María Camila Medina Montes

Laticíferos em Sapindaceae

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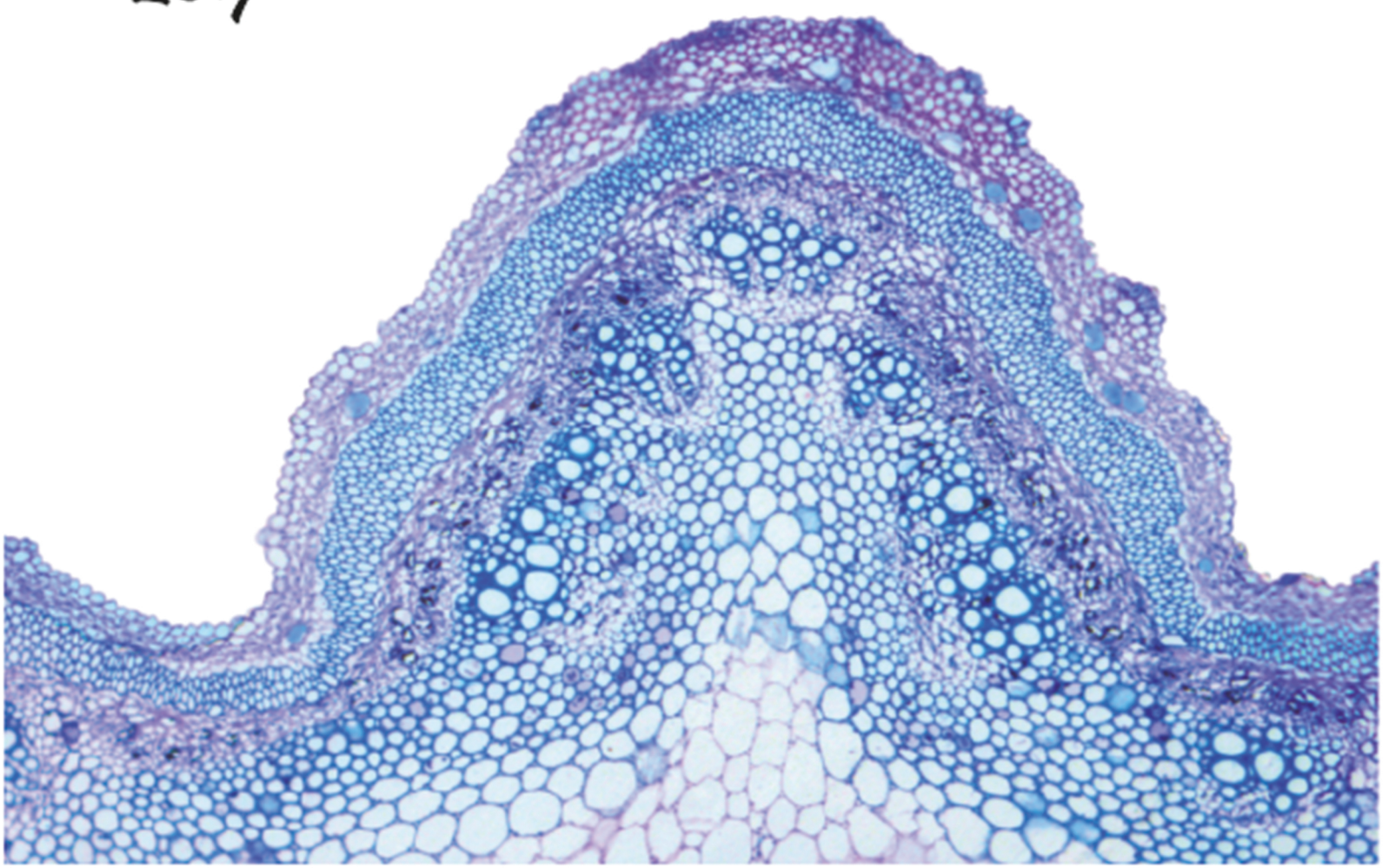

MARÍA CAMILA MEDINA MONTES

LATIFICEROS EM SAPINDACEAE

SÃO PAULO
2017

María Camila Medina Montes

Laticíferos em Sapindaceae

Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para obtenção do título de Mestre em Ciências, na área de Botânica

Orientação: Prof. Dr. Diego Demarco

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COMISSÃO JULGADORA

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Prof. Dr. **Prof. Dr.**

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Prof. Dr. **Prof. Dr.**

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Prof. Dr. Diego Demarco

“El día que te llamo, vienes a mí, y me dices: «No tengas miedo.»”

Lamentaciones 3:57.

Dedico

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CONTENTS

Abstract

Laticifers are poorly known among Sapindaceae species. They have only been cited in few works, without a description of its structure, ontogeny and histochemistry, which is of great importance to classify and distinguish them from idioblasts that are cells usually observed in the same organs. Therefore, this work aimed to verify the presence of laticifers in 64 species belonging to 21 genera of Sapindaceae, and to analyze their anatomical aspects, latex composition and evolution within the family. The material obtained from herbaria was rehydrated and embedded in methacrylate, according to the usual techniques in plant anatomy. For histochemical analysis fresh or Paraplast-embedded shoots were used. The presence of articulated nonanastomosing laticifers was confirmed for 15 genera from two subfamilies, being described for the first time in some genera. Apparently, this secretory structure arose six times during evolution of the family. They originate early in the development of shoot apex when the tissues are still in meristematic phase and distributed generally in cortex, phloem and pith. The histochemical tests allowed the observation of lipids, carbohydrates, proteins, alkaloids, and phenolic compounds in the latex. Callose and suberin was also detected in laticifer walls of some species, helping in the interpretation of the infratribal relationships. In general laticifers in the family are small, short and narrow in comparison with other families, and for this reason, for a long time, remained unrecorded in the literature. Presence or absence of laticifers can be used as a character that can help in taxonomic resolution and establishment of relations among groups within Sapindaceae.

Keywords: distribution, evolution, laticifers, ontogeny, Paulliniodae, Sapindaceae.

Resumo

Os laticíferos são pouco conhecidos em espécies de Sapindaceae. Eles só foram citados em alguns trabalhos, sem uma descrição da sua estrutura, ontogenia e histoquímica que permita classificá-los e distingui-los dos idioblastos que geralmente são observados nos mesmos órgãos. Portanto, o objetivo deste trabalho foi verificar a presença de laticíferos em 64 espécies pertencentes a 21 gêneros de Sapindaceae e analisar seus aspectos anatômicos, da composição de látex e evolução dentro da família. O material obtido a partir de herbários foi reidratado e incluído em metacrilato de acordo com as técnicas usuais de anatomia vegetal. Para a análise histoquímica foram utilizados ápices frescos e incluídos em Paraplast. A presença de laticíferos articulados não anastomosados foi confirmada para 15 gêneros de duas subfamílias, tendo sido descrita pela primeira vez em alguns destes gêneros. Aparentemente, essa estrutura secretora surgiu seis vezes na família. Eles são originados no início do desenvolvimento no meristema apical quando os tecidos ainda estão na fase meristemática e são distribuídos geralmente no córtex, no floema e na medula. O uso de testes histoquímicos permitiu observar lipídeos, carboidratos, proteínas, alcaloides e compostos fenólicos no látex. Calose e suberina também foram observadas na parede do laticífero de algumas espécies, ajudando na interpretação das relações infratribais. Em geral, os laticíferos da família são pequenos, curtos e estreitos em comparação a outras famílias e, por esse motivo, não se encontram registros na literatura. A presença ou ausência de laticíferos pode ser usada como caráter para ajudar na resolução taxonômica e estabelecimento de relações entre grupos dentro de Sapindaceae.

Palavras-chave: distribuição, evolução, laticíferos, ontogênese, Paulliniodae, Sapindaceae.

General Introduction

The laticifers are the cells that contains the latex, this being an emulsion with different index of refraction, which is exuded from the plant when it suffers some type of damage (Fahn, 1988; Konno, 2011). Latex is most commonly a milky white liquid, but in some cases it may be colorless or have other colors. This is due to latex composition, containing a variety of biologically active compounds, which can be several primary and secondary metabolites (Fahn, 1979; Demarco, 2015). Compounds such as terpenoids (Nemethy et al., 1983; McGarvey and Croteau, 1995; Famuyiwa et al., 2014; Demarco, 2015), cardenolids (Malcolm, 1991; Rasmann et al., 2009), alkaloids (Sacchetti et al., 1999; Weid et al., 2004; Hirayama et al., 2007), various proteins and enzymes (Ruperti et al., 2002; T. Freitas et al., 2007; Wasano et al., 2009), and protein inhibitors (Murdock et al., 1987; Gruden et al., 2003; Rodrigues Macedo et al., 2011) are common in latex (Ryan, 1990; Fürstenberg-Hägg et al., 2013).

As for the type, the laticifers are classified as nonarticulated, when they are formed by individualized cells that can remain indivisible (unbranched) or bifurcate many times (branched), and as articulated, when they consist of rows of cells that maintain their walls intact (nonanastomosed) or dissolve the terminal contact (anastomosed), forming a continuous secretory system (Fahn, 1979)

According to studies (Fahn, 1979; Evert, 2006), laticifers are generated from initial cells that are present in the cotyledon node, whose number can vary depending on the type of laticifer, but also within the species. Initials typically appear on the cotyledon node, forming a ring at the periphery of the young vascular tissue (Mahlberg, 1993). Thus, in *Nerium oleander* the usual number of initials found is 28 (Mahlberg, 1961), while in *Euphorbia* there are only four (Mahlberg and Sabharwal, 1968).

However, laticifers are found from the beginning of plant formation, originating in cells of the fundamental meristem and/or procambium in Apocynaceae and Euphorbiaceae (Demarco et al., 2006, 2013; Demarco and Castro, 2008). The growth form of the laticifers in the body of the plant is due to the addition of new meristematic cells followed by cellular elongation without intrusive penetration (Demarco et al., 2006; Santos Rubiano et al., 2017).

The differentiation of meristematic cells into laticifers occurs rapidly, and their development is sometimes difficult to observe. For this reason some species have been erroneously described as having nonarticulated laticifers (Metcalfe, 1967; Serpe et al., 2001), because of the early dissolution of the transverse walls, being actually an articulated anastomosing type (Demarco et al., 2006, 2013; Demarco and Castro, 2008).

In some families, the type of laticifer is not constant. In Euphorbiaceae, for example, *Euphorbia* presents nonarticulated laticifers, while *Hevea* has articulated laticifers, *Jatropha* has both articulated and nonarticulated types and *Sapium* has two types of articulated laticifers in the same organ (Evert, 2006; Demarco et al., 2013).

Regarding the presence of laticifers in plants, they may be in aquatic herbs, shrubs, large trees or even lianas, since latex is not restricted to only one type of habitat or specific habit (Metcalfe, 1967). However, regarding the abundance, the largest number of latescent plants belongs to several families mainly in tropical areas. This can be explained by the fact that tropical forests have been modeled by strong ecological and evolutionary interactions between plants and herbivores, with a high diversity of natural enemies and high rates of herbivory (Lewinsohn, 1991; Coley and Barone, 1996).

The strategies employed by plants to defend themselves against herbivorous insects are very diverse. At first, these strategies may be related to a set of traits present in

plants, which could include aspects such as the nutritional quality of the plant, physical characteristics, toxicity, phenology, and indirect defenses (Agrawal and Fishbein, 2006). These characteristics could somehow affect insect preference, such as host plant selection, and food behavior, and other species affect their performance, such as growth rate and development. These attributes may be morphological and are used for the physical defense or production of compounds for chemical defense (Fürstenberg-Hägg et al., 2013), which can be expressed constitutively or can be induced and developed only after the attack. The biological importance of latex in plants has not yet been well understood. So far, no function has been known in primary metabolism, but due to the variable amount of secondary metabolites stored, it has been related to defensive functions, converting latex into a chemical barrier against herbivorous insects (Nishida, 2014).

Latex is confined within the laticifer and is released only when the organ is crushed, acting at first as chemical defense but also as a physical defense that can trap and intoxicate the insect that tries to feed on the plant (Hébant and De Fay, 1980; D'Auzac et al., 1989; Agrawal and Konno, 2009; Souza et al., 2010; Konno, 2011; Fürstenberg-Hägg et al., 2013).

Another important aspect to be highlighted that could strengthen latex defense properties is the amount of its production by the plant. Besides the anatomical type of the laticifer, latex volume can be strongly influenced by biotic and abiotic factors of the habitat. These conditions may be drought, soil moisture and fertility, and also herbivory, as demonstrated by studies performed with *Ipomea batatas*, and species of the genus *Asclepias* (Tupy, 1989; Data et al., 1996; Raj et al., 2005; Agrawal and Konno, 2009). In studies carried out with *Hevea brasiliensis*, the authors concluded that the duration

and intensity of sunlight directly influence the levels of sucrose in latex, which influence the metabolic activity of the laticifers (Tupy, 1989; Raj et al., 2005).

