, and the composition of its secretion (Demarco 2017).

Resin ducts are similar to laticifers in their predominantly terpenoid composition as well as in the color and appearance of the secretion, but differ in origin, structure and storage mode. Resin ducts are formed by an epithelium that delimits an intercellular space called lumen, in which the secretion produced will be stored (Fig 45 b, f-h, Prado and Demarco 2018).

Secreting idioblasts are specialized cells that secrete their compound internally (Fahn 1979, Evert 2006) and because of their different morphology in some families, they have been confused with laticifers (Metcalfe and Chalk 1950, Lewinsohn 1991, Williamson and Schneider 1993, Balaji *et al.* 1996, Gomes *et al.* 2005, Leme

2019). Idioblasts will differ significantly regarding the composition of their secretion, producing a phenolic compound, essential oils or mucilages (Demarco 2017).

We have used Sudan black in our study to determine the presence of lipidic substances verified in resin ducts and laticifers (Fig 21b-c; Fig 25 f-g; Fig 37 c, Fig 39 c; Fig 41 d-e) and ferric chloride for the detection of phenolic compounds found in idioblasts (Fig 45 d, l). Such tests produced best results when performed on dried material, thus making it possible to analyze the compound in these structures.

8. Ecological Implications

Latescent plants demonstrate to have greater evolutionary success over non-latescent plants, which is a characteristic that increases plant resistance in environments where herbivores are present (Lewinsohn 1991, Karban & Baldwin 1997, Agrawal & Konno 2009). However, although it has a wide variety of compounds that can be toxic or dissuasive as food, latex does not fully protect against herbivory (Wink 2008), since the evolution of latescent plants took place in parallel with the evolution of herbivores. Some herbivores became specialists in avoiding the deleterious effects of latex ingestion and some even manage to use it in their own defense (Dussourd and Denno 1987, Konno 2011, Dussourd 2017).

Strategies such as vein-cutting and vein-trenching with precise cuts made to the plant to cause the latex to overflow reduce exudate intake by up to 90% (Dussourd and Eisner 1987, Dussourd and Denno 1991, Dussourd and Denno 1994, Agrawal and Konno 2009, Konno 2011, Dussourd 2017). Specialist herbivores also use different strategies to feed on latescent leaves, depending on the structure of the laticiferous system. When laticifers are unbranched, insects break only one vein to cause the latex to

seep and then feed from a distal part in relation to the cut, where the amount of latex is minimal. However, in plants with branched laticiferous systems they need to furrow through the leaf, damaging several veins and all regions of the laticiferous system to stop the latex from flowing (Dussourd 1990, Dussourd and Denno 1991). The architecture of the laticiferous system also influences the volume of extruded latex. When a cut is made on a plant that has non-anastomosed laticifers, there is a small overflow of latex since few cells are ruptured, but when a cut is made on a plant with branched anastomosed laticifers, the entire latex in the interconnected laticifer system drains through the cut (Demarco 2015).

On the other hand, some herbivore species have physiological strategies to protect themselves from toxic latex compounds and even use them in their own defense, as seen particularly in *Danaus plexippus* L. (Lepidoptera: Danaidae), whose larvae eat cardenolides present in the latex for subsequent use against predators (Malcolm and Zalucki 1996, Kekwick 2001), since it renders them unpalatable (Brower and Moffitt 1974).

Both latescent plant lines and expert herbivore groups evolved independently into several plant and insect lines, with a predominance in tropical habitats (Lewinsohn 1991, Prado and Demarco 2018), evidencing predation as a driving factor in the evolution of this defense system (Prado and Demarco 2018). Latescent plants demonstrate to have greater evolutionary success over non-latescent plants, which is a characteristic that increases plant resistance in environments where herbivores are present (Lewinsohn 1991, Karban & Baldwin 1997, Agrawal & Konno 2009). However, although it has a wide variety of compounds that can be toxic or dissuasive as food, latex does not fully protect against herbivory (Wink 2008), since the evolution of latescent plants took place in parallel with the evolution of herbivores. Some herbivores

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9. *Evolution*

Our analyses show that latex is present in 22 orders distributed through 34 families (Fig 1), with no phylogenetic relationship among the vast majority of them. In 14 orders, only one family has laticifers, while only 13 families share a common latescent ancestor and the entire clade carries latex, as observed in the urticalean clade. Our analysis shows that laticifers emerged independently at least 36 times.

