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Plant anatomy: history and future directions

Leaf secretory structures in *Ceiba* (Malvaceae - Bombacoideae): ontogeny, anatomy and histochemistry

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Abstract

The description of secretory structures in Malvaceae is controversial, and results in conflicting interpretations. Amid conflicting interpretations, therefore, the present study aims to describe the ontogeny diversity and histochemistry in *Ceiba*, emphasizing the secretory structures in leaves of *C. erianthos*, *C. jasminodora*, *C. pentandra*, and *C. speciosa*. All analyzed species present mucilaginous, crystalliferous, and phenolic idioblasts. These structures are randomly arranged in the mesophyll, epidermis, petiole cortex, and parenchymatic tissue of the midrib. However, in *C. jasminodora* and *C. pentandra*, secretory structures are only found in the midrib area. The development of mucilaginous idioblasts is asynchronous and, when mature, they coalesce forming large structures full of mucilage. Clavate-type pluricellular glandular trichomes were also detected scattered randomly on both leaf surfaces. Their ontogeny is described, and histochemical tests showed the presence of lipophilic substances. Extrafloral nectaries were observed in the middle third of the midrib, on the abaxial side of the leaflets. They originate from the protoderm and ground meristem of the midrib cortex. It is expected that these results will help consolidate knowledge of secretory structures in Malvaceae, leading, in turn, to the elucidation of phylogenetic relationships.

Key words: Bombaceae, extrafloral nectaries, mucilaginous idioblasts.

Resumo

A descrição das estruturas secretoras em Malvaceae não é consenso e resulta em interpretações conflitantes. Em meio a interpretações conflitantes, o presente estudo tem como objetivo descrever a diversidade ontogenética e histoquímica em *Ceiba*, enfatizando as estruturas secretoras em folhas de *C. erianthos*, *C. jasminodora*, *C. pentandra* e *C. speciosa*. Todas as espécies analisadas apresentam idioblastos mucilaginosos, cristalíferos e fenólicos. Tais estruturas estão dispostas aleatoriamente no mesofilo, epiderme, córtex do pecíolo e no tecido parenquimático da nervura central. Porém, em *C. jasminodora* e *C. pentandra*, encontram-se apenas na região da nervura central. O desenvolvimento de idioblastos mucilaginosos é assíncrono e quando maduros, coalescem formando grandes estruturas repletas de mucilagem. Tricomas glandulares pluricelulares do tipo clavado também foram detectados distribuídos aleatoriamente em ambas as superfícies foliares. Sua ontogenia é descrita e testes histoquímicos evidenciaram a presença de substâncias lipofílicas. Nectários extraflorais foram observados no terço médio da nervura central, na face abaxial dos folíolos. Estes se originam da protoderme e do meristema fundamental do córtex da nervura central. Espera-se que estes resultados ajudem a consolidar o conhecimento das estruturas secretoras em Malvaceae, levando, por sua vez, à elucidação das relações filogenéticas.

Palavras-chave: Bombaceae, nectários extraflorais, idioblastos mucilaginosos.

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Introduction

Ceiba Mill. is a neotropical genus from Bombacoideae (Malvaceae) with 18 species, 11 of which occur in Brazil, and six are endemic (Gibbs & Semir 2003; Carvalho-Sobrinho & Queiroz 2008; Carvalho-Sobrinho 2023). The genus is represented by medium to large trees, reaching up to 50 m in height (*Ceiba pentandra* (L.) Gaertn.), with aculeate stems, usually ventricose and with greenish bark, deciduous leaves at flowering (Gibbs & Semir 2003; Carvalho-Sobrinho & Queiroz 2008). Carvalho-Sobrinho *et al.* (2016) carried out phylogenetic studies based on molecular and morphological data by sampling representatives of all genera and subfamilies from Malvaceae and delimited three different tribes, namely Adansonieae Horan., Bernoullieae Carv.-Sobr. and Bombaceae Kunth., making the group more concise. More recently, Pezzini *et al.* (2021) performed a phylogenetic and biogeographical analysis based on molecular data, using 30 accessions representing 14 species, which resulted in the recovery of *Ceiba* as monophyletic. The genus is represented by three main clades: (i) Central and South American rain forest lineage, sister to the remaining species, (ii) lineage Central American and Mexican seasonally dry tropical forests, and (iii) lineage South American seasonally dry tropical forests.

It is well known that morphological analyses are important for understanding phylogenetic relationships throughout the evolutionary course of Angiosperms, and for clarifying relationships among families and genera (*e.g.*, Fougère-Danezan *et al.* 2010; Nogueira *et al.* 2013). Particularly, secretory structures provide strong taxonomic and ecological relevance (Solereder 1908; Webber 1938; Metcalfe & Chalk 1957). Morphological descriptions related to such structures have also been used in ontogeny studies, aiming to clarify the relationships among taxa (*e.g.*, family, genus, and species) (Luna *et al.* 2019; Silva *et al.* 2019).

In Malvaceae, secretory canals/ducts and cavities, idioblasts, trichomes, and nectaries are very frequent and, in general, have mucilage as the main exudate, which is observed in both vegetative and reproductive organs (Metcalfe & Chalk 1957; Scott & Bystrom 1970; Gregory & Baas 1989; Sawidis 1991, 1998). Some studies point out that secretory structures constitute synapomorphies for the order Malvales (Alverson *et al.* 1998, 1999; Judd *et al.* 2009); the which first anatomical description was made for *Althaea* (L.) (Malvaceae) by Meyer in 1837 (Gregory & Baas 1989).

However, studies reporting on the secretory structures in *Ceiba* are scarce. Kuruvilla & Anilkumar (2018) and Perrotta *et al*. (2007) reported the presence of secretory cavities in the genus, and Ferreira (2016) cited the presence of extrafloral nectaries in some Bombacoideae genera, including *Ceiba*. Despite this, ontogenetic studies have not been carried out for the genus.

Considering the anatomical, taxonomic, ecological, and economic importance of secretory structures (Metcalfe & Chalk 1957; Lackey 1978), the present study aimed to describe the anatomy, ontogeny, and histochemistry of the leaf secretory structures in *Ceiba* species, to help to consolidate knowledge of secretory structures in Malvaceae, leading, in turn, to the elucidation of phylogenetic relationships.

Materials and Methods

Plant material

Leaf primordia and both young and completely expanded leaves were collected from the fifth node of *Ceiba erianthos* (Cav.) K. Schum., *Ceiba jasminodora* (A. St.-Hil.) K. Schum., *Ceiba pentandra* (L.) Gaerth. and *Ceiba speciosa* (A. St.-Hil.) Ravenna were collected (Fig. 1). Samples were carried out at the Arboretum of the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Collected species and voucher information are summarized in Table 1.