As previously mentioned, the composition of the latex is variable and complex. A clear example is the one observed in *Euphorbia tirucalli*, in which latex contains important precursors of highly toxic secondary metabolites (Souza et al., 2010). This suggests that depending on the species, latex has one or more compounds that make it more or less toxic, as demonstrated by Sethi et al. (2008) using two lettuce varieties "Valmine" and "Tall Guzmaine", where the total deterrence coefficient of Valmine latex was 3.9 times higher than that of Tall Guzmaine latex, in contrast to the pest *Diabrotica balteata*, by the presence of moderately polar chemicals.

Not only the products of secondary metabolism are responsible for the defense function of latex. Studies have showed that primary metabolites such as the proteins present in the latex have a relevant enzymatic activity against pathogens correlated with deleterious effects on their development (Ramos et al., 2007; Souza et al., 2010). Many defense-related proteins such as arginase, ascorbate oxidases, lipoxygenases, polyphenol oxidases, and peroxidases may have anti-nutritional properties, and others such as chitinases, cysteine proteases, chitin-binding proteins, lectins and leucine aminopeptidases may have a toxic effect (Felton, 2005; Zhu-Salzman et al., 2008; Buerki, 2010).

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LATICIFERS IN SAPINDACEAE: STRUCTURE, DISTRIBUTION AND PHYLOGENETIC IMPORTANCE

INTRODUCTION

Laticifers have a wide distribution in plant kingdom and are present in 36 families without taxonomic relationship (Lewinsohn, 1991; Judd et al., 2007). Latescent species are found in almost all major groups of vascular plants, from ferns as *Regnellidium* (Gouvêa Laboriau, 1952), "Gymnosperms" as *Gnetum* (Behnke and Herrmann, 1978; Tomlinson and Fisher, 2005), to angiosperms that can be annual herbaceous plants, including many species of *Euphorbia,* even large trees as *Hevea* (Metcalfe, 1967). This suggest that these structures may have evolved many times along the evolutionary history of these groups (Demarco et al., 2006). Although laticifers origin is considered to be recent during evolution, fossil records indicate that they were already present in an arborescent plant of the Eocene (Mahlberg et al., 1984).

Comparative systematics studies of laticifers are scarce and the possible meaning of phylogenetic variations in the degree of specialization has not yet been revealed, however the presence of these structures in some species indicate their great taxonomic importance in families such as Araceae, Asteraceae, Moraceae, Nelumbolaceae and Papaveraceae (Simpson, 2010). In the specific case of Araceae, a systematic study about the occurrence of articulated anastomosing laticifers in leaves and inflorescences of 75 genera showed that the laticifers were found only in the subfamily Colocasoideae and in *Zomicarpa*, a genus of the subfamily Aroideae (French, 1988). In addition, the chemical composition of latex has also a potential application as an aid in the delimitation of taxa and in the interpretation of the evolutionary life history

of a group (Rudall, 1987).This is the case of Sapindales, a large and diverse order that has some representatives which exude a white secretion, usually interpreted as latex.

Within Sapindales the presence of latex has been observed only in Sapindaceae, considered an uncommon character present in some species (APG, 2016). This family is one of the largest families of the order, comprising ca. 144 genera and 1900 species distributed in 4 subfamilies: Xanthoceroideae, Hippocastanoideae, Dodonaeoideae, and Sapindoideae. The latter, is the most diverse with approximately 1340 species (Acevedo-Rodríguez, 2011; Muellner-Riehl et al., 2016), of which about 475 belong to the tribe Paullinieae. This tribe of herbaceous or woody vines is characterized by the presence of compound leaves with a distal leaflet well developed and the presence of stipules, an exclusive feature of Paullinieae within Sapindaceae.

There are reports of latex in taxonomic works for some genera of Paullinieae (Sapindoideae) (Weckerle and Rutishauser, 2005) and described as branched in *Paullinia carpopodea* (Ferraz and Gonçalves C., 1985). Nevertheless, there is no anatomical confirmation that they are laticifers (Milanez, 1959). For Hippocastanoideae the presence of latex for some species of *Acer* and *Dipteronia* was also reported (Benedict, 1961; Amini et al., 2008; APG, 2016), without confirmation of any kind. Another factor that arise questions on the description of laticifers in this family is the fact that there are few anatomical works using representatives of this group, and for most of them the secretory cells have been described as idioblasts, a secretory structure abundant in all Sapindales. Considering the co-occurrence of these two secretory structures, only a histochemical analysis can distinguish them undoubtedly, once the difference between a secretory idioblast and a laticifer sometimes resides only in the nature of their secretion (Fahn, 1979). Milanez (1959) recognized the resemblance between the ontogeny of the “secretory tubes” in *Paullinia* and the laticifers but

concluded that an ontogenetic study with an adequate fixation to confirm that would be necessary.

Considering that the presence of laticifers, their type and the latex composition have taxonomic and systematic importance for many groups (Demarco et al., 2013), a comprehensive investigation of Sapindaceae is necessary to verify the occurrence of laticifers in the genera described as having latex and their allies. The objectives of this work are to study 1) the occurrence of laticifers in Sapindaceae, 2) the ontogeny and distribution of the laticifers and 3) the identification of the latex composition. This could provide taxonomic resolution to clarify the relation among several genera.

MATERIAL AND METHODS

This work was carried out in the Instituto de Biociências of the Universidade de São Paulo, in the Laboratório de Anatomia Vegetal in São Paulo, Brazil. Some species were collected in the campus of the Universidade de São Paulo (Reserva Florestal and Fitotério) in São Paulo, Brazil. However, the majority of the material was acquired from herbarium samples (Table 1).

The study comprised 64 species belonging to 21 genera of two subfamilies:

1. Sapindioideae: This subfamily is most species-rich with various tribe and groups and in this work the following tribes were analyzed: Paullinieae, Thouinieae, Brigdesieae, Athyaneae and the groups: Melicoccus, Cupania, Litchi, and Bloomia.

2. Hippocastanoideae This subfamily is small and comprises five genera, and in this work *Acer* and *Dipteronia* were analyzed.

To test hypotheses of laticifer evolution, a maximum likelihood analysis was performed using Mesquite v3.04 (Maddison and Maddison., 2017). Each genus was identified as a unit to construct a character matrix, which was made only with the

presence or absence of laticifers. This was based on the phylogeny of Buerki et al. (2009) and Acevedo-Rodríguez et al. (2017)

Laticifer detection

The occurrence of laticifers was detected from dried specimens. Fragments of young stem followed the protocol of herborization reversal (Smith and Smith, 1942). Subsequently, the fragments were dehydrated in a gradual series of alcohol and embedded in methacrylate (Meira and Martins, 2003). The material was transverse and longitudinal sectioned in a Microm HM340E rotary microtome (Microm International, Walldorf, Germany) and the sections were stained with toluidine blue (O'Brien et al., 1964). The photomicrographs were obtained in a Leica DMLB light microscope.

Latex and cell wall composition

Free-hand sections obtained from fresh and fixed material were submitted to different treatments for detection of the main chemical classes of compounds that constitute the latices, as follows: Sudan black B and Sudan IV (Pearse, 1985) and Neutral red (Kirk, 1970) for lipids in bright field and under UV light, respectively; Aniline blue for calose under UV light (Smith and McCully, 1978); Nile blue (Cain, 1947) for neutral and acidic lipids; Nadi reagent (David and Carde, 1964) for terpenoids; copper acetate and rubeanic acid (Ganter and Jollés, 1969, 1970) for fatty acids; ferric chloride (Johansen, 1940) and potassium dichromate (Gabe, 1968) for phenolic compounds; vanillin – hydrochloric acid (Mace and Howell, 1974; Gardner, 1975) for tannins; Dragendorff's reagent (Svendsend and Verpoorte, 1983) and Wagner's reagent (Furr and Mahlberg, 1981) for alkaloids; periodic acid – Schiff reaction (PAS) (Jensen, 1962) for polysaccharides; and ruthenium red (Johansin, 1940;

Gregory and Baas, 1989) for acidic mucilage and pectins. The control for hydrophilic substances and lipophilic substances were carried out according to Demarco (2017). All photomicrographs were taken using an Olympus BX51 microscope (Melville, USA).

Histochemical tests of Sudan Black B and Ferric chloride were realized to confirming the presence of laticifers and idioblast respectively, and autofluorescence in UV light for analyze the laticifers walls for material of herbarium specimens.

Ontogeny

Three species of tribe Paullinieae were selected for development study of laticifers on the shoots (*Paullinia seminuda, Serjania lethalis, and Urvillea ulmacea)*, collected in the campus of Universidade de São Paulo. The material collected was deposited in the SPF herbarium (USP).

For the developmental analysis of the laticifers, shoots were fixed in formalin-acetic acid-alcohol 50% (FAA) for 24 hours (Johansen, 1940) or in buffered neutral formalin (Lillie, 1965) and stored in ethanol 70%. Subsequently, shoot apices were isolated, dehydrated in an ascending butyl series (Johansen, 1940) and embedded in Paraplast (Leica Microsystems, Heidelberg, Germany). The material was longitudinal and transversely sectioned using a Microm HM340E rotary microtome (Microm International, Walldorf, Germany) and the sections were stained with astra blue and 1% safranin in ethanol 50% (Gerlach, 1984). The slides were mounted in Permount resin (Fisher Scientific, Pittsburgh, PA) and photographed under a Leica DMLB LM (Leica Microsystems). Photographs were processed using Adobe Photoshop.

Table 1. Sapindaceae species sampled grouped according to Buerki et al. (2009) and Acevedo-Rodriguez et al. (2017).

SUBFAMILY/ TRIBE	GENUS	COLLECTOR NUMBER	VOUCHER NUMBER	COLLECTION LOCALITIES
Sapindoideae/ Tribe Paullinieae	*Cardiospermum corindum* L.	Urdampilleta J. D. 328	UEC 153247	Campinas, SP, Brazil
		Harley, R. M. 16254	UEC 41658	Bahia, Brazil
	C. grandiflorum Sw.	Obando S., et al. 5350	UEC 171513	São João Batista do Glória, MG, Brazil
		Martins, A. B., et al. 31419	UEC 67565	Águas da Prata, SP, Brazil
		Firetti, F., et al. f5	UEC 145213	Mogi-Guaçu, SP, Brazil
		Obando S., et al. 5350	UEC 171513	São João Batista do Glória, MG, Brazil
	C. halicacabum L.	Urdampilleta J. D. 263	UEC 145127	Imbaú, PR, Brazil
		Urdampilleta J. D 288	UEC 153300	
	C. heringeri Ferrucci	Urdampilleta 363	UEC 153245	
		Urdampilleta J. D., et al. 369	UEC 171423	Santa Teresa, ES, Brazil
	C. cf. *integerrium* Radlk.	A. C. Kin et al 30014	UEC 066163	
	C. oliveirae Ferrucci	Urdampilleta J. D. 344	UEC 153291	
		Urdampilleta 338	UEC 153248	
	C. pterocarpum	Urdampilleta, J. D. 321	UEC 153275	
	C. urvilleoides	Urdampilleta, J. D. 425	SFP 193867	Itaobim, MG, Brazil
	Lophostigma plumosum Radlk.	Ferrucci M. S., et al. 2681	UEC 168401	Narciso Campero, Cochabamba, Bolivia
	Paullinia bicorniculata Somner	Melis, J. V. 544	UEC 168566	Ubatuba, SP, Brazil
		Kim A. C., et al. 30056	UEC 66166	Ubatuba, SP, Brazil
	P. carpopodea Cambess.	Obando S. & Urdampilleta, J. D. 330	UEC 153203	Ubatuba, SP, Brazil
		Urdampilleta J. D. 435	UEC 153198	
	P. coriacea Casar.	Obando S., et al. 239	UEC 1399646	Ubatuba, SP, Brazil