After corrections to the descriptions in the literature, although few families have latex in all their limbs, we observe that latescent species/genera are generally closely related. Latex can be considered as a synapomorphy for infrafamily clades in 13 lines. The emergence of laticifers is discriminated per order below.

The first record of laticifers was made in "ferns", where they emerged only in Marsileaceae (Salviniales), being described in all its genera.

In Gnetales, "gymnosperms", laticifers emerged specifically in Gnetaceae, genus *Gnetum*.

In angiosperms, in Nymphaeales, laticifers emerged only once in the Nymphaeaceae + Cabombaceae clade, with a secondary loss in genus *Cabomba*.

In monocotyledons, laticifers emerged independently at least six times:

In Alismatales, laticifers emerged independently three times – in Araceae, Juncaginaceae and Aponogetonaceae. In Araceae they emerged only in subfamily Aroideae (French 1988). In Juncaginaceae, they were only described in genus *Triglochin,* and in Aponogetonaceae it is a synapomorphic character of the family, described in *Aponogeton*.

In Pandanales, laticifers occur in Cyclanthaceae, but only in *Cyclanthus,* subfamily Cyclanthoideae.

In Asparagales, laticifers emerged only once in Amaryllidaceae, subfamily Allioideae, occurring only in the Allieae tribe.

In Zingiberales, laticifers occur exclusively in Musaceae, being a family synapomorphy.

In eudicotyledons, the first emergence occured in Ranunculales, where of the three derived subfamilies, only Papaveraceae presents laticifers, with a single appearance.

In the Fabids clade, laticifers appear in four orders – Celastrales, Malpighiales, Fabales and Rosales:

In Celastrales, laticifers occur only in *Tontelea*, family Celastraceae.

In Malpighiales, it emerged three times in Malpighiaceae and once in Euphorbiaceae, demonstrating that it occurs independently in the families. In Malpighiaceae, laticifers occur in three genera of the Galphimieae tribe, and there are confirmed records for *Stigmaphyllon* and *Tetrapterys* (Pace *et al.* 2019).

A study by Davis & Anderson (2010) shows the phylogeny of Malpighiaceae, where in the Galphimieae tribe, *Spachea*, which has no laticifers (present study) is a sister genus of *Lophanthera*, which has latex, along with *Verrucularia* and *Galphimia* (Vega *et al.* 2002). From the phylogeny presented it is possible to state that there were at least three independent emergences in the family.

Several studies involving the evolution of laticifers in Euphorbiaceae have been carried out; however, they did consider the existence of two types of laticifers – articulated and non-articulated (Mahlberg 1975, Biesboer and Mahlberg 1981, Mahlberg *et al.* 1987, Rudall 1987, 1994). In this work we confirmed the presence of laticifers in the two most derived subfamilies, Crotonoideae and Euphorbioideae, which

have only articulated laticifers. This indicates that there was a single emergence in the family.

In Fabales, laticifers emerged only once in Fabaceae, but only in some isolated taxa of the family (Agrawal & Konno 2009).

In Rosales, laticifers emerged in Cannabaceae, Moraceae and Urticaceae. According to Leme (2018), laticifers are also present in *Celtis Pteroceltis* and *Trema.* Thus, we can confirm a concurrent emergence for the three families.

In the Malvids clade, laticifers emerged in two orders – Sapindales and Brassicales:

According to Montes (2017), laticifers emerged in Sapindaceae at least six times during the evolution of the family. They are present only in subfamily Sapindoideae.

According to Miller (1982), there is latex in all species of the Brassicales family, which confirms a concurrent appearance.

Latex appears in at least three independent emergences in Santalales – Aptandraceae, Coulaceae and Erythropalaceae.

In Asterids, laticifers emerged in two orders – Cornales and Ericales:

In Cornales, they appeared twice independently – in Alangiaceae and Nyssaceae. However, according to phylogenies by Xiang *et al.* (1998 a) and Xiang (1999), the positioning of genera in Nyssaceae and Cornaceae is still uncertain, and further studies are needed.

In Ericales, laticifers emerged only once – in Sapotaceae – and according to Swenson *et al.* (2018), latex is a synapomorphy in the family.