Light microscopy

Collected samples were fixed for 48 hours in 2.5% glutaraldehyde with 0.05 M sodium phosphate buffer, pH 7.2 (Gabriel 1982), at room temperature and under vacuum. Fixed samples were then dehydrated in a gradual ethanol series, embedded in hydroxyethylmethacrylate (Gerrits & Smid 1983), transversal and longitudinal sectioned in a semi-automated Leica RM2245 rotary microtome and, finally, stained with 0.05% Toluidine Blue (O'Brien & McCully 1981).

Some fixed samples were processed by diaphanization with 5% sodium hydroxide at 60 °C and bleached with 100% sodium hypochlorite solution between 1 and 14 days, depending on the sample, and 5% hydrated chloral. Finally, samples were stained with 1% safranin (Johansen 1940) and mounted on 50% glycerin.

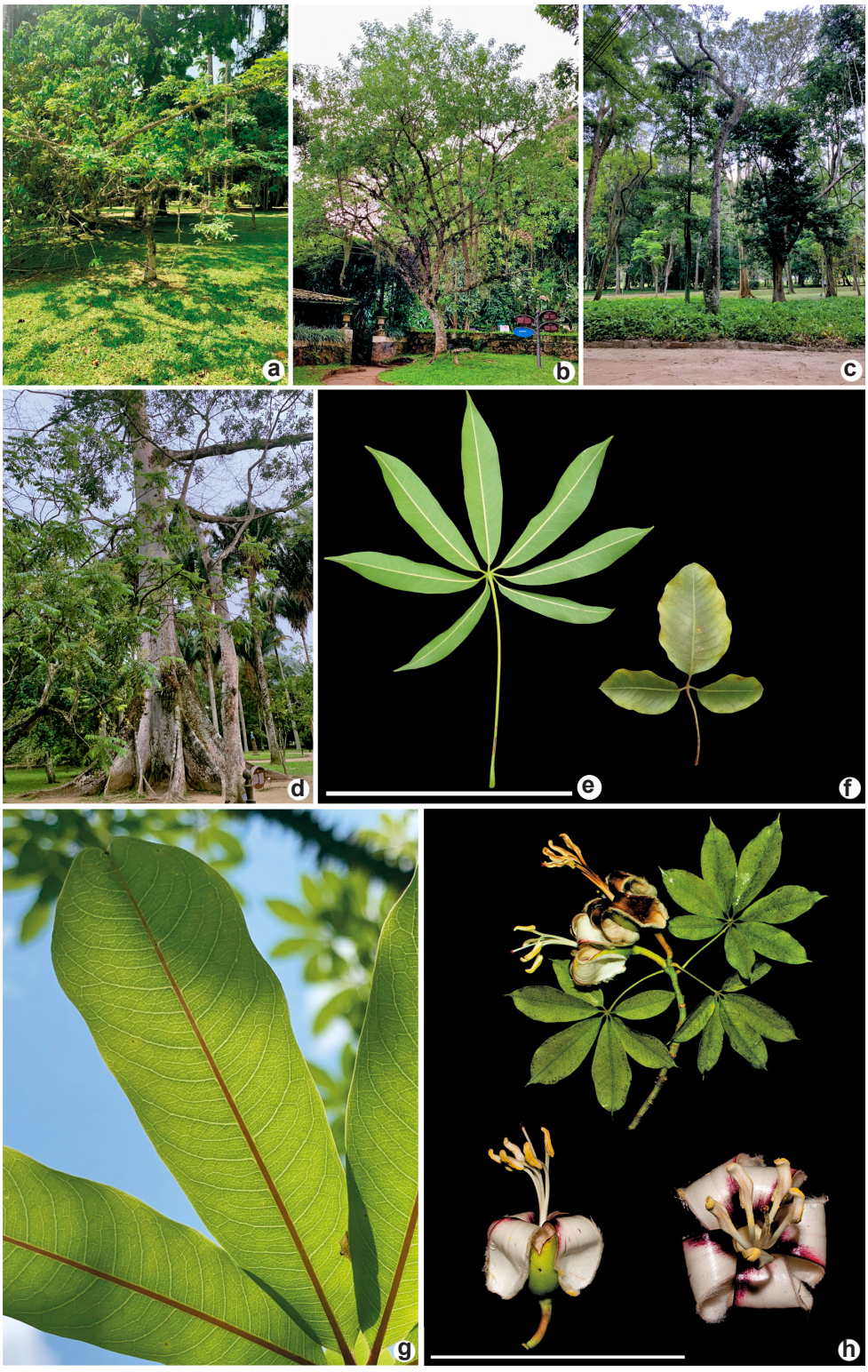

Figure 1 – a-h. General aspects of the species analyzed in this work – a, g-h. *Ceiba erianthos*; b, f. *Ceiba jasminodora*; c. *Ceiba speciosa*; d-e. *Ceiba pentandra*. a-d. habit of individuals in the Arboretum of the Rio de Janeiro Botanical Garden Research Institute; e-f. fully expanded leaf; g. detail of the adult leaf, showing the veins; h. inflorescences and flowers. Scale bar = 20 cm.

Table 1 – Selected species and vouchers information.

Species	Herbarium	Arboretum
Ceiba erianthos	RB646494	RBV 741
Ceiba jasminodora	RB354350	RBV 3140
Ceiba pentandra	RB841529	RBV 936
Ceiba speciosa	RB645506	RBV 6824

Histochemical analyses were performed on fresh leaves sectioned with a razor blade or on fixed and embedded samples, as described above. Nile blue (Cain 1947) was used for the detection of neutral and acidic lipids, Sudan III (Johansen 1940) for total lipids, ferric chloride for phenolic compounds (Johansen 1940), ruthenium red (Johansen 1940) for polysaccharides, Alcian blue (Pearse 1980) for acid mucopolysaccharides, Oil red (Pearse 1968) modified by Jayabalan & Shah (1986) for rubber, periodic acid-Schiff's reagent (McManus 1948) for neutral polysaccharides and NADI reagent for resin and essential oils (David & Carde 1964). The controls were carried out accordingly.

All slides were examined and documented with an Olympus BX 50 light microscope equipped with an Olympus DP73 digital camera in bright field or fluorescence microscopy.

Scanning electron microscopy

Fresh segments of fully developed leaves were excised from the middle portion of leaf specimens, mounted on stubs with carbon tape, and observed under a Hitachi TM4000 Plus II low vacuum scanning electron microscope.