		Obando S., et al. 286	UEC 139946	Ubatuba, SP, Brazil
	P. cristata Radlk.	Obando S., et al. 300	UEC 153209	Guaraqueçaba, PR, Brazil
		Hatschbach, G., et al. 50549	UEC 45098	Antonina, PR, Brazil
	P. cupana Kunth	Obando S., et al. 353	UEC 171416	Ubatuba, SP, Brazil
		Urdampilleta J. D. 281	UEC 138838	Piracicaba, SP, Brazil
	P. elegans Cambess.	M. Solís 81	UEC 52792	
		Francisco, E. M., et al. 28528	UEC 157783	Sertaneja, PR, Brazil
	P. micrantha Cambess.	Leitão Filho, H. F., et al. 34483	UEC 80218	Ubatuba, SP, Brazil
		Leitão Filho, H. F., et al. 34474	UEC 80225	Ubatuba, SP, Brazil
	P. meliifolia Juss.	Obando S., et al. 352	UEC 578	Piracicaba, SP, Brazil
		Urdampilleta J. D., et al. 452	UEC 171432	Parati, RJ, Brazil
	P. pinnata L.	Macedo M. & Assumpção, S. 1768	UEC 36092	Mato Grosso, Brazil
		Prado, A. L. 3264	UEC 136476	Poconé, MG, Brazil
	P. rhomboidea Radlk.	Spina, A. P. 270	UEC 99646	Campinas, SP, Brazil
		Urdampilleta J. D. & Obando, S. 353	UEC 153171	Ubatuba, São Paulo, Brazil
	P. spicata Benth.	Silva, G. P. & Pereira, J. B. 4522	UEC 169169	Cavalcante, GO, Brazil
		Silva, G. P., et al. 4762	UEC 169172	Cavalcante, GO, Brazil
	Serjania caracasana (Jacq.) Willd.	Obando S. 295	UEC 139951	Ubatuba, SP, Brazil
		Carmello-Guerrero, S. M., et al 212	UEC 139949	Botucatu, SP, Brazil
		Martins, E. 18401	UEC 85603	São Sebastião, SP, Brazil
	S. communis Cambess.	M. T. Grombone-Guaratini, M. T., et al. 138	UEC 108259	Campinas, SP, Brazil
		M. T. Grombone-Guaratini, M. T., et al 138	UEC 108254	Campinas, SP, Brazil
		Grombone-Guaratini, M. T., et al 136	UEC 108336	Campinas, SP, Brazil
	S. erecta Radlk.	J. A. Raher 4003	UEC 14429	Brasília, Brazil

		S. M. Salis 144	UEC 052764	Brotas, SP, Brazil
	S. fuscifolia Radlk.	F. C. Passos, et al. FP83	UEC 79195	Gália, SP, Brazil
		Scotigma V. A. 810	UEC 188506	Nova Odessa, SP, Brazil
	S. gracilis	Obando S., et al. 306	UEC 153179	Paraná, Brazil
		Urdampilleta J. D., et al. 301	UEC 153296	Ponta Grossa, PR, Brazil
	S. laruotteana Cambess.	N. Tarodo et al. 18597	UEC 43379	Campinas, SP, Brazil
		F. C. Passos, et al. FP62	UEC 79170	Gália, SP, Brazil
	S. lethalis A. St.-Hil.	Alencar, M. E. 369	UEC 119855	Piripiri, PI, Brazil
		Urdampilleta J. D. 355	UEC 153222	São Carlos, SP, Brazil
		Obando S., et al. 348	UEC 171509	São João Batista do Glória, MG, Brazil
	S. multiflora Cambess.	Spina, A. P. 414	UEC 99878	Campinas, SP, Brazil
		Leitão Filho H. F., et al. 1670A	UEC 29757	Poços de Caldas, MG, Brazil
	S. pinnatifolia Radlk.	Urdampilleta J. D, et al. 408	UEC 153270	Rio de Contas, BA, Brazil
		Bernacci 1825	UEC 78809	São Roque de Minas, MG, Brazil
	S. reticulata Cambess	Hernandes-Bicudo, L. R., et al. 672	UEC 44213	Botucatu, SP, Brazil
		Makino, H. 35	UEC 14499	Ibiuna, SP, Brazil
	Thinouia compressa Radlk.	Harley, R. M. 21701	UEC 42508	Correntina, BA, Brazil
		Harley, R. M. 21997	UEC 42518	Tabovas, BA, Brazil
	T. mucronata Radlk.	Leitão Filho, H. F., et al. 23238	UEC 181267	Jundiaí, SP, Brazil
		Urdampilleta 230	UEC 153193	Londrina, PR, Brazil
	T. paraguayensis (Britton) Radlk.	Ferruci M. S., et al. 2267	UEC 165249	Chiquitos, Santa Cruz, Bolivia
	T. scandens	Vervlvet, R. R., et al. 3024	UEC 171528	Governador Lindenberg, ES, Brazil
	T. ventricosa Radlk.	Hatschbach, G. 42750	UEC 50876	Morretes, PR, Brazil

		Magnago, L. F. S., et al. 629	UEC 171542	Água Branca, ES, Brazil
	U. andersonii Ferruci	Urdampilleta, J. D. & Obando, S. 345	UEC 153290	Brumado, BA, Brazil
		M. Hatschbach 56644	UEC 63502	Imbituba, SC, Brazil
	Urvillea chacoensis Hunz.	At. Hunziker 22940	UEC 133045	
		Ferrucci M. S., et al. 2701	UEC 167548	Santa Cruz, Bolivia
	U. filipes Radlk.	Ferrucci, M. S., et al. 2595	UEC 168381	Chiquitos, Santa Cruz, Bolivia
	U. glabra Cambess.	Torres R. B., et al. 335	UEC 151416	Angatuba, SP, Brazil
		Urdampilleta, J. D. & Obando, S. 293	UEC 153309	Parati, RJ, Brazil
	U. laevis Radlk.	Melis J. van., et al. 3749	UEC 168536	Campinas, SP, Brazil
		Ferrucci, M. S., et al. 2534	UEC 165225	Chiquitos, Santa Cruz, Bolivia
	U. rufescens Cambess.	Wilson Hoenhe 6094	UEC 14477	Araruama, RJ, Brazil
		Urdampilleta, J. D. & Obando, S. 291	UEC 153310	Parati, RJ, Brazil
	U. stipularis Ferrucci	Urdampilleta 443	UEC 171424	Linhares, ES, Brazil
	U. triphylla Radlk.	Urdampilleta, J.D. & Obando, S. 349	UEC 153165	Ubatuba, SP, Brazil
		Urdampilleta, J. D. & Obando, S. 348	UEC 153166	Ubatuba, SP, Brazil
	U. ulmacea Kunth	Melo, E., et al. 2849	UEC 131872	Jaguaquara, BA, Brazil
		Obando, S., et al. 310	UEC 153175	Jaguariaíva, PR, Brazil
	U. uniloba Radlk.	Jarenkow, J. A. 696	UEC 81495	Capão do Leão, RS, Brazil
Sapindoideae/ Tribe Thouinieae	*Allophylus sericeus* Radlk.	Urdamilleta J. D. & Ferrucci, M. S. 438	UEC 171428	Santa Teresa, ES, Brazil
		Martins, F.R. 10056	UEC 14295	Santa Rita do Passa Quatro, SP, Brazil
	Allophyllastrum frutescens Acev.-Rodr.	Perdiz, R. O. 1310	MIRR 10057	Boa Vista, Roraima, Brasil
	Thouinia tomentosa DC.	Avecedo-Rodriguez 12867	US	Dominican Republic
Sapindoideae/ Tribe Bridgesieae	*Bridgesia incisifolia* Bertero ex Cambess.	Killip, Pisano 39778	US	Aconcagua, Chile

Sapindoideae/ Tribe Athyaneae	*Athyana weinmanniifolia* (Griseb.) Radlk.	Acevedo-Rodriguez 11166	US 3579590	Santa Cruz, Bolivia
	Diatenopteryx sorbifolia Radlk.	Tamashiro, J. Y., et al. 4718	UEC 60881	Campinas, SP, Brazil
		Jarenkow J. A. 945	UEC 81468	Marcelino Ramos, RS, Brazil
Sapindoideae/ Melicoccus group	*Melicoccus lepidopetalus* Radlk.	Ferrucci, M. S. 1539	SPF 149916	Bella Vista, Amambay, Paraguay
	Talisia angustifolia Radlk.	L. C. Bernacci 20850	UEC 50176	Itirapina, SP, Brazil
		J. Semir et al. 11551	UEC 25443	São Carlos, SP, Brazil
	Talisia esculenta (A. St.-Hil.) Radlk.	Leitão Filho H. F. 6051	UEC 14486	Campinas, SP, Brazil
Sapindoideae/ Cupania group	*Cupania zanthoxyloides* Cambess.	Galvão J. C. 27111	UEC 77227	Atibaia, SP, Brazil
	C. vernalis Cambess.	Caselli, C. B. & Schramm, J. E. 42008	UEC 152339	Jundiaí, SP, Brazil
	Matayba elaeagnoides Radlk.	Tamashiro, J. Y. & Goularti A. M 1320	UEC 25650	Poços de Caldas, MG, Brazil
	M. guianensis Aubl.	Oliveira, M. M. A. 1993	UEC 193321	Aracruz, ES, Brazil
	M. juglandiflora Radlk.	Leitão Filho, H. F., el tal. 1162	UEC 85413	
	Vouarana guianensis Aubl.	Nascimento J. R & Silva, C. F. 624	UEC 112908	Itacoatiara, AM, Brazil
Sapindoideae/ Litchi group	*Pometia pinnata* J. R. Forst. & G. Forst.	Kadir A664	US 3223206	Kabili, Borneo, Malaysia
Sapindoideae/ Bloomia group	*Guindilia cristata* (Radlk.) Hunz.	Ferrucci, MS. et al. 2930	US 3628155	San Juan, Argentina
Hippocastanoideae	*Acer palmatum* Raf.	Medina, M.C. 0112	SPF	São Paulo, SP, Brazil
	Dipteronia sinensis Oliver	W.V. Chun 4455	US	Hupeh, China

Abreviations: MBM, Museu Botânico Municipal; MIRR, Herbário do Museu Integrado de Roraima; UEC, Universidade Estadual de Campinas; SPF, Herbário da Universidade de São Paulo; US, Smithsonian Institution.

RESULTS

1. LATICIFER DISTRIBUTION

Several species belonging to Sapindioideae and Hippocastanoideae were analyzed to observe the presence and absence of laticifers and to establish comparisons. Laticifers were observed not only in the Paullinieae tribe, as initially thought, but also in most of the Supertribe Paullinodae and in the external groups analyzed. The presence of laticifers in the two analyzed genera of Hippocastanoideae was also verified.

In all species studied articulated nonanastomosing laticifers were observed, whose walls have a thickness similar to that of the adjacent parenchyma cells or in some cases may be slightly thicker. Depending on the genus, variations were observed concerning the composition of the laticiferous wall, frequency, diameter, length and distribution.

1. 1. SUBFAMILY SAPINDOIDEAE

1.1.1 Subertribe Paulliniodae

1.1.1.1 Tribe Paullinieae

Cardiospermum

From the six species of *Cardiospermum* that were analyzed, four do not have laticifers. These species are *C. corindum, C. grandiflorum, C. halicacabum,* and *C. heringeri* (Figure 1. A, B, C, D) and presented large narrow of secretory idioblasts of large size, mainly in the secondary phloem and the cortex where they are near to the sclerenchyma ring. In longitudinal and transversal section, it is noted a parenchymatous sheath that in this case is crystalliferous and in this species was very evident. These cells

are large and can be easily misinterpreted with laticifers, when they are empty. However, the Sudan Black B and Ferric chloride tests shows that these cells do not have secretion. The specie *C. heringeri* presented thelargest idioblasts observed in this study (Figure 1. A, B).

Laticifers were observed in *C. integerrimum* and *C. oliverae* (Figure 1. E, F), occurring in the cortex and phloem and classified as articulated nonanastomosing type. This was confirmed with Sudan Black B test. In appearance, laticifers and idioblasts are very similar in the sections with Toluidine blue, but histochemical tests helped to identify correctly (Figure 3. D).

Lophostigma

Lophostigma plumosum presented laticifers medium-shape forming large rows in the pith, and in the phloem, they are more narrow and long (Figure 1. G, H). The secretory idioblasts are present mainly in the secondary phloem.

Paullinia

Laticifers are present in the cortex, pith and secondary phloem of the 12 species of *Paullinia* analyzed. Laticifers in *Paullinia* can vary in size (Figure 2. A, B), being some very narrow and short (*P. coriacea, P. micrantha, P. pinnata* and *P. rhomboidea* (Figure 2.B)), or medium-size (*P. melifolia* and *P. spicata),* or largest (*P. bicornulata* (Figure 2.A)*, P. carpopodea, P. cristata P. cupana, P. melifolia* and *P. pinnata)* when observed in longitudinal section. With respect to the abundance, there are also important differences between species.

Serjania

Seven species of *Serjania* genus were analyzed. In general, the laticifers located in the cortical zone are small or medium and scarce (*S. fuscifolia, S. gracilis, S. multiflora, S. pinnatifilia* and *S. reticulata*) in comparison with those in the secondary phloem (Figure 2.C, D). Laticifers abundance and shape had low variation between species. Using Ferric chloride test large secretory idioblasts forming long rows was observed (Figure 5. A, B, C).

Thinouia

Five species were studied. In general, the laticifers in this genus are broad and long and in the cortex they are near of the crystalliferous sheath when are abundant (Figure 2. E, F). Histochemical tests were positive for laticifers (Figure 5. E) and idioblasts.

Urvillea

Ten species of *Urvillea* genus were analyzed. In the cortex, laticifers are adjacent to the sclerenchymatous ring, which is present in all species of the family. Laticifers are long and narrow, except in *U. filipe* (Figure 2. G, H) and *U. laevis,* which present long and broad laticifers in the cortex and pith and medium laticifers in the secondary phloem. The secretory idioblasts are more evident surrounding the pith and the phloem.