In the Lamiids clade, latex emerged in three orders – Garryales, Gentianales and Solanales:

In Garryaceae, it emerged only once, in Eucommiaceae. However, in Gentianales, it emerged at least twice – in Rubiaceae and in Apocynaceae. According to the literature, Apocynaceae contains all latescent representatives (Solereder 1908, Metcalfe and Chalk 1950, Demarco 2017).

In Solanales, laticifers emerged only in the most derived subfamily of Convolvulaceae – Convolvuloideae – in the Convolvuleae and Ipomoeeae tribes, demonstrating not to be an ancestral trait.

The last clade to present laticifers is Campanulids, where it emerged specifically in *Cardiopteris* of Aquifoliaceae (Aquifoliales), and Asterales, whose latex emerged at least three times; once in Campanulaceae, and twice in Asteraceae, in subfamily Cichorioideae and in some Asteroideae genera.

We can suggest a convergent evolution of laticifers, where its polyphyletic origin is observed by the similarity of its compounds in the latices of phylogenetically distinct groups (Agrawal & Konno 2009, present study – histochemistry section) and by its function against herbivory and pathogens (Prado & Demarco 2018, present study), especially in groups that evolved in a tropical environment.

It has been found that in some families, such as Apocynaceae, Sapotaceae and Campanulaceae, latex demonstrates to be an ancestral condition, but in others such as Fabaceae and Malpighiaceae, latex originated and evolved in isolated taxa.

10. Conclusions

Our study has found the occurrence of laticifers in vascular plants by reevaluating previous descriptions and investigating the existence of laticifers in lines never before studied for this purpose. We confirmed the presence of laticifers in 34

families of 22 orders, with unpublished descriptions for some lines, and refuted 18 families considered latescent. According to the actual distribution of laticifers, we found that this secretory structure appeared at least 36 times during the evolution of vascular plants, being homoplasic in most clades. We also observed that laticifers usually occur only in part of family representatives, and can be used as a taxonomic and, in some cases, a phylogenetic trait.

The comprehensive analysis carried out as part of this work indicates the inexistence of non-articulated laticifers. Earlier descriptions of these laticifers, especially for Apocynaceae, Euphorbiaceae and Moraceae, stem from the lack of ontogenetic analyses or the difficulty of observing a single row of cells in a sinuous path. Thus, the distinction between articulated and non-articulated laticifers is not supported. Thus, laticifers always consist of rows having two or more cells and we propose a simplified classification by dividing laticifers solely into anastomosed or non-anastomosed, branched or unbranched. The only other possibility would be a unicellular laticifer. Although we do not rule out this possibility, such structure has never been observed in a latescent plant.

Our study has also made important contributions regarding laticifer growth. The description of intrusive autonomous apical growth in laticifers described as non-articulated and, more recently, also in some articulated laticifers is not supported when faced with the analysis of all groups. There is no evidence of this type of growth in any latescent groups and numerous disputes are found in the literature suggesting the impossibility of this type of growth. However, more specific studies are needed. Our comprehensive analysis opens new questions involving the various latescent groups, which may be answered in future studies.

Future Prospects

Much has been presented about laticifers regarding their structure, distribution and composition, but much still remains to be studied.

By focusing our objectives on studies using cell growth markers, gene expression, enzymatic and cytoskeletal analysis, we intend to further investigate the evolution of laticifers within families, ascertaining their single or multiple appearance. We also intend to assess the redundancy of laticifer protection with other protection structures and to analyze the survival rate of latescent plants in different environments.

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General Conclusions

In our study, we have established that, although at times laticifers and resin ducts are similar in relation to the color of their secretion and the diversity of secretion compounds, latex is a secretion produced and stored inside the cell, both in the vacuole and in the cytoplasm. The latex is considered to be the entire laticifer protoplast, because when the plant is injured the entire cell content – that is, both the metabolites produced and the laticifers' membranes, organelles and nuclei – is released. On the other hand, resin ducts produce an extracellular secretion that has no structural components in its composition and is stored in an intercellular space called lumen. In addition, laticifers are conceptually rows of cells, while resin ducts are formed by an epithelium that limits the lumen.