Anatomical description and identification of the secretory structures were based on specialized literature (general aspects of the secretory structures - Fahn 1979; idioblasts - Baas & Gregory 1985; glandular trichomes - Payne 1978; extrafloral nectaries - Pacini *et al.* 2007).

Results

Digitate composite leaves of *Ceiba erianthos*, *C. jasminodora*, *C. pentandra*, and *C. speciosa* (Fig. 1) present idioblasts, glandular trichomes, and nectaries. Table 2 presents a summary of the secretory structures found in each analyzed species, as well as their location and origin. The identified secretory structures are detailed below.

Idioblasts

Idioblasts with a mucilaginous content were observed in mature leaves of all analyzed species. They are randomly distributed over the leaf blade, midrib, and petiole (Fig. 2a-c). Particularly, idioblasts occur: (i) on both adaxial and abaxial sides of the epidermis, intrusively interrupting the hypodermic stratum (Fig. 2b), (ii) on the parenchyma (chlorenchyma and cortex) (Fig. 2a-c), and (iii) in association with vascular tissues (Fig. 2d) of the midrib and petiole (Fig. 2c).

The development of mucilaginous idioblasts is asynchronous; in the same leaf primordium, idioblasts are found in early stages of development, while others are fully mature (Fig. 2e). It is noteworthy that idioblasts differentiate first in the midrib and then in the rest of the leaf blade (Fig. 2e). Idioblasts originate from the protoderm, ground meristem and procambium cells. In the first stage of development, precursor cells of idioblasts differ from the others because they are more voluminous, having evident nuclei and denser cytoplasm (Fig. 2f). Subsequently, the secretion starts to accumulate in the peripheral portion of the cytoplasm (Fig. 2g-h). Then, as a result of this continuous and progressive accumulation of secretion, the cytoplasm is gradually reduced and confined to a small portion of the cell (Fig. 2i-j). At maturity, the cell is then filled with secretion (Fig. 2k). During the development of idioblasts, many appear very close to each other (Fig. 3a), in all parts of the leaf. This proximity causes adjacent idioblasts to unite, finally coalescing between two or more of them (Fig. 3b), forming a single structure (Fig. 3c-d), that resembles the morphology of mature secretory cavities.

Mature idioblasts are characterized as voluminous, predominantly isodiametric in shape, and containing dense mucilaginous secretion (Figs. 3a; 4a).

Table 2 – Secretory structures, location in the leaf and origin. * = non-analyzed ontogeny.

Secretory structure	Location	Origin	Species			
			Ceiba erianthos	*Ceiba jasminodora*	*Ceiba pentandra*	*Ceiba speciosa*
Mucilaginous idioblast	epidermis, mesophyll, cortex and central portion of the midrib and petiole	protoderm, ground meristem and procambium	(+)	(+)	(+)	(+)
	inserted close to the vascular bundles		(-)	(+)	(+)	(-)
Druse-containing idioblast	mesophyll, cortex and central portion of the midrib	*	(+)	(+)	(+)	(+)
Phenolic idioblasts	cortex and central portion of the midrib	*	(+)	(+)	(+)	(+)
Clavate-type glandular trichome	randomly scattered on both sides of the leaf epidermis	protoderm	(+)	(+)	(+)	(+)
Extrafloral nectaries	abaxial surface of the leaf, in the middle third of the midrib	protoderm and ground meristem	(+)	(+)	(+)	(+)

Nonetheless, they may contain other substances such as polysaccharides (Fig. 4b), essential oils (Fig. 4c), total (Fig. 4e), and neutral lipids (Fig. 4f). In all analyzed species, note that mucilaginous idioblasts are more frequent and numerous than druse-containing and phenolic idioblasts.

In addition to mucilaginous idioblasts, idioblasts with druses (Fig. 2b) and idioblasts with phenolic content (Figs. 3b; 4d) are also found among the analyzed species. Idioblasts with phenolic compounds are more frequently found in the parenchyma tissue of the midrib (Fig. 2e). Details regarding the presence or absence of exudates are shown in Table 3.

Glandular trichomes

Clavate-type glandular trichomes were also observed on the leaves of all studied species. They are randomly dispersed on both surfaces of the epidermis, occurring singly or in pairs (Fig. 5a-c). Trichomes are herein classified as clavate, and show a stalk and a pluricellular head. They are formed by a basal cell, a uniseriate stalk, and a pluricellular apical portion (Fig. 5c). Young and fully developed trichomes are observed on young leaves, whereas on adult leaves, only fully developed trichomes were detected.

The ontogeny of clavate-type glandular trichomes starts in the leaf primordium. The first step involves an outward projection of a protodermal cell (Fig. 5d) and its subsequent periclinal division, resulting in a basal and apical cell (Fig. 5e-f). Then, the apical cell undergoes one more periclinal division (Fig. 5g), defining its apical portion and the stalk. This new apical cell undergoes about four to five periclinal divisions (Fig. 5h-i). Of these cells, the most apical one stands out, which undergoes about two anticlinal divisions (Fig. 5j), establishing the mature trichome, with a basal cell, one stalk cell, and several club-shaped apical cells (Fig. 5c,j).

Histochemical tests indicate lipophilic substances in mature trichomes, as detailed in Table 3. In *C. speciosa*, in addition to lipophilic substances, the secretion is also marked by the presence of terpenes (Tab. 3).

Extrafloral nectaries

The extrafloral nectaries (ENs) are located on the abaxial surface in the middle third portion of the midrib of leaflets (Fig. 6a-a'). Not all leaflets on the same leaf have ENs. In the secretory phase they are tumescent (Fig. 6b-c). In all analyzed species, the ENs are structurally composed of two distinct regions: (i) clavate-type glandular trichomes (Fig. 6d-h) on the surface; and (ii) subepidermal tissue (Fig. 6e-h) organized in a longitudinal groove along the median portion of the midrib (Fig. 6a).