1.1.1.2 Tribe Thouinieae

Allophylus

The species *Allophylus sericeus* was evaluated. Laticifers are evident in pith (Figure 3. A). The secretory idioblasts are present mainly in the secondary phloem.

Thouinia

Thouinia tomentosa was evaluated. Laticifers are broad and short forming narrows of few cells. The Sudan Black B test shows an unusual thickness in the laticifers wall (Figure 3. B, C) and autofluorescence with UV light revealed the suberin presence in the wall, fluorescing in blue (Figure 3. D, E) and the secretion was also observed (Figure 3. C).

The secretory idioblasts are very small compared with laticifers, and they are present mainly in the secondary phloem (Figure 6. A, B).

1.1.1.3 Tribe Bridgesieae

Bridgesia

Monospecific *Bridgesia incisifolia* does not have laticifers, but scarce secretory idioblasts were observed forming short narrows (Figure 3. F) (maximum 2 cells), which was confirmed by histochemical tests (Figure 5. F).

1.1.1.4 Tribe Athyaneae

Athyana

The single species *Athyana weinmanniifolia* was evaluated. This species does not have laticifers. However, smallest secretory idioblasts are present (Figure 8. e, f). The ferric chloride test was positive for idioblasts.

Diatenopteryx

This genus has two species from which *Diatenopteryx sorbifolia* was evaluated. Laticifers form short narrows in the cortex and secondary phloem being scarce (Figure 3. G, H). Observed with Sudan Black B test the walls of laticifers look thicker (Figure 6. C), and suberine was observed in the fluorescence with UV light (Figure 3. G). Secretory idioblasts are abundant in both cortex and pith forming long narrows.

1.1.2 Melicoccus Group

Talisia

The species *T. angustifolia* and *T. esculenta* were evaluated. In particular, *T. angustifolia* the laticifers are exceptionally large compared with the rest of family (Figure 4. A).

1.1.3 Cupania Group

Matayba

The three species of this genus have laticifers (Figure 4. f) in both cortex (Figure 4. C) and phloem. This was observed using fluorescence techniques with Sudan Black B

test, due to the sections with Toluidine blue did not allow to clearly observing the presence of laticifers (Figure 6. D, E). They are smaller and the narrows are very short (maximum 2 cells) (Figure 4. D). The idioblasts are distributed in the cortex, phloem and pith.

Vouarana

One species of *Vouarana* genus was evaluated. In the phloem, the laticifers are more abundant and smaller (Figure 4. E, F). In this species idioblasts were not observed, neither by Toluidine blue nor Ferric chloride tests.

Cupania

Two species of *Cupania* genus were evaluated. Both phloem and cortex laticifers are closely located on the edge of sclerenchymatous ring, although being few in the phloem; they are longest (Figure 6. F).

1.1.4 Litchi Group

Pometia

The *Pometia pinnata* species was studied. Laticifers are longest and scarce (Figure 4. G). For this species, Sudan Black B test was positive for both laticifers and parenchymatous rays, which indicates its secretory nature.

1.1.5 Bloomia group

Guindilia

The species studied was *Guindilia cristata.* These species present largest secretory idioblasts in the phloem that can be confused with laticifers. However this cells reacted only with Ferric chloride test (Figure 6. G).

1.2 SUBFAMILY HIPPOCASTANOIDEAE

Dipteronia

Laticifers occur in the phloem of *Dipteronia sinensis* (Figure 10. e, f). Sudan Black B and Ferric chloride tests were used to confirm the laticifers and idioblasts presence (Figure 4. H, 5. H).

1. CHEMICAL COMPOSITION OF LATEX AND LATICIFER CELL WALL

The histochemical tests identified a great variety of compounds that constitute the latex, however the main component is the lipid fraction, from which terpenes (essential oils and resins) and fatty acids were mainly identified. In addition, carbohydrates, including mucilage, proteins and phenolic compounds were detected in all species. Alkaloids were identified only in the latex of *Paullinia* observed in some orange cells distributed both in the cortical zone as in pith (Figure 8).

The latex composition is very different from that of idioblasts, since they produce phenolic compounds exclusively in all genera studied identified as being of tannins type in fresh material analyzed. Although the phenolic compounds are also present in the latex, they are minority and detected by a weak staining. The laticifer wall is primary and rich in pectin in all species analyzed, however some genera were

distinguished by having callose or suberin in the wall. This was possible to observe under fluorescence and autoflourescence UV light respectively (Table 2).

Table 2. Histochemical tests for detection of compounds present in the latex of Paullinieae.

TEST	**COMPOUND**	**LATICIFERS**			**IDIOBLASTS**		
Sudan Black B	**Lipids**	***P***	***U***	***S***	***P***	***U***	***S***
Sudan IV	**Lipids**	+	+	+	-	-	-
Neutral red	**Lipids**	+	+	+	-	-	-
Nile blue	**Acidic and neutral Lipids**	+	+	+	-	-	-
Nadi reagent	**Essential oils and resins**	+	+	+	-	-	-
Copper acetate and rubeanic acid	**Fatty acids**	+	+	+	-	-	-
Tannic acid and ferric chlorid	**Mucilage**	-	-		-	-	-
Ruthenium red	**Acidic mucilage**	+	+	+	-	-	-
PAS reaction	**Carbohydrates**	+	+	+	-	-	-
Aniline blue black	**Proteins**	+	+	+	-		-
Aniline blue	**Callose**	+		+	-	-	-
Wagner's reagent	**Alkaloids**	+	-	-	-	-	-
Dragendorff's reagent	**Alkaloids**	+	-	-	-	-	-
Vanillin-hydrochloric acid	**Tannins**	-	-	-	+	+	+
Ferric chloride	**Phenolic compounds**	+	+	+	+	+	+
Ferrous sulfate in formalin	**Phenolic compounds**	-	+	+	+	+	+
Potassium dichromate	**Phenolic compounds**	-	-	-	+	+	+
Lugol's reagent	**Starch**	-	-	-	-	-	-

Abbreviations: P, Paullinia; S, Serjania, U, Urvillea.

2. DEVELOPMENT OF LATICIFERS

3.1 *Primary laticifers*

Laticifers and idioblasts were observed on leaves and stems (Figure 17). In the latter, both are distributed in the cortical and in some species in pith areas. Laticifers differ from idioblasts on the cell diameter, shape, color, distribution, and aspect of secretion (Figure 7. B). The idioblasts form extensive rows through shoot apical meristem and in general, in the tissues with primary growth. These secreting cells are observed abundantly (Figure 7. G).

This family present only articulated nonanastomosing laticifers type. Eventually can some anastomosing can be seen but they are few and only observed in *Paullinia* genus (Figure 7. I). Both laticifers and idioblasts are observed early in the development of shoot apex when the tissues are still in the meristematic phase (Figure 7. A, D). In the studied species, laticifers are originating from ground meristem and procambium when new meristematic cells are added at the apex of those already formed. Depending on the species, the idioblasts can be formed first than the laticifers, or vice versa. Laticifers start their secretory activity from de beginning of the formation, and the secretion inside can be observed. (Figure 7. E, G)

The two types of cells are distributed in both the cortex (Figure 7. H) and pith. The first laticifers formed, present in the ground meristem area, are mainly located in the cortical area showing a large sized compared with neighboring cells. In the primary growth was particularly observed laticifers accompanying the vascular system, with large nuclei very evident (Figure 7. D). The idioblasts are found in great amount, in both the cortical area, as in pith. Comparing to idioblasts, the size of laticifers is obviously greater (figure 7. A). In cross section they were observed distributed in both the cortical

area and pith, having a great size (Figure 7. B).

In cross section it can be observed that the cortical laticifers are formed first, and when they reach full differentiation, it can be seen laticifers in the pith having a smaller size in the successive sections. These are less frequent and the cells are shorter and narrower showing nuclei with various nucleoli (Figure 7. F). This particularity was also observed in parenchyma cells adjacent to laticifers. In the species that form several peripheral vascular cylinders, laticifers are observed in the cortex of each cylinder. In the leaf primordia, the location of laticifers is mainly in the adaxial side.

3.2 *Secondary laticifers*

In secondary growth, the cambium originates secondary laticifers in the phloem. They are located in the periphery of the phloem in the limits of sclerenchyma ring. The secondary laticifers do not join the laticifers originated in the primary growth and in fact, in advanced stages of secondary growth it is hard to observe primary laticifers.

Laticifers originate in the secondary growth are more narrow and form rows of few cells in comparison with those that occur in the cortex and pith. Due to structural similarity between laticifers and idioblasts, test for lipids or autoflourescence were necessary, which helped in the confirmation of the secretory cell type.

FIGURES

Figure 1. (Page 41). Morphology and distribution of laticifers in the Supertribe Paulliniodae **A.B.** *Cardiospermum heringeri* **C.** *C. halicacabum* **D.** *C. corindum* **E.F.** *C. integerrimum.* **G.H.** *Lophostigma plumosum.* Toluidine blue. Laticifers (White arrow).

Figure 2. (Page 42). Morphology and distribution of laticifers in the Supertribe Paulliniodae **A.** *Paullinia bicornulata* **B.** *P. rhomboidea* **C.** *Serjania reticulata* **D.**. *S. fuscifolia* **E.** *Thinouia paraguaiensis* **F.** *T. micrantha* **G. H.** *Urvillea filipes* Toluidine blue. Laticifers (white arrow). Sclerenchymatous ring (black arrow).

Figure 3. (Page 43). Morphology and distribution of laticifers in the Supertribe Paulliniodae **A.** *Allophylus sericeus* **B.C.D.E.** *Thouinia tomentosa* **F.** *Bridgesia incisifolia* **G.H.** *Diatenopterix sorbifolia.* **A.B.C.F.H.** Toluidine blue**. D.E.G.** Autofluorescence under UV light. Laticifers (white arrow). Idioblast (black arrow).

Figure 4. (Page 44). Morphology and distribution of laticifers in the Meliccocus group, Cupania group, Litchi group and subfamily Hippocastanoideae. **A.** *Talisia angustifolia* **B.** *Matayba elaeagnoides* **C.** *M. juglandiflora* **D.** *M. guianensis* **E.F.** *Vouarana guianensis* **G.** *Pometia pinnata* **H.** *Dipteronia sinensis.* **A.** Melicoccus group. **B.C.D.E.F.** Cupania group. **G.** Litchi group. **H.** Hippocastanoideae. **A.C.D.E.F.G.H** Toluidine blue**. B.** Autofluorescence under UV light. Laticifers (white arrow).

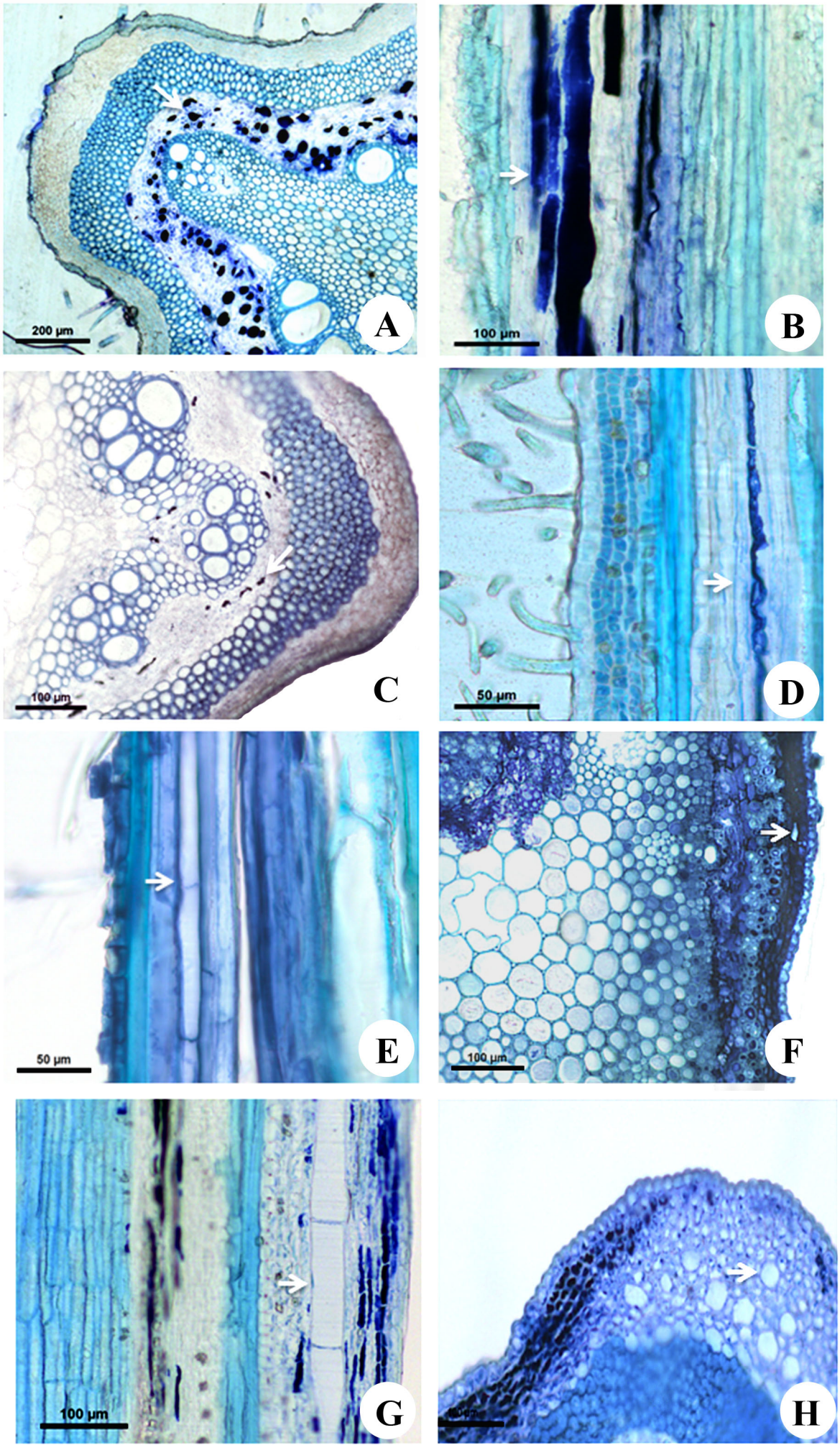
Figure 5. (Page 45). Histochemical test for confirmation of laticifers and idioblasts in Sapindaceae. **A.** *Serjania caracasana.* **B.C**. *S. laruotteana.* **D.** *Cardiospermum corindum.* **E.** *Thinouia paraguaiensis.* **F.** *Bridgesia incisifolia.* **A.E.** Sudan Black B test. **D. F.** Ferric chloride test. **B.** Astra Blue and Safranin. **C.** Neutral red test. Laticifers (White arrow). Idioblasts (black arrow).