The histochemical analysis of latex from plants of different families has also shown an important evolutionary convergence with latescent plants of different strains of vascular plants, and that the latex from any plant is composed of lipids (especially terpenes), proteins, phenolic compounds, carbohydrates and alkaloids, the latter being the only compound that might be absent.

Based on the correct identification of laticifers and the detection of latex, we have identified laticifers in 34 vascular plant families, distributed into 22 orders, often without phylogenetic relationship, showing that laticifers have emerged multiple times in the evolution of vascular plants. We also refute the occurrence of laticifers in 18 families considered latescent, which have only resin ducts or secretory idioblasts. Further, we have ascertained that all laticifers are articulated and can be classified as anastomosed or non-anastomosed, branched or unbranched, refuting the existence of laticifers classified as non-articulated. Finally, we also contest the intrusive autonomous growth theory since there is no evidence of its occurrence in any of the latescent families.

Appendix

TableS1. Species studied and vouchers

Acalypha sp. L.	Pirani JR – SPF 100590
Acourtia carpholepis D. Don	Tenorio P 7999 - MBM 200170
Aesculus hippocastanum L.	Smith AE 16A - MBM 97474
Agapanthus africanus L'Hér	Kummrow R 776 - MBM 75015
Alangium Platanifolium Lam.	Ming T. 95581- MBM 239012
Allium cepa L.	Prado E. 001
Allium schoenoprasum L.	Pereira B. 650 IX-97 – UEC 121397
Amorphophallus sp. Blume ex Decne	Prado E. 002
Angophora costata Cav.	Constable, EF 19379 - SP58716
Aponogeton elongatus L. f.	Henshall, TS 3857 - SP 175984
Aponogeton japonica L. f.	Shaffer-Fehre, M 600 - MBM 314595
Argemone mexicana L.	Scardino 098 – UEC 28857
Aucuba japonica Thunb.	Kanai H. Tateishi Y - SP 188863
Azolla filiculoides Lam.	Sasaki D *et al.* 1503 – SPF 207522
Billia columbiana Peyr.	Davidse G 21328 - MBM 89790
Bixa orellana L.	Lopes C.M. 03- UEC 14200
Brasenia schreberi Schreb.	Stanard B.L. 1031- MBM 254407
Byrsonima sp. Rich ex. Kunth	Alves M 2127 – SPF 142832
Cabomba aquatica Aubl.	Costa S.M. 763- UEC 154809
Cabomba furcata Aubl.	Vasconcellos M.B. 13530 - UEC 28443
Calystegia occidentalis R. Br.	Utech FH 86097 - MBM 114793
Camptotheca acuminata Decne.	Tamashiro J.Y. 2010 - UEC 163702
Cannabis sativa L.	Dias U.S. 19749 - UEC 47000
Carica papaya L.	Prado E. 009
Caseria decantra Jacq.	Yamamoto L.F. 488 - UEC 149702
Centaurea cyanus L.	Kellog, JH 10 – SP 26295
Cichorium sp. L.	Garcia MA 23 – UEC 167491
Citronella megaphylla D. Don	Pedroni F. 1538 - UEC 174455
Citronella panicullata D. Don	Meireles L.D. - UEC 120123
Chelidonium majus L.	Ahles, HE 78821 - SP 131018
Chelidonium majus L.	Ininteligível s.n. - SP 224189
Clusia sp. Lindl.	Pirani JR 6341 – SPF 221441
Convolvulus crenatifolius L.	Schinini A 6325 - MBM 27802
Cornus racemosa L.	Nee M. 14526a -SP 217367