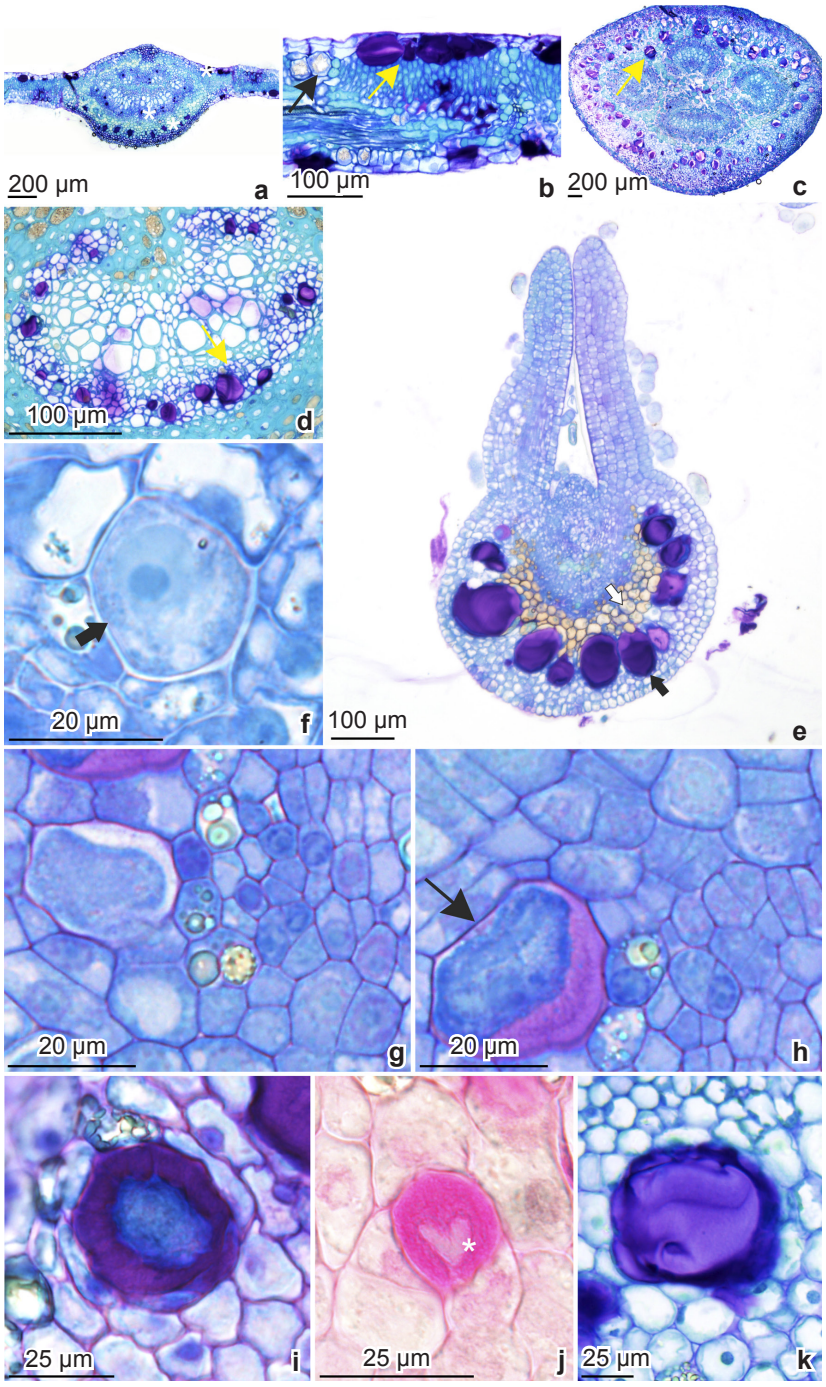

Figure 2 – a-k. Anatomy and ontogeny of mucilaginous idioblasts in *Ceiba* leaves – a-b. *C. jasminora*; d. *C. pentandra*; e-j. *C. speciosa*; c, k. *C. erianthos*. a-b, d. Adult leaves; c. petiole; e-k. young leaves. a. general aspect of midrib area, showing mucilaginous idioblasts (white asterisks); b. detail of the leaf mesophyll, showing idioblasts with druses (black arrow) and mucilage (yellow arrow); c. general aspect of the petiole with mucilaginous idioblasts (yellow arrow); d. detail of the mucilaginous idioblasts (yellow arrow) in the vascular tissues of the midrib; e. leaf primordium with mucilaginous idioblasts (black arrow) in different stages of development and phenolic idioblasts (white arrow); f. idioblast precursor cell (black arrow); g-h. early mucilage accumulation in the portion of the cytoplasm of idioblast (black arrow); i-j. progressive accumulation of mucilage in the idioblast – j. mucilage secretion (white asterisk) indicated by positive reaction to ruthenium red; k. mature idioblast with mucilage occupying the entire cytoplasm. (a-k. cross sections – a-i, k. toluidine blue; j. ruthenium red).

The ontogenesis of ENs involves the differentiation of protodermal cells that are precursors of glandular trichomes and cells of the ground meristem, which will give rise to the subepidermal tissue (Fig. 6i-m). The ontogeny of clavate trichomes in EN follows the same developmental steps as those already described for the same trichomes found in other regions of the leaflet. Initially, the protodermal cells divide in a periclinal direction (Fig. 6j-k), each of the resulting cells then originating a basal cell and an apical cell (Fig. 6k). The basal cell remains at the same level as the other epidermal cells (Fig. 6j-k), while the apical cell undergoes successive periclinal divisions and, finally, anticlinal divisions to form the stalk and a multicellular apical portion (Fig. 6l-m).

In cross section, the subepidermal tissue is formed by isodiametric collenchyma cells, in which plasmodesmata are observed (Fig. 6f). It should also be noted that numerous crystalliferous idioblasts containing druses surround the nectariferous parenchyma (Fig. 6b, f).

Discussion

Secretory structures have been widely studied and prospected for their utility as diagnostic