Figure 6. (Page 46). Histochemical test for confirmation of laticifers and idioblasts in Sapindaceae. **A.B.** *Thouinia tomentosa.* **C.** *Diatenopteryx sorbifolia.* ***D.*E.** *Matayba elaeagnoides.* **F.** *Cupania zanthoxyloides.* **G.** *Guindilia cristata.* **H.** *Dipteronia sinensis.* **A.B.C.D.E.F.H.** Sudan Black test. **G.** Ferric Chloride. Laticifers (White arrow). Idioblasts (black arrow).

Figura 7. **(Page 47).** Distribution and ontogeny of laticifers**. A** *Paullinia carpopodea.* **B-D.** *Serjania* sp. **E.** *Urvillea ulmacea.* **F.** Laticifer with dense content)**. G.** *Paullinia seminuda.* **H.** *Serjania* sp. **I.** *Urvillea ulmacea.* **J.** *Paullinia carpopodea.* Laticifers (black arrow). Anastomosing laticifer (black arrow). Astra blue and Safranin.

Figure 8. (Page 48). Histochemical tests for chemical composition of latex and laticifer cell wall **A.C.D.H.I.L.** *P. seminuda.* **B.F.J.K.** *Serjania* sp**. E.G.M.N.O.** *Urvillea ulmacea.*