Corymbia ptychocarpa K.D. Hill & L.A.S. Johnson	Santos AA *et al.* 829 - SP 379021
Croton fuscescens L.	Joly AB 174 – SPF 84327
Croton sphaerogynus L.	Thomas WW 6131 – SPF 63725
Croton lundianus L.	Joly AB snº - SPF 16588
Cyclanthus bipartidus Poit. ex A. Rich.	Morawets W 390 – SPF 91002
Dahlstedtia araripensis Malme	Tozzi AMGA 97-49 - UEC 91074
Dendrobangia boliviana Rusby	Boswesen B 6014 - MBM 286975
Dichondra macrocalyx J.R. Forst. & G. Forst.	Lozano ED 2815 – MBM 394290
Discophora guianensis Miers	Carvalho AMV *et al.* - SP 392339
Dodonaea viscosa Mill.	Flores T.B. 2406 – SPF 152852
Euonymus sp. L.	Boechat C.S. 47613
Erythrina speciosa L.	Rossi L snº- SPF 61242
Eucalyptus globulus L'Hér.	Flores T.B. 570 - UEC 196578
Eucalyptus gummifera L'Hér.	Dietrich A.M. 2585 - MBM 102340
Eucalyptus gummifera L'Hér.	Ford NC snº - SP 55544
Eucommia ulmoides Oliv.	Feng-Lie Z. 135 - MBM 361488
Euphorbia milii Des Moul.	Prado E. 004
Ficus benjamina L.	Prado E. 005
Garrya elliptica Douglas ex Lindl.	Thorne RF 31653 - SP 85265
Gnetum nodiflorum L.	Mc Daniel S 29926 – MBM 141865
Heisteria silvianii Jacq.	Stefani E. Jr. 122010 - UEC 160976
Helwingia japonica (Thunb.) F. Dietr.	Tateishi Y, *et al.* 10299 - SP 203401
Hippobroma longiflora G. Don	Pirani JR 6375 – SPF 204649
Hippocratea volubilis L.	Loefgren A CGG1307 - SP 13937
Humulus lupulus L.	Uotila P. 32963 - MBM 102811
Hura crepitans L.	Amorim AMA 3928 – SPF 194513
Hydnocarpus sp. Gaertn.	Leitão Filho H.F. 8899 - UEC 5217
Ilex cerasifolia L.	Yamamoto L.F. 84 – UEC 136987
Ipomoea tricolor L.	Prado E. 012
Kielmeyera sp. Mart. & Zucc.	Silva ASL – SPF 178834
Laetia americana Loefl. ex L.	Pott A. 1453 - UEC 75189
Laportea aestuans Loefl. ex L.	Kinupp V 4969 - SP 465050
Lactuca indica L.	Ohashi H 10811 - SP 210809
Lilaea scilloides Bonpl.	Shirata MT 721 - HUCP 7856
Limnocharis laforestii Bonpl.	Peixoto A.L. 1684 - UEC 32903
Lonchocarpus sp. Kunth	Tamashiro J.Y. 95 – UEC 185807

Lophanthera lactescens Raf.	Pianaro A snº - UEC 143475
Mammilaria sp. Haw.	Prado E. 010
Mandevilla splendens Lindl.	Prado E. 011
Manilkara sp. Adans.	Gomes FS 1321 - MBM 393944
Mimosa laticifera L.	Anderson WR 8328 - MBM 100470
Marsilea sp. L.	Anajde Leme do Prado 2916 - UEC 138327
Minquartia guianensis Aulb.	Wide7837 - MBM 110755
Morus nigra L.	Queiroz EP 1341 – SPF 174366
Musa paradisiaca L.	Prado E. 003
Mussaenda erythrophylla L.	Delprete PG 9445 – SPF 189594
Myrianthus holstii P. Beauv.	Ithe MM 366 - SP 465042
Nelumbo nucifera Gaertn.	Cordeiro J. 4753 – MBM 391590
Nothoscordum sp. Kunth.	Dutilh JHA snº UEC 170608
Olax mannii Oliv.	Wide 612– MBM 157481
Peridiscus sp. Benth.	Fróes R.L. 22097 - UEC 006550
Philodendron sp. Schott.	Sano PT *et al.* CFSC13844 – SPF 211334
Phthirusa podoptera Mart.	Stanard B.L. 1031 - MBM 254407
Phyllanthus acidus L.	Cardoso PR – UEC 143516
Phyllanthus urinaria L.	Figueiredo JO snº - UEC 66656
Pilularia globulifera L.	Durer M. 0 - UEC 34609
Pourouma guianensis Aulb.	Melo MMRF 870 - SP 245878
Pouteria caimito (Ruiz & Pav.) Radlk.	Rochelle A.L.C.J. 686 - UEC170034
Protium sp. Burm. f.	Prado E. 007;
Ptychopetalum olacoides Benth.	Wide 7952 – MBM 163416
Sagittaria guayanensis Schltdl.	Barbosa T.M.D. 1553 - UEC 175387
Sagittaria montevidensis Cham. & Schltdl.	Tanaka C. 0 - UEC 115194
Salacia crassifolia (Mart.) G. Don	Gomes B.M. 50 - UEC 127795
Salacia elliptica (Mart.) G. Don	Marchori 450 - UEC 160355
Salix babylonica L.	Ikemoto E. 04-17 - UEC 144919
Salvinia auriculata Aubl.	Viana G 710 – SPF 205133
Sanguinaria canadensis L.	Ahles HE 86693 - UPCB 11137
Schoepfia brasiliensis Scherb.	Santos K. 243 - UEC 103090
Schinus sp. L.	Prado E. 008
Spachea sp. A. Juss.	Macedo M. Assumpção S. 1806 - UEC 36099
Struthanthus concinnus Mart.	Tozzi A.M.G.A. 98-185 - UEC 103142
Struthanthus vulgaris Mart.	Leitão Filho H.F. 32867 - UEC 90322