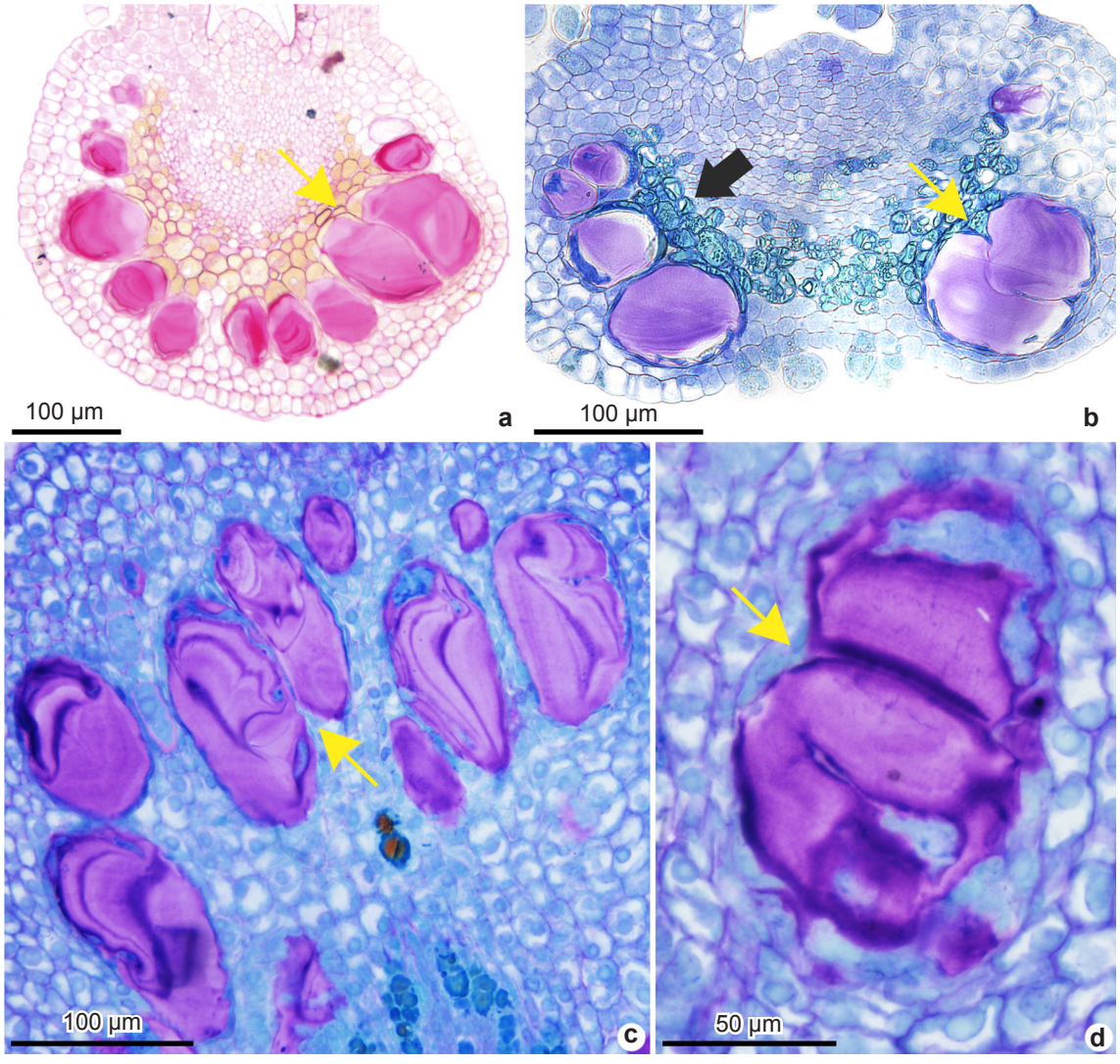

Figure 3 – a-d. Coalescence of idioblasts (yellow arrows) in tleaves of *Ceiba speciosa* – a-b. mature idioblasts in the midrib and phenolic idioblast (black arrow); c-d. idioblasts forming a unique structure. a-b. cross sections; c-d. longitudinal sections. (a. ruthenium red; b-d. toluidine blue).

characters (*e.g.*, for Rutaceae, Muntoreanu *et al.* (2011); Malvaceae, Rocha *et al.* (2011); Lamiaceae, Salmaki *et al.* (2009)). The structural organization of both cells and tissues, as well as the chemical nature of the secretion, allows the use of secretory structures for the classification and circumscription of taxa (Luna *et al.* 2013).

In Malvaceae, the presence of idioblasts containing mucilage is one of the main distinguishing features of the family (Metcalfe & Chalk 1957). Secretory canal/ducts, and cavities, glandular trichomes, and nectaries also occur in Malvaceae (Metcalfe & Chalk 1957; Rocha & Neves 2000; Lattar *et al.* 2009; Rocha *et al.* 2011), including Bombacoideae (Metcalfe & Chalk 1957; Kuruvilla & Anilkumar 2018). However, for *Ceiba*, only a few studies describe the types of secretory structures, *e.g.*, *Ceiba glaziovii* (Kuntze) K. Schum. (Medeiros 2022), *C. pentandra* (Kuruvilla & Anilkumar 2018), *C. chodattii* (Hassl.) Ravenna and *C. speciosa* (Perrotta *et al.* 2007) from ducts/ducts and secretory cavities, idioblasts, and glandular trichomes.

Nonetheless, the secretory structures identified in the species of *Ceiba* studied here included mucilaginous, crystalliferous and phenolic idioblasts, clavate-type glandular trichomes, and extrafloral nectaries. Such structures are ordinary and follow the records found in the literature, both for Malvaceae and Bombacoideae (Gregory & Baas 1989; Pimentel *et al.* 2011; Rocha *et al.* 2011), except for extrafloral nectaries, which, to the best of our knowledge, is the first record for the genus.