A. Sudan Black (lipids). **B.** Sudan IV (lipids). **C.** Neutral red (Lipids). **D.F.** Nile blue (Acidic and neutral lipids). **e.** Nile blue- Fluorescence (Acidic and neutral lipids). **G.H.** Nadi reagent (Essential oils and resins). **I.** Copper acetate and rubeanic acid (Fatty acids). **J.** Aniline blue black (Proteins). **K.** Aniline blue (Callose). **L.** Dragendorff's reagent (Alkaloids). **M.** Ruthenium red (Acidic mucilage). **N.** PAS reaction (Carbohydrates). **O.** Ferrous sulfate in formalin (phenolic compound). Laticifers (White arrow). Idioblast (black narrow).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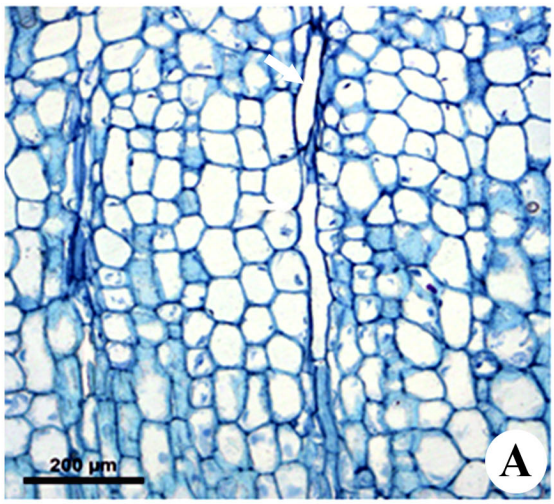
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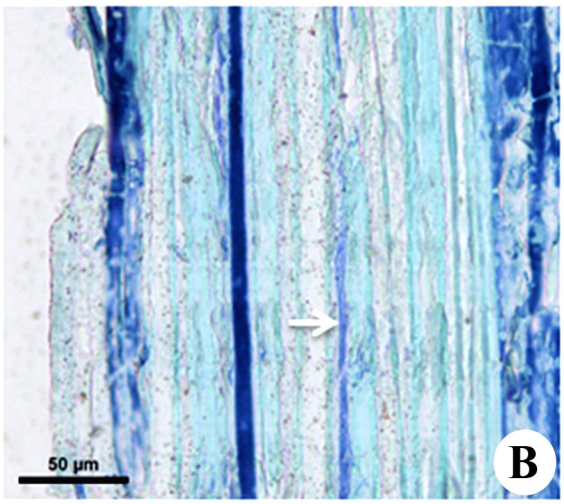
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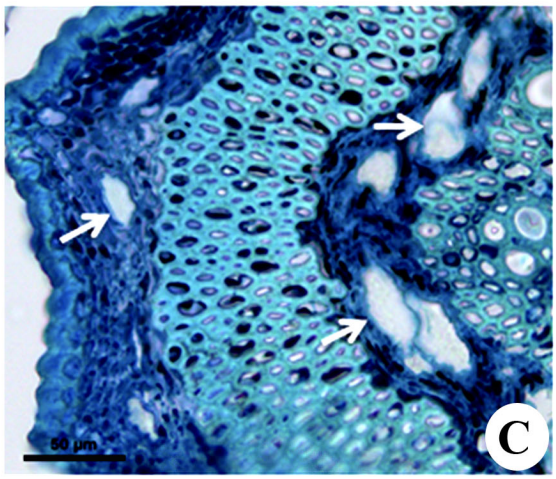
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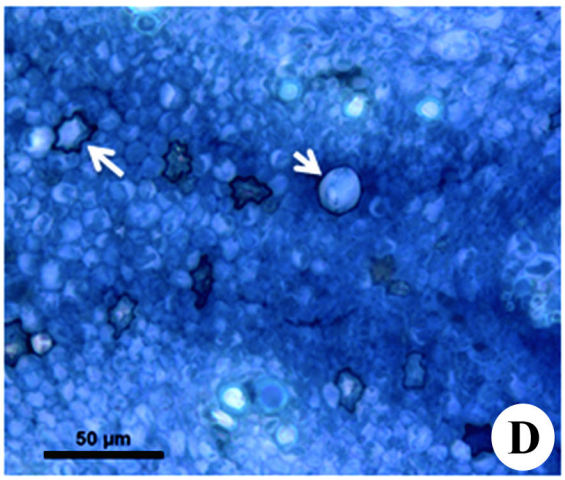
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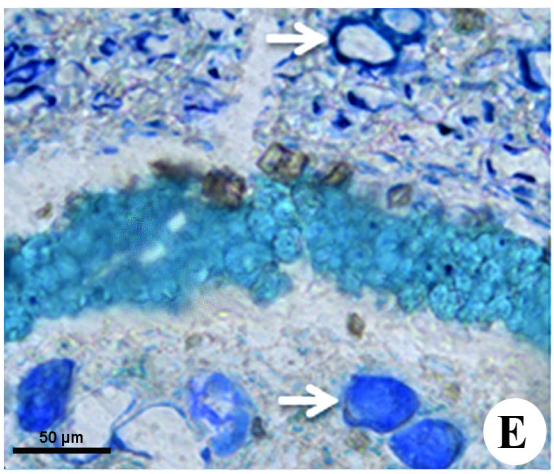
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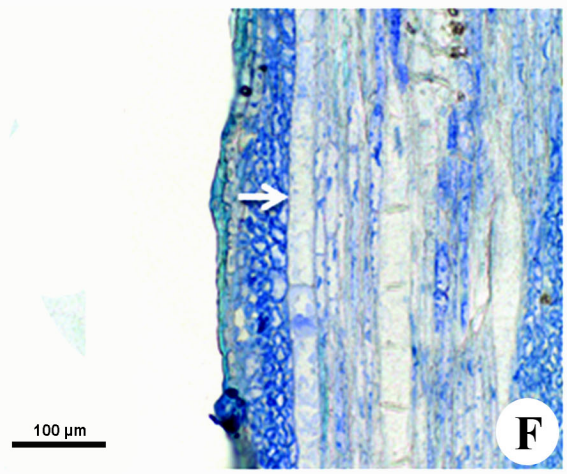
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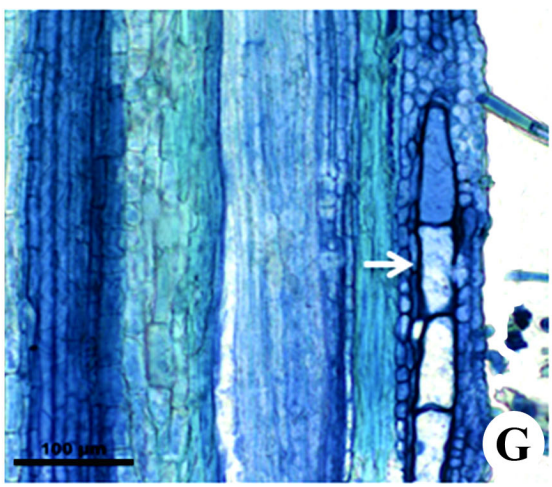
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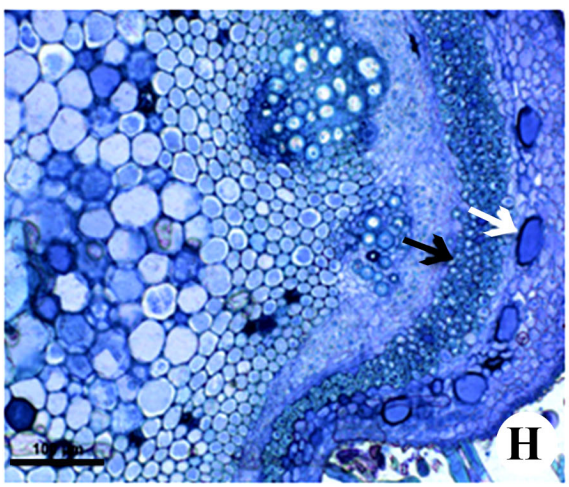