Taraxacum officinale Zinn	Prado E. 013
Tetrastylidium engleri Engl.	Rochelle A.L.C. J911
Tetroncium sp. Willd.	– Libsch D 022/013 - HUCP 15584
Tontelea micrantha Miers	Kinoshita L.S. 00-148 – UEC 125303
Tontelea miersii Miers	Lombardi J.A. 12008 – UEC 168608
Triglochin striata Ruiz & Pav.	Yano O 891 - SP 154674
Tristagma uniflorum (Lindl.) Traub	Pederson F.M.16-58 – MBM 202085
Tropaeolum pentaphyllum Lam.	Siqueira EL 1106 - MBM 397201
Ulmus alata L.	Sherman S 110 - SP 234632
Ulmus glabra L.	Schuhwerk F 9215 – MBM 346659
Urera baccifera Gaudich.	Prado E. 006
Ximenia americana L.	Leitão Filho H.F. 34822 – UEC 80594
Xylosma sp. G. Forst.	Mendonça RC *et al.* 5848 – UEC 146708

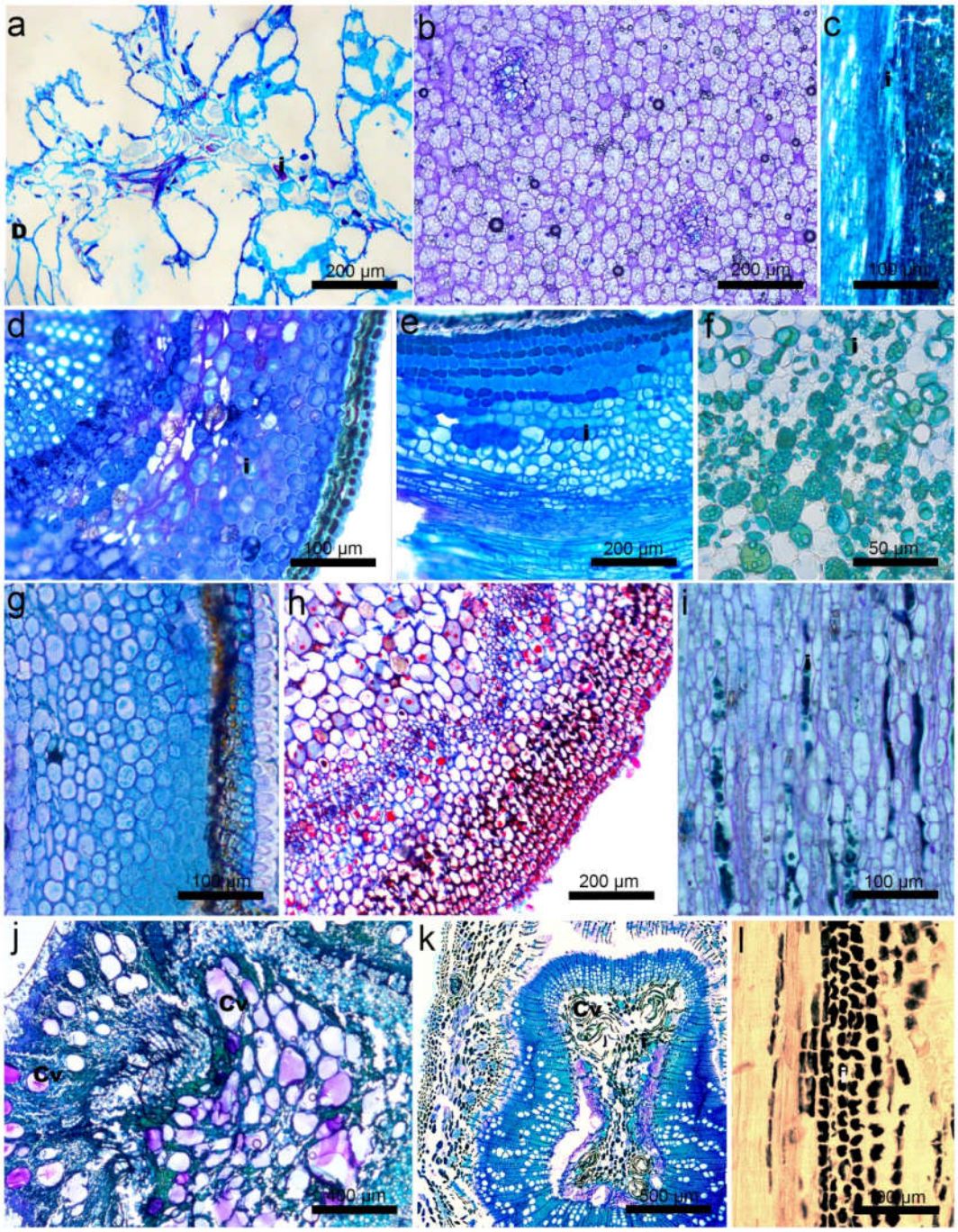

Fig S1: Non-latescent genera – Salviniaceae (Salviniales) – *Azolla* sp.: a. Longitudinal section indicating the presence of phenolic idioblasts; Amaryllidaceae (Asparagales) – *Nothoscordum* sp.*:* b. Longitudinal section; Peridiscaceae (Saxifragales) – *Peridiscus* sp.*:* c. Longitudinal section indicating the presence of phenolic idioblasts; Celastraceae (Celastrales) - *Eounymus* sp.: d. Longitudinal section indicating the presence of phenolic idioblasts; *Salacia crassifolia* e. Longitudinal section indicating the presence of phenolic idioblasts; Malpighiaceae (Malpighiales) – *Byrsonyma* sp.: f. Cross section indicating the presence of phenolic idioblasts; *Spachea* sp.: g. Cross section indicating the presence of phenolic idioblasts; Euphorbiaceae (Malpighiales) – *Acalypha*: h. Cross section; Fabaceae (Fabales) – *Erythrina speciosa:* i. Longitudinal section indicating the presence of phenolic idioblasts; Urticaceae (Rosales) – *Myrianthusholstii:* j. Cross section indicating the presence of oil cavities; Myrtaceae (Myrtales) – *Eucalyptus gummifera*: k. Cross section indicating the presence of phenolic idioblasts and oil cavities; l.

Ferric chloride test – Longitudinal section indicating phenolic composition; (i – Idioblasts; D – Resin duct; Cv – Oil cavity).