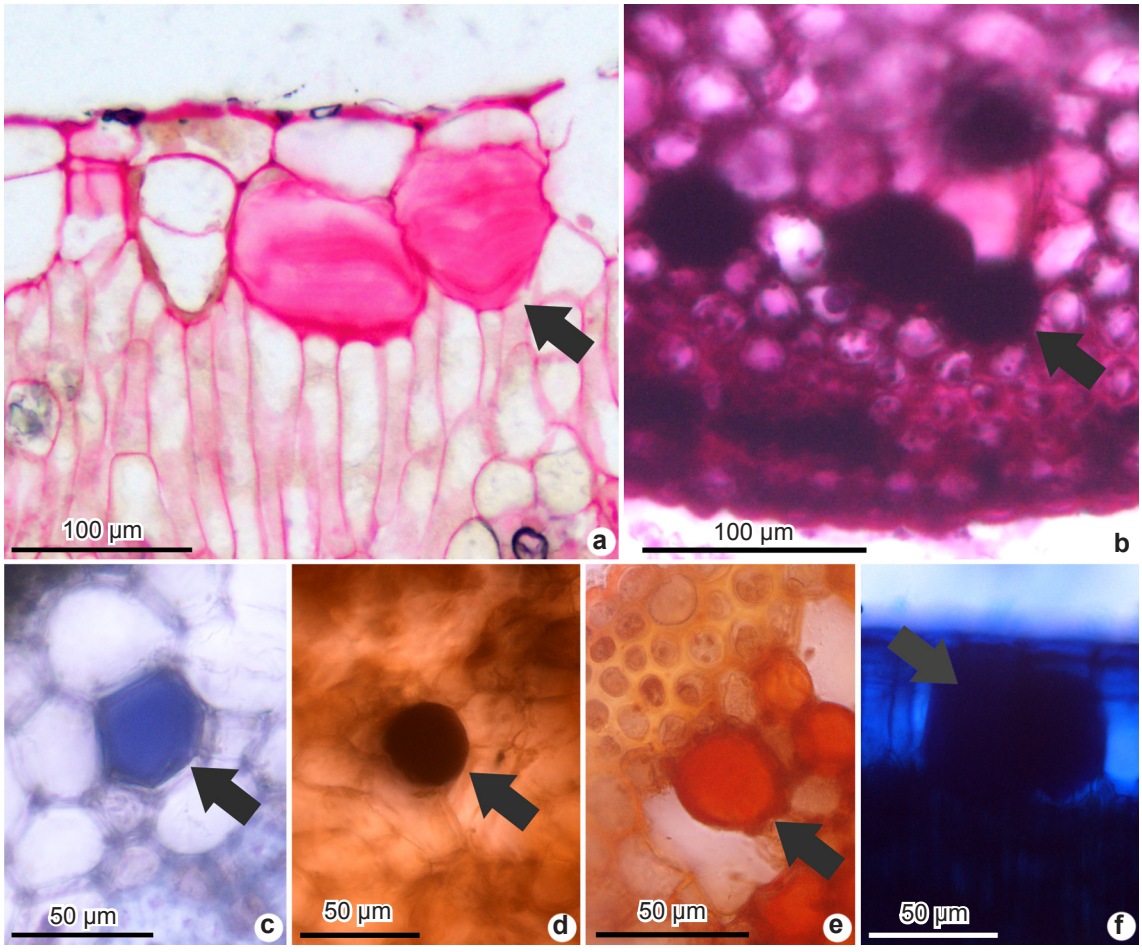

Figure 4 – a-f. Histochemical tests on idioblasts from expanded *Ceiba* leaves – a-b, e. *C. jasminodora*; c-d, f. *C. erianthos*. a. mucilage stained with ruthenium red; b. general polysaccharides stained with PAS; c. essential oils (terpenoids) stained with NADI; d. phenolic compounds stained with ferric chloride; e. total lipids stained with Sudan III; f. neutral lipids stained with Nile blue. (a-f. cross sections).

Table 3 – Histochemistry of leaf secretory structures in *Ceiba erianthos*, *C. jasminodora*, *C. pentandra* and *C. speciosa*. (+) = positive reaction; (-) = negative reaction.

Histochemical tests		Secretory structures and analyzed species							
		Ceiba erianthos		***Ceiba jasminodora***		***Ceiba pentandra***		***Ceiba speciosa***	
Detected substances	Reagents	Idioblasts	Glandular trichomes	Idioblasts	Glandular trichomes	Idioblasts	Glandular trichomes	Idioblasts	Glandular trichomes
Total and neutral lipids	Sudan III and Nile blue	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Phenolic compounds	Ferric chloride	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
Essential oils and resins	NADI	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)
Terpens	Oil red	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
Acidic and neutral polysaccharides	Alcian blue and PAS	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
Mucilage	Ruthenium red	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)

The term idioblast defines cells that differ from others by their shape, size, or content (Fahn 1979; Crang *et al.* 2018). Among the different types of idioblasts, mucilaginous idioblasts stand out. Some authors use the term mucilage cells to describe idioblasts that contain mucilaginous content (Fahn 1979; Baas & Gregory 1985). However, in the context of functionality and morpho-anatomical characteristics, both terms mean and are used to designate the same structure. Mucilaginous idioblasts occur randomly, isolated, in pairs, or groups (Bakker *et al.* 1991; Bakker & Gerritsen 1992). However, according to Gregory & Baas (1989), they may occur more frequently on the adaxial side or, more rarely, on the abaxial side, close to the mesophyll and the ground tissue of the midrib and petiole. Nevertheless, in the species analyzed here, contrary to Gregory & Baas (1989), the occurrence of these cells seems to be equal on both leaf surfaces and also occur in the mesophyll, cortex, and central portion of the midrib and petiole. These findings corroborate the work of Spegg (1959), who recognized and described three diagnostic distribution patterns of mucilage cells in Malvaceae leaves, such as those localized (i) in the epidermis of adaxial surface, (ii) in the epidermis of both leaf surfaces, and (iii) in the mesophyll. Therefore, the pattern of distribution of these structures on the leaves of the *Ceiba* species in the present study follows the characters considered to be diagnostic of Malvaceae.

It is suggested that mucilage may be related to changes in transpiration, as well as water loss (Volkens 1887; Gregory & Baas 1989; Rocha *et al.* 2002). Fahn (1979) also points out that mucilages, as they constitute a complex of polysaccharides, can increase the water retention capacity of the cells since these complexes constitute a hydrophilic substance that becomes viscous upon any contact with water (Scott & Bystrom 1970; Eames & MacDaniels 1925). Mucilaginous idioblasts contain general polysaccharides, essential oils, and both general and neutral lipids. Supporting this, Rocha *et al.* (2011) report histochemical tests showing that the mucilage of the idioblasts found in *Hibiscus pernambucensis* (Malvaceae) consists predominantly of acidic and neutral polysaccharides, lipids, and phenolic substances, which is in line with the findings in our study.

Our results also showed the development and accumulation of mucilage in idioblasts based on the classification proposed by Baas & Gregory (1985). These structures progressively accumulate mucilage, until they are filled, resulting in the

complete obliteration of the cytoplasm. This pattern of development and accumulation of secretion is similar to that already reported by Bakker & Gerritsen (1992) for the mucilage cells found in *Hibiscus schizopetalus* (Dyer) Hook. f. Bakker *et al.* (1991) also described the accumulation of mucilage, noting that it occupies the entire periplasmic space, producing multidirectional forces, which push and compress the protoplast in the center of the cell, which is subsequently degenerated. Baas & Gregory (1985) and Bakker *et al.* (1991) reported that the accumulation of mucilage occurs, in most cases, from vesicles filled with polysaccharides from the Golgi complex. These polysaccharides freely move towards the plasmalemma, merging with it, thereby accumulating and retaining mucilage between plasmalemma and cell wall.