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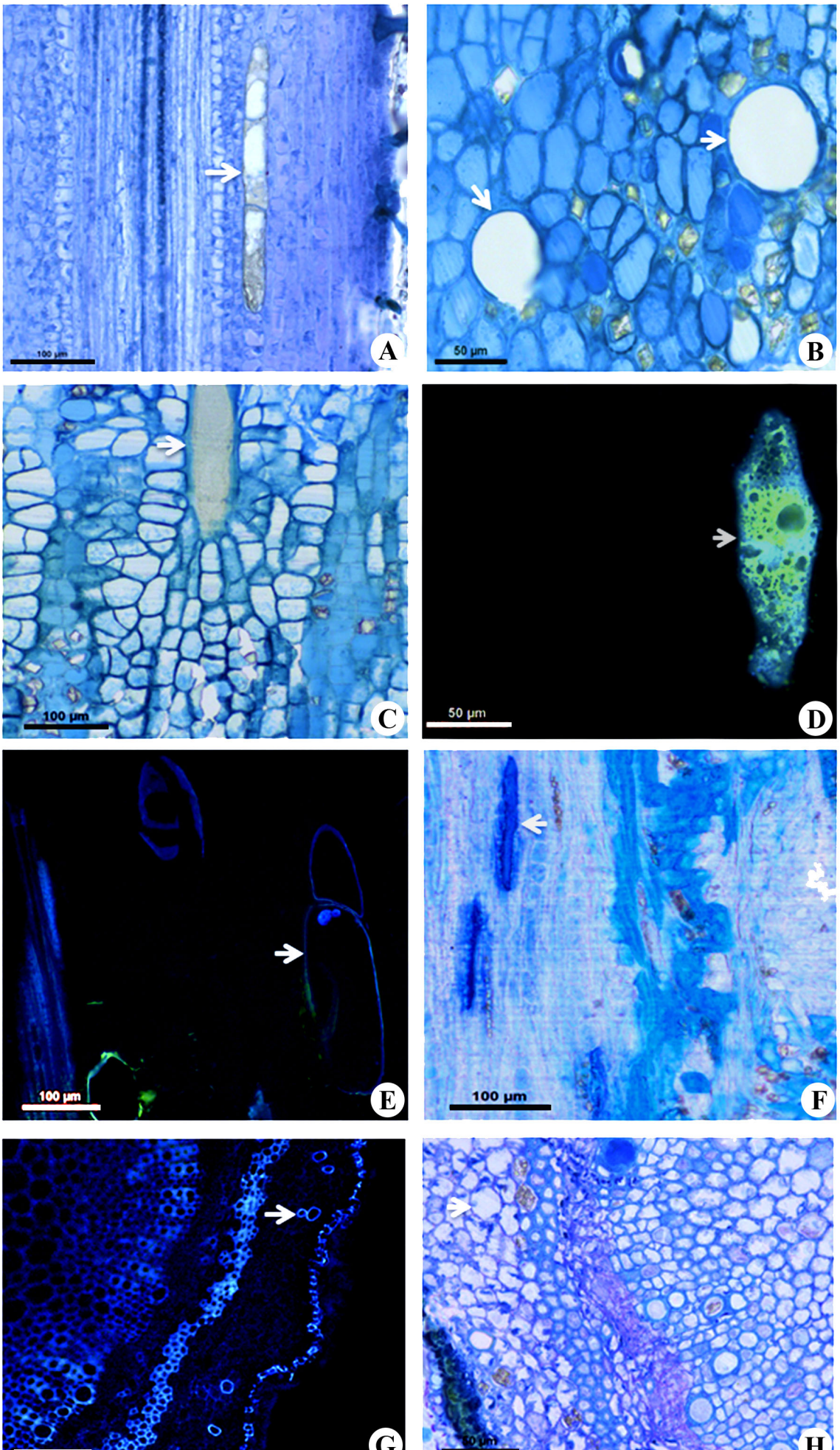
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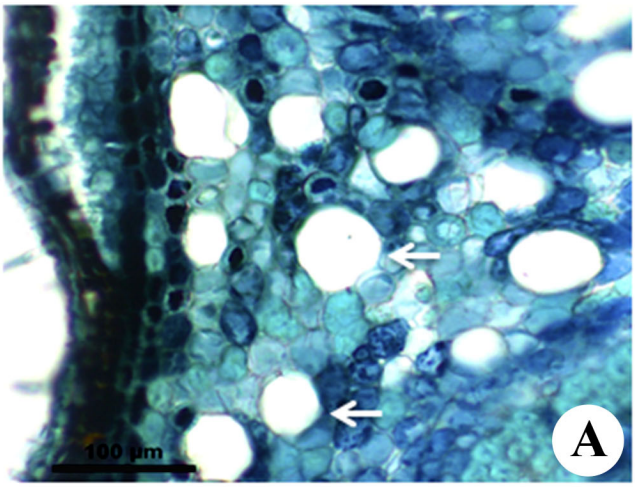
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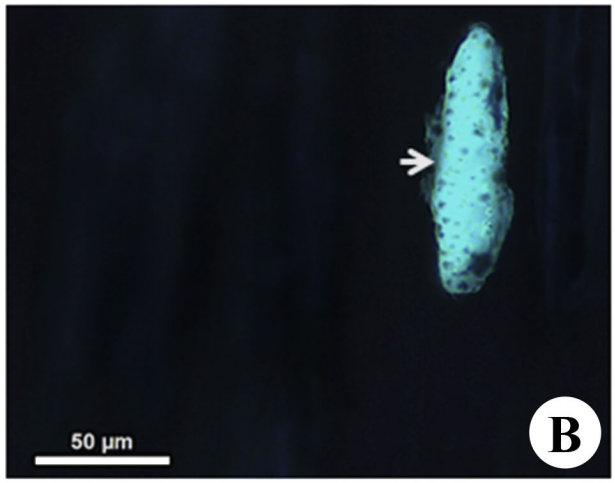
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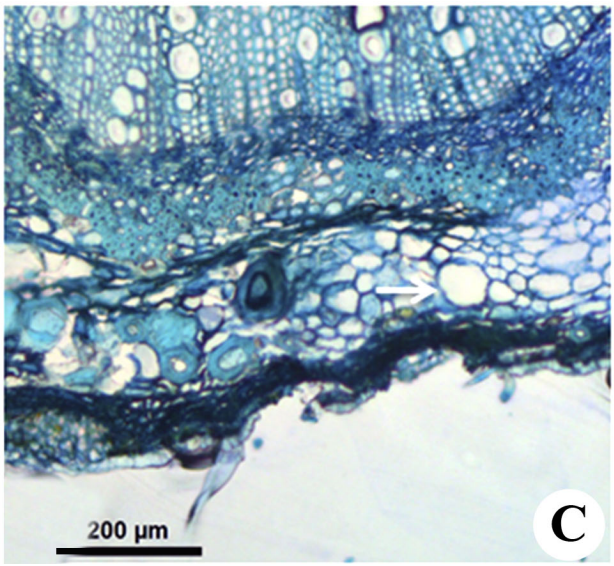
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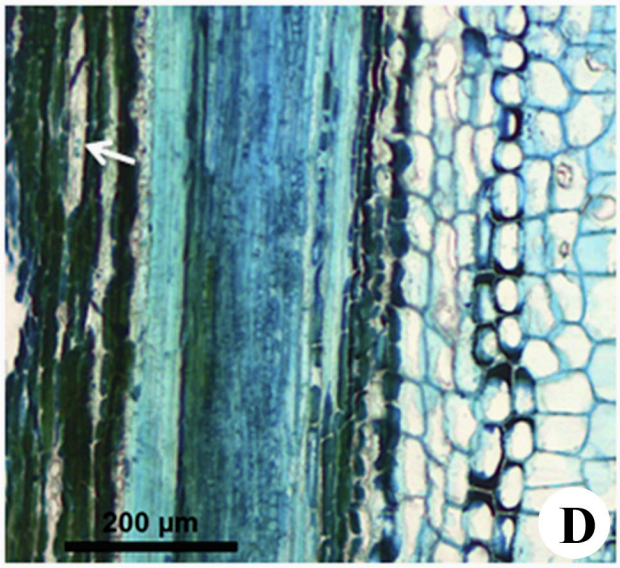
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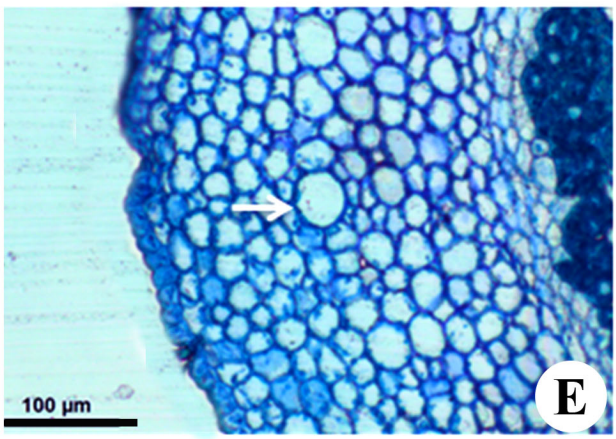
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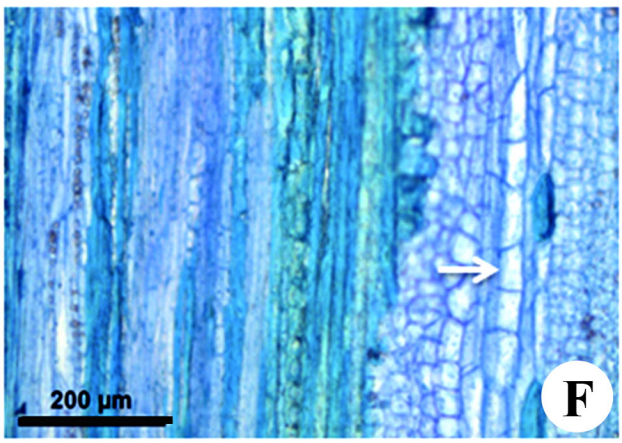
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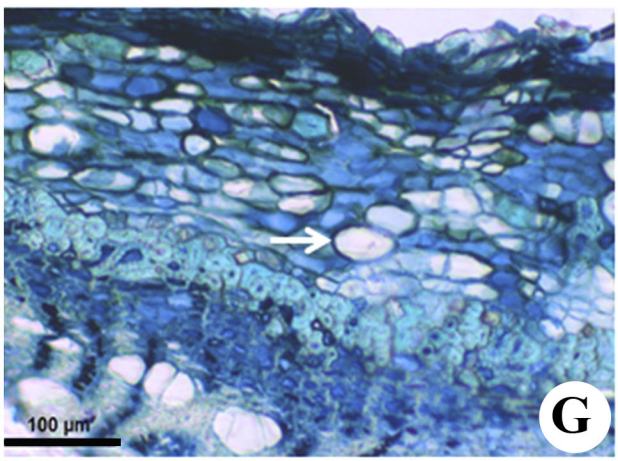
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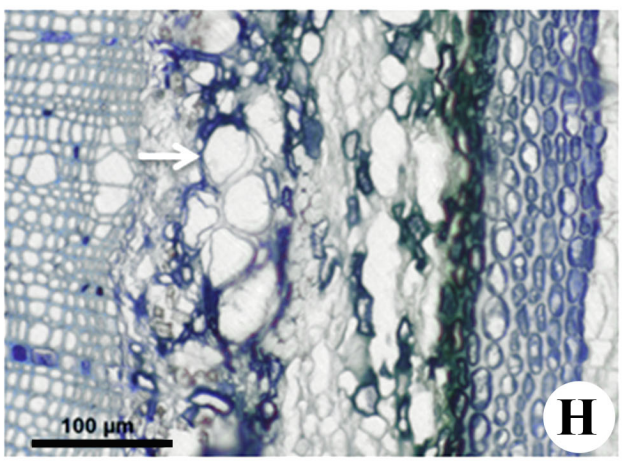
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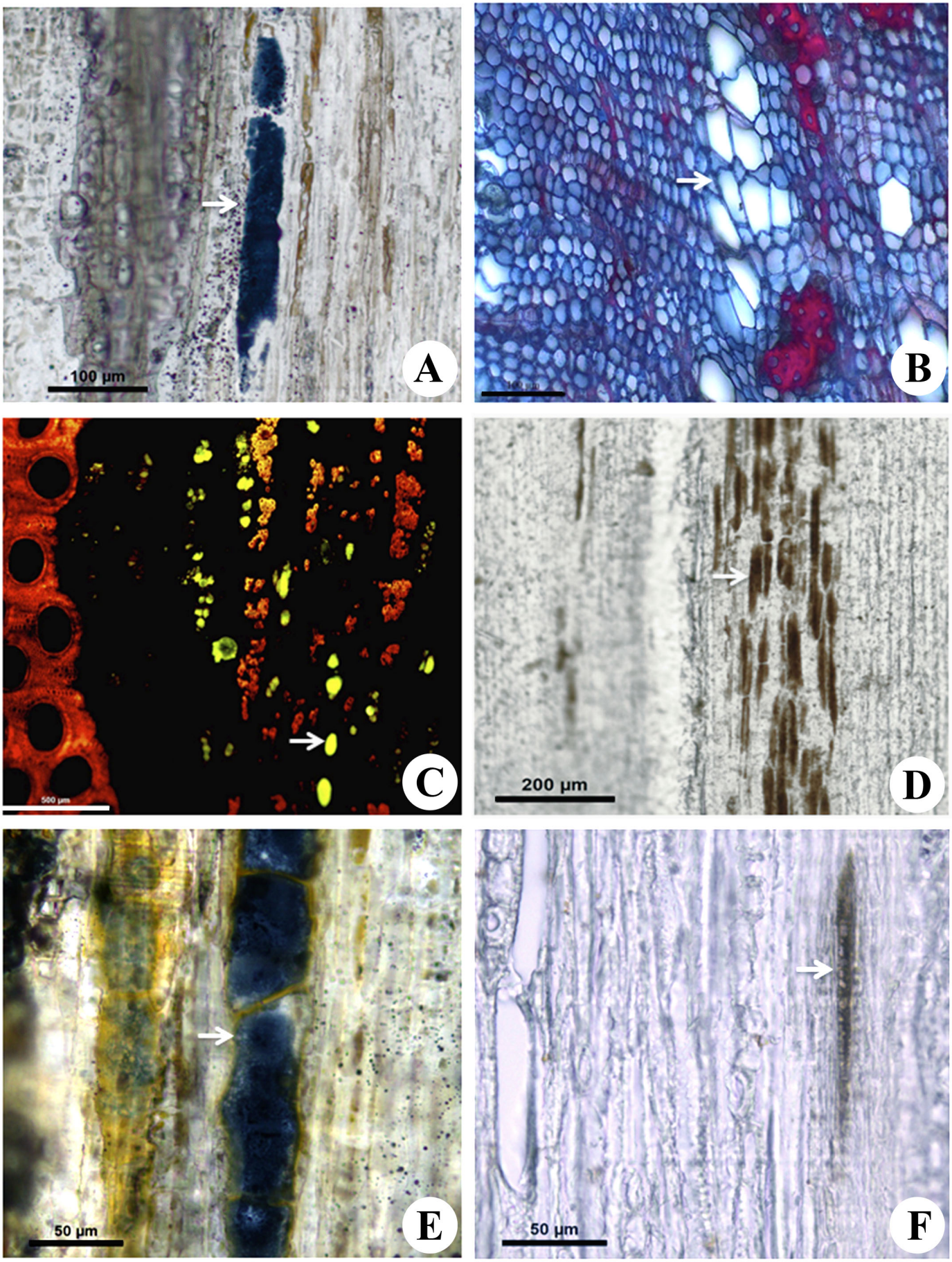
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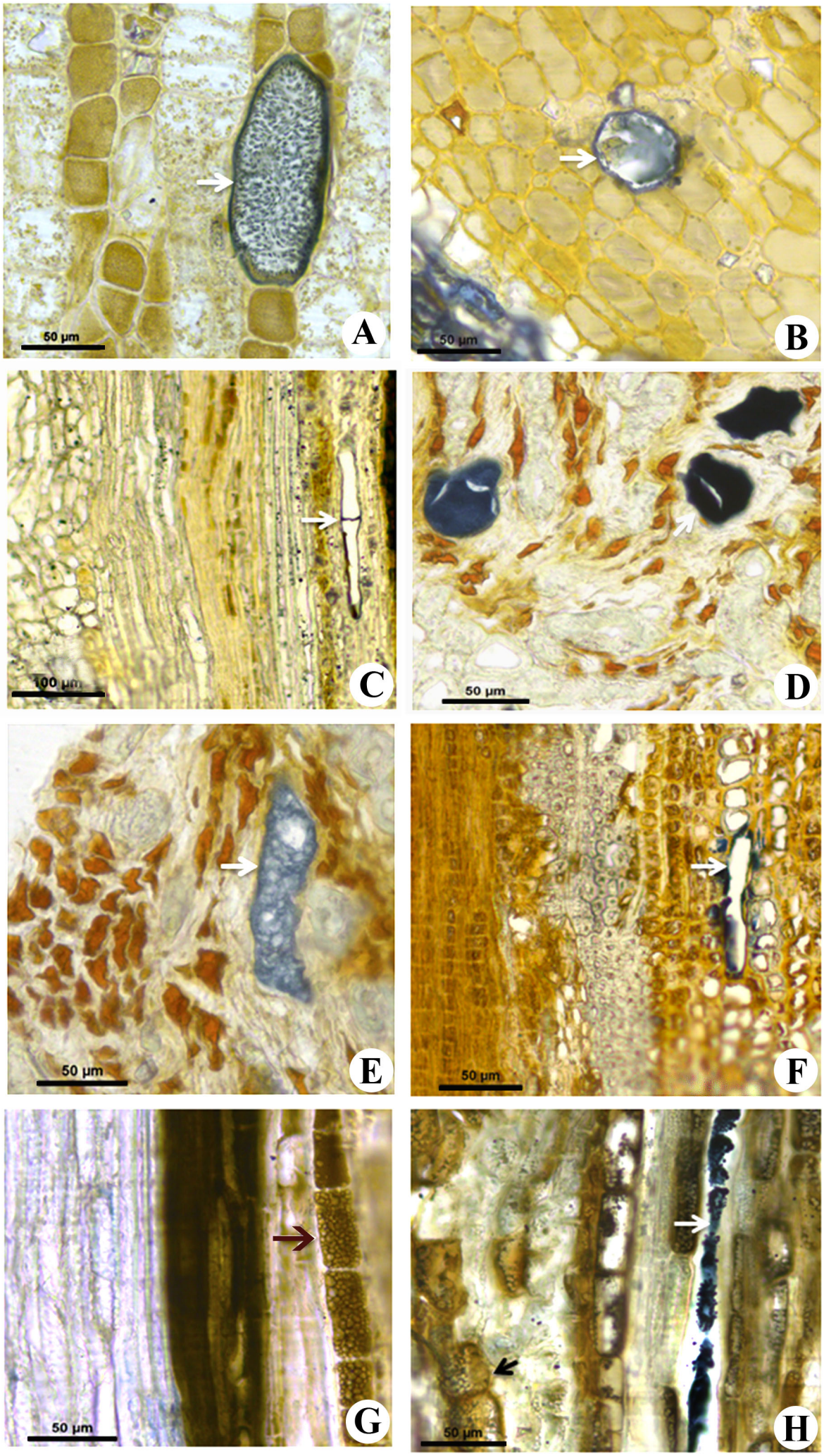
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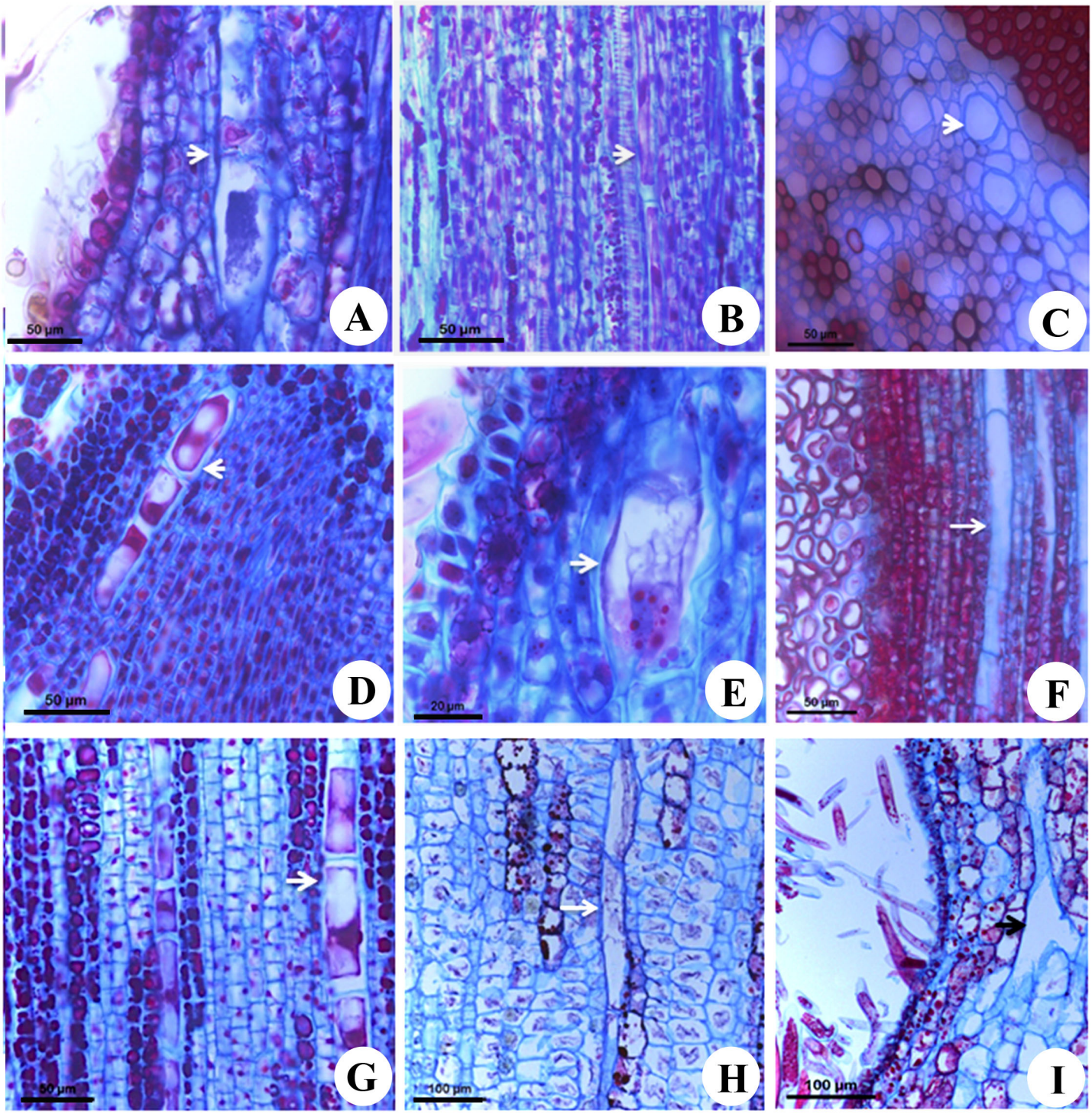
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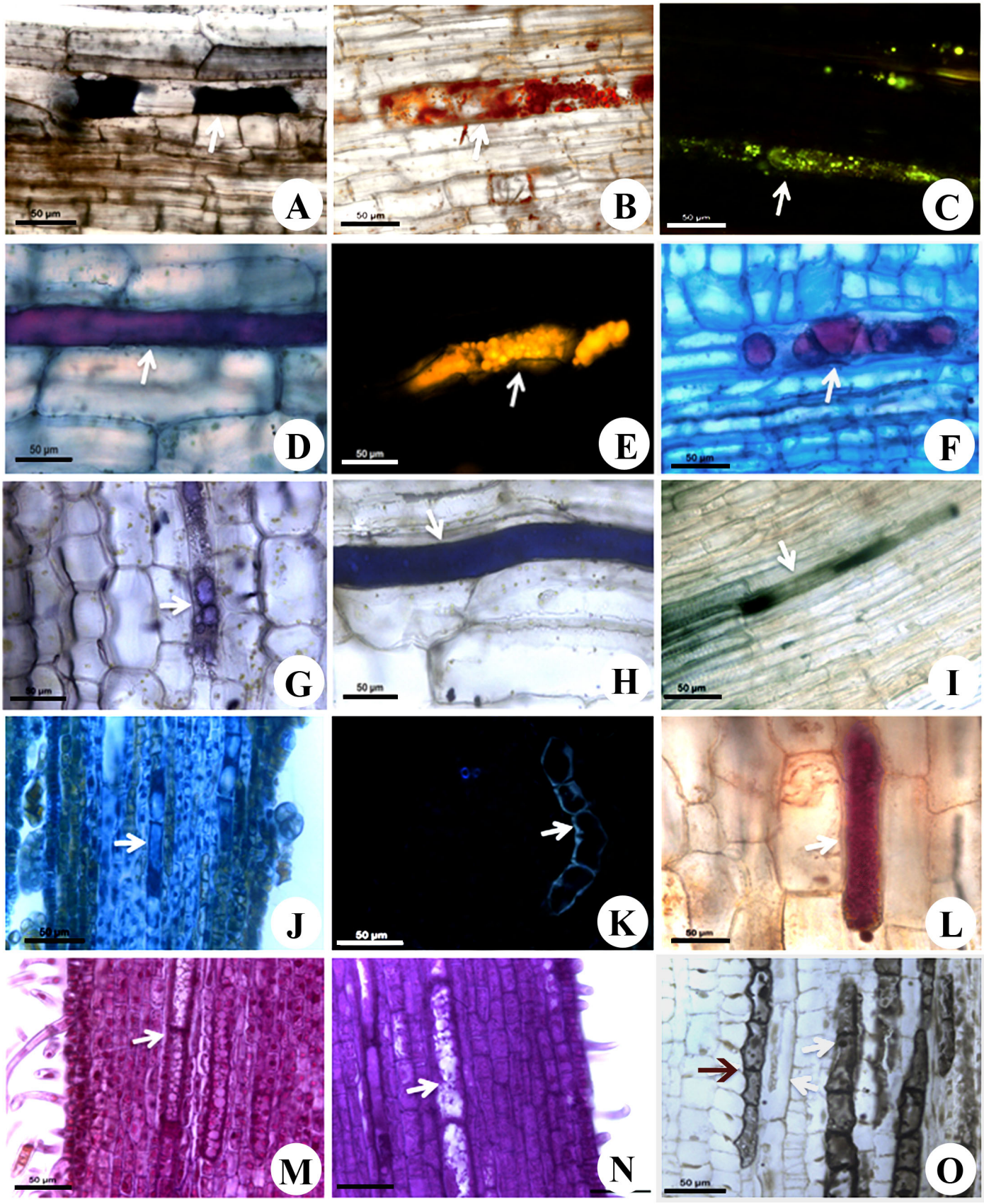
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DISCUSSION