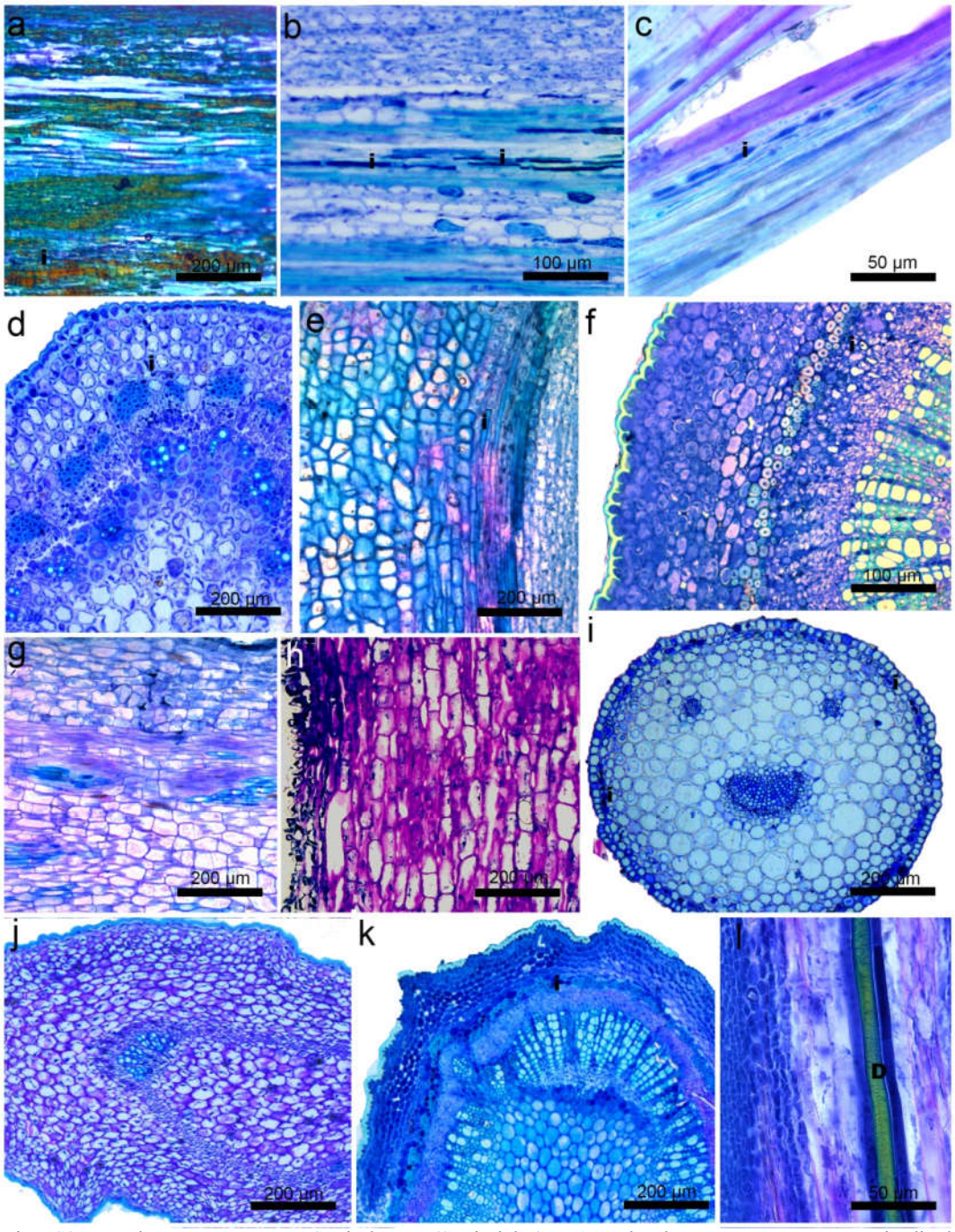

Fig FS2: Non-latescent genera – Sapindaceae (Sapindales) – *Aesculus hippocastanum*: a. Longitudinal section indicating the presence of phenolic idioblasts; *Billia columbiana:* b. Longitudinal section indicating the presence of a phenolic idioblasts; Tropaeolaceae (Brassicales) – *Tropaeolum pentaphyllum:* c. Longitudinal section indicating the presence of phenolic idioblasts; Loranthaceae (Santalales) – *Struthanthusvulgaris:* d. Cross section indicating the presence of phenolic idioblasts; Schoepfiaceae (Santalales) – *Schoepfia brasiliensis*: e. Longitudinal section indicating the presence of phenolic idioblasts; Strombosiaceae (Santalales) – *Tetrastyllidium engebri*: f. Cross section indicating the presence of phenolic idioblasts; Ximeniaceae (Santalales) – *Ximenia americana*: g. Longitudinal section; Garryaceae (Garryales) – *Aucuba japonica*: h. Cross section; Convolvulaceae (Solanales) – *Dichondra*

sp.*:* i. Cross section indicating the presence of phenolic idioblasts; Aquifoliaceae (Aquifoliales) - *Ilex:* j. Longitudinal section; Helwingiaceae (Aquifoliales) – *Helwingia japonica*: k. Cross section indicating the presence of phenolic idioblasts; Asteraceae (Asterales) – *Centaurea cyanus:* l.– Longitudinal section indicating Resin duct; (i – Idioblasts; D – Resin duct).