In all species studied herein, the ontogeny of idioblasts takes place from cells of the ground meristem, protoderm, or procambium, as already described for vegetative organs of Eudicots

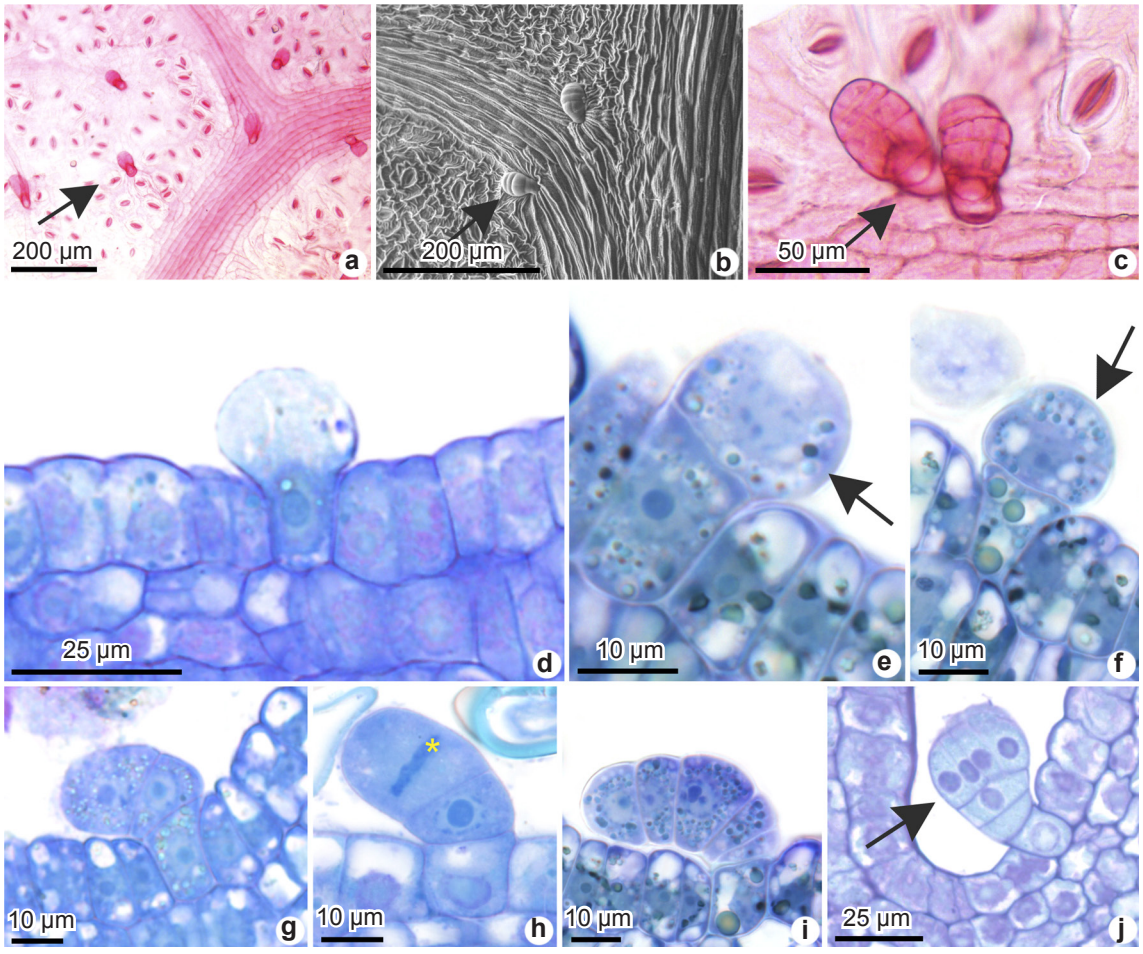

Figure 5 – a-j. Anatomy and ontogeny of pluricellular clavate glandular trichomes in *Ceiba* – a,c. *C. jasminodora*; d, g-h, j. *C. speciosa*; b, e-f, i. *C. erianthos*. a-c. mature leaves; d-j. young leaves. a. clavate – trichomes (black arrow) distributed randomly along the leaf blade on the abaxial side; b. detail of clavate trichomes (black arrow) on the abaxial surface of the leaf; c. clavate trichomes (black arrow) on the abaxial surface; d. establishment of the trichome with the protrusion of the protodermal cell; e-f. result of the first periclinal division with formation of the basal cell and apical cell (black arrow); g. trichome with three cells, after the periclinal division of the apical cell, and establishment of the stalk cell; h. mitosis in the apical cell (yellow asterisk); i. trichome with four apical cells, after periclinal divisions; j. trichome with apical portion with four cells (black arrow) generated from anticlinal divisions. (a, c. diaphanized leaf, safranin; b. SEM analyses; d-j. cross sections, toluidine blue).

(Gregory & Baas 1989). Variations of this type of accumulation can occur, for example, in idioblasts containing raphides wherein mucilage is accumulated in a manner surrounding the crystals, which are stored and located in a large central vacuole (Baas & Gregory 1985; Kausch & Horner 1984).

We also reported that mature idioblasts may coalesce and form a large structure with mucilage. At maturity, they state the cell walls of idioblasts that occur side by side can disintegrate resulting in the formation of large structures similar to "cavities" surrounded by elongated cells that contain dense mucilaginous secretion. However, the use of the term "cavity" for such a structure is dubious because it is designed to describe another type of secretory structure, which is also very common in Malvaceae. This structure differs from idioblasts in that they are made up of epithelial cells and have a lumen, formed by the arrangement of these cells (Bakker & Gerritsen 1992). Perrotta *et al.* (2007) reported the presence of secretory cavities in *C. speciosa*, though results indicate that they are mucilaginous idioblasts. The development of ducts and secretory cavities is well established in the literature by the unique pattern of mitotic divisions, lysis between cell walls, and cell separation and/or lysis (Fahn 1988; Evert 2006; Luna 2013). Still this is completely different from the ontogeny of idioblasts and their tendency to coalesce and form a large structure with mucilage, as we reported.

Thus, despite the anatomical and histochemical description of secretory structures, some results are conflicting. Often, most secretory structures are difficult to interpret, leading to the use of different terms to refer to the same type of structure, or the same terms to refer to different structures. Only ontogenetic studies can resolve such conflicts as they allow the complete observation of origin and development, establishing distinct homologies or not. In Malvaceae, as well as *Ceiba*, studies with an ontogenetic approach are rare, and indicate the presence of cavities, causing uncertainty in the application of conceptual terms.

In addition to idioblasts, clavate glandular trichomes were also observed in the analyzed species. These occur singly or in pairs on both leaf surfaces, scattered across the leaf blade, and/or close to the midrib and sides. Trichomes are morphologically quite diverse structures and they can be classified as unicellular or multicellular, simple or compound, secretory or non-secretory (Metcalfe & Chalk 1957; Fahn 1988). The morphological diversity of these structures is a valuable taxonomic attribute in the circumscription of taxa, as, for example, observed for species of Rubiaceae (Kocsis *et al.* 2004), Leguminosae (Jordão *et al.* 2020), and Primulaceae (Luna *et al.* 2017), for example. In Malvaceae, as well, studies describe trichomes and emphasize their relevance as a valuable character to species identification. In some cases, when trichomes are fully developed, morphoanatomy alone is not enough, and only ontogenetic studies allow correct validation of trichome types (Ma *et al.* 2016).