This study confirmed the presence of laticifers in two subfamilies of Sapindaceae (Hippocastanoideae and Sapindoideae), for which the study of the structure and morphology has shown that they are of articulated non-anastomosing type, whose latex is composed of many chemical classes of compounds with the prevalence of terpenoids. Eventual anastomosing can occur as observed in *Paullinia* but this is rare in the laticifer system. In addition, two laticifer systems occur in the genera analyzed: one constituted by primary laticifers in the cortex or cortex and pith of stems, and other constituted by secondary laticifers present in secondary phloem. Both systems are independent from each other.

Since Radlkofer's recognition in 1931 and despite of molecular analyses made until today, the family is largely unresolved (Kubitzki, 2011). The study of secretory structures has been of great help for a better resolution of the phylogenetic relationships within this group at genus and species levels (Monteiro-Scanavacca et al., 1979; Maleci and Marchi, 1983; Bini Maleci and Servettaz, 1991; Doaigey and Harkiss, 1991; Castro et al., 1997) due to conservatism of these structures of a high metabolic complexity (Solereder, 1908; Fahn, 1979). In general, the distribution of laticifers and idioblasts in Sapindaceae has a constant pattern, and there is no variation within the same genera. The morphology of the laticifers in the family is also little variable within genus but there are differences between genera, such as wall composition and size.

For the Paullinodae supertribe, laticifer has been described for three genera (Weckerle and Rutishauser, 2005). We described the presence of laticifers for the first time for genera that had not been recognized as latescent. Even in *Paullinia, Serjania* and *Urvillea*, where some species have a milky secretion described as latex (Alves de

Areia, 1966; Weckerle and Rutishauser, 2005), not all species of the genera were considered latescent. This work showed that the presence or absence of laticifers is constant within the genera of Sapindaceae. This results corroborate the actual circumscription of *Cardiospermum* and segregation of species suggested by Acevedo-Rodríguez et al. (2017) from molecular data.

Our results demonstrate that all Paullinodae supertribe have laticifers, except *Athyana, Bridgesia* and *Cardiospermum sensu stricto* lineages. In addition, species of Melicoccus group, Cupania group, and Litchi group analyzed as external groups, also have laticifers and idioblasts. Observed in transverse section, laticifer morphology in these outgroups of Paullinodae is markedly different, being shorter and wider. Although latex has never been reported to non-Paullinodae groups in Sapindoideae, there are reports of a red exudate for *Pometia* (Litchi group) (Kubitzki, 2004) that we confirm that they are indeed laticifers. Additionally, we observed parenchymatous rays with phenolic content in the present study. For Cupania group, secretory idioblasts producing saponins (Suárez et al., 2004) and lipids (Mark, 2003) have been described but we also observed laticifers in this group.

The supposed absence of latex in various species and even in genera is due to laticifer type, cell volume and frequency. In some species, the continuity of these secretory cells that are generated by the dissolution of the transverse or oblique walls and by the lateral anastomoses between different laticifers, allows a better flow of latex at the wound site. For this reason the latex produced in several interconnected cells is released simultaneously (Demarco and Castro, 2008), which does not occur in plants whose laticifers are articulated nonanastomosing like those of Sapindaceae. The water flow is another important factor in the amount of latex released (Downton, 1981). When the laticifers wall does not have any type of hydrophobic substance, they are permeable

to water, which implies that have a decrease of turgor pressure of laticifer and the water of adjacent tissues flow through the wall. This contributes to a great amount of latex exudation (Pickard, 2008). The walls of laticifers from families as Convolvulaceae are impregnated by a suberine layer within the wall surrounding each cell (Fineran et al., 1988) and until now it was considered as the only family with these characteristic. However, suberin was detected in the cell wall in basal groups within supertribe Paullinoidae (*Diatenopterix* and *Thouinia*) and this can explain why they have not been observed before.

The time of the year is very important in the water flow, since when there is little quantity of water in the soil, the latex exudate is drastically reduced. In the case of genera as *Cardiospermum s.s., Lophostigma Thinouia,* and *Urvillea,* the quantity of latex observed when the stem is cut is less in comparison with other studied species due to nonanastomosing, short and scarce cells. For *Lophostigma* secretory canals was described (Acevedo-Rodriguez, 1993), but in this work was confirmed to be laticifers very similar to other species of tribe Paullinieae.

Idioblasts that have saponins have been already described, as well as mucilage cells that occur in the epidermis of the leaves, and the unconfirmed presence of laticifers, being common in Sapindaceae (Metcalfe and Chalk, 1950b; Kubitzki, 2011; APG, 2016). It is difficult to differentiate idioblast from laticifers in this group (Milanez, 1959), due to short and narrow cells in some laticifers have, whereas in some genera the idioblasts are large and long, give rise to an overlap when trying to differentiate only morphologically. Nevertheless, in our study, the histochemical analysis clearly demonstrated the presence of phenolic idioblasts in all species of the family, identified as tannins in at least three species, very distinct from latex which is composed of different classes of terpenoids, fatty acids, carbohydrates, proteins,

phenolic compounds and eventually alkaloids. In addition, the composition of the laticiferous wall is distinct from the idioblasts wall in some genera, indicating a distinct evolutionary origin. .

For the subfamily Hippocastanoideae latex has been reported in *Acer* structures called as "secretory sacs" present in phloem of leaf veins and axis and less frequently in the mesophyll (Metcalfe and Chalk, 1950a). In taxonomical works, latex was described in just one out of four species of *Acer* (Amini et al., 2008). In *Dipteronia,* a genus with two species, laticifers have also been cited in phloem of the fruit (Benedict, 1961) . In this work was proved the existence of laticifers in both genera, which is an exception in Hippocastanoideae.

Latex

Milanez (1959) described the presence of secretory cells in fruits of *Paullinia cupana* var. *sorbilis,* which he called secretory tubes with resinous content. However, we detected many different compounds in the latex of *P. carpopodea* and *P. seminuda,* including essential oils, resins, fatty acids, carbohydrates and mucilage, proteins, alkaloids and, in less amount, phenolic compounds. Several works have demonstrated that the genus *Paullinia* presents a wide variety of chemical compounds where prevail alkaloids as the cafein, theophylline and theobromine; polyphenols, saponins, condensed tannins, cyanogenic compounds (Otobone et al., 2005; Pérez-Dávila et al., 2011). Some species are widely used in various regions of Amazon as medicinal, in the croops, veterinary and pest control (Iannacone et al., 2007).

Serjania may produce aqueous or milky exudates (Acevedo-Rodriguez, 1993) and secretory cells have been described in the pith and cortex of *S. caracasana* (Tamaio, 2001). Nevertheless, the nature of secretion is unknown. Our analysis evidenced that

latex of *Serjania* and *Urvillea* is as complex as *Paullinia* latex, except the presence of alkaloids.

Phylogenetic inference of laticifer evolution

The laticifers were identified in a much larger number of genera in relation to the expected one, from taxonomic data of collection. Its presence in the studied species reveals important generic relationships within the family, corroborating the current circumscription and indicating future perspectives of study.

Considering previous works and the most recent phylogeny made for the supertribe Paulliniodae (Acevedo-Rodríguez et al., 2017), *Cardiospermum* is interpreted as a polyphyletic group. Based on molecular data, some species are located close to *Serjania* and others close to *Urvillea* or as a monophyletic group, forming a clade with *Paullinia*. Acevedo-Rodríguez et al. (2017) proposed new combinations or new genera for the former species of *Cardiospermum.* In our work, *Cardiospermum s.s.*, (*C. corindum, C. grandiflorum, C. halicacabum* and *C. heringeri)* does not have laticifers. Moreover, species of *Cardiospermum* that are more related with *Serjania* and *Urvillea* (*C. integerrimum, C. oliverae)*, undoubtedly, have laticifers. The close relationship between *Cardiospermum* and *Urvillea* is strengthened by the presence of anemochorous fruits which are septifragal with papery, inflated capsules and herbaceous stems (vines) in both genera (Weckerle and Rutishauser, 2005).

Optimizing our data in the phylogeny of Sapindaceae (Buerki et al., 2009; Acevedo-Rodríguez et al., 2017), we observed that the laticifers evolved at least six times throughout the evolutionary history of the family (Figure 9). Probably, the ancestor of Sapindaceae does not have laticifers (0.01% likelihood), and this character

emerged multiple times in different lineages of Sapindaceae. For example, the ancestor of Paullinieae + Thouinieae could have had laticifers (95% likelihood) but within Paullinieae this character was secondarily lost in *Cardiospermum s.s.* For the supertribe Paullinodae the probability of the ancestor having had laticifers was 47% (likelihood), however is important to keep in mind that the tribes in which the character of having laticifers is absent are tribes with few species or, in some cases, monotypic. The ancestor of Cupania group could not have had laticifers (0.02% likelihood) and in Melicoccus group could have had laticifers (93% likelihood). In the case of Hippocastanoideae, which is a monophyletic group (Buerki, 2010), the genera studied (*Acer* and *Dipteronia)* present laticifers and this character could have been present in the ancestral of these genera (88% likelihood) but not in the ancestral of the subfamily (14% likelihood).

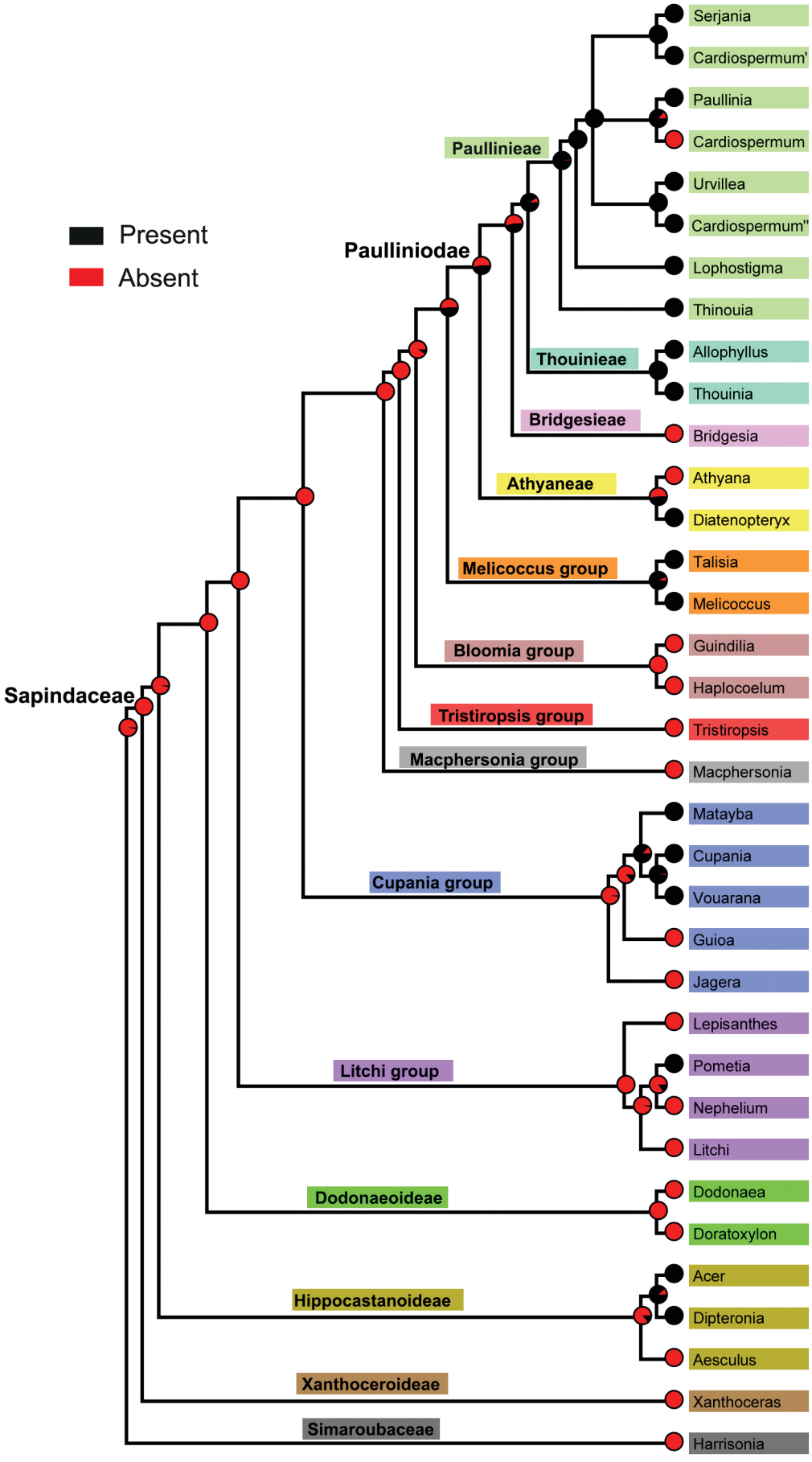

Figure 9. Distribution of laticifers in Sapindaceae and maximum likelihood analysis based on a phylogenetic framework (Buerki et al., 2009; Acevedo-Rodríguez et al., 2017).

CONCLUSIONS

This was the first detailed description of laticifers in the family and the presence of this secretory structure was reported for several genera for the first time. This is due to the type of laticifer present in the family, which does not exude profuse latex when the plant is sectioned. Laticifers have had multiple origins in the family and present particular characteristics of cell wall or different colors of latex depending on the genus analyzed, indicating a metabolic diversity of this structure in the family. Presence or absence of laticifers can be used as a character that can help in taxonomic resolution and establishment of relationships between groups within Sapindaceae. New studies including more species are needed to expand the knowledge about the evolution of these structures within the family.

Acknowledgements

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