Clavate glandular trichomes in the studied species have a protodermal origin and appear at the beginning of leaf primordia development, remaining functional also in the adult leaves. The ontogeny of the glandular trichomes described here follows the pattern observed by Ramírez-Díaz *et al.* (2019) for *Tilia caroliniana* subsp. *floridana* (Sm.) A.E. Murray (Malvaceae) in which a cell of the early protoderm expands, forming a bulge, which subsequently undergoes periclinal division. These results may suggest a pattern for the development of such trichomes in Malvaceae. Clavate trichomes have also been reported on the leaf surfaces of other Malvaceae taxa. For instance, studying *Dombeya*, Lersten (1997) observed that the clavate trichomes were accompanied by non-glandular trichomes on both leaf surfaces and that the clavate form occurred in at least 69 species of the genus. On the other hand, the clavate trichomes observed in the species selected for this work can occur isolated or accompanied by other trichomes also clavate. Garcia *et al.* (2014) also reported the occurrence of these trichomes in the primordia and expanded leaves of *Theobroma grandiflorum* Schum. and *T. subincanum* Mart.

Extrafloral nectaries located on the abaxial face of the midrib or lateral veins were described in Malvaceae by Solereder (1908), Metcalfe & Chalk (1957), Lewton (1925), Cogni & Freitas (2002), Rocha *et al.* (2002) and Vogel (2000). However, given the high representativeness of Malvaceae in tropical ecosystems (Rocha & Machado 2009), few works have investigated the anatomy of extrafloral nectaries in the family, and those include studies on *Abutilon* (Findlay *et al.* 1971; Gunning & Hughes 1976), *Gossypium* (Wergin *et al.* 1975; Eleftheriou & Hall 1983), and *Hibiscus* (Santos 1959; Sawidis 1998).

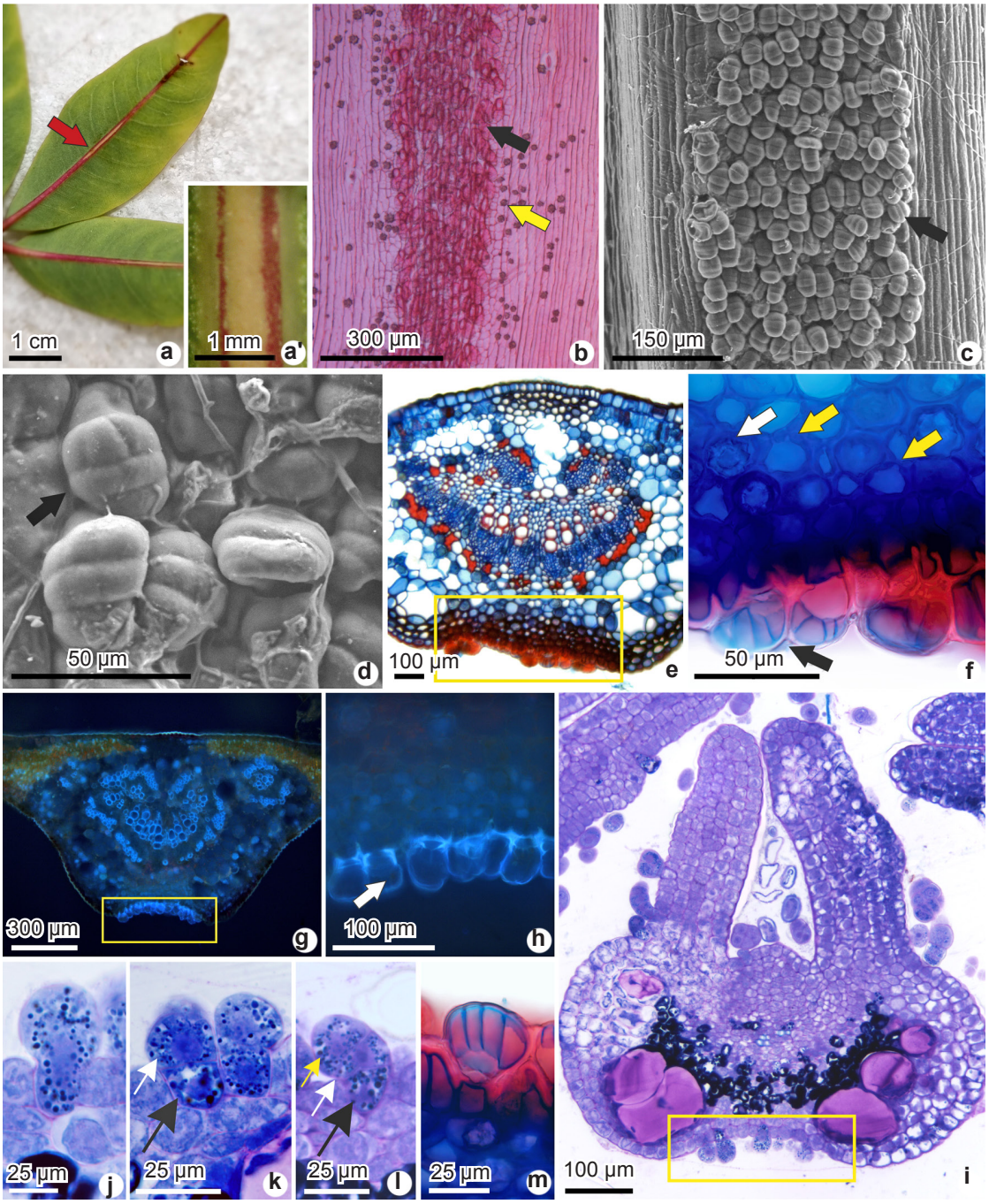

Figure 6 – a-m. Anatomy and ontogeny of extrafloral nectaries (EN) in *Ceiba* – a-a', c-h, k-m. *C. erianthos*; b. *C. jasminodora*; i-j. *C. speciosa*. a-h, m. mature leaves; i-l. young leaves. a-a'. EN on the middle third of the midrib; b. glandular trichomes (black arrow) of the EN with druses idioblasts (yellow arrow); c-d. EN showing clavate trichomes (black arrow); e. midrib with isodiametric cells of the ground meristem and epidermis with glandular trichomes; f. detail of the abaxial surface of midrib showing glandular trichomes, plasmodesmata (yellow arrow) and druse idioblast (white arrow); g. EN (yellow rectangle); h. detail of clavate trichomes (white arrow); i. EN in leaflet primordium; j-k. protodermal cells in periclinal division, giving rise to a basal cell (black arrow) and an apical cell (white arrow); l. apical cell (white arrow) with periclinal divisions (yellow arrow) and black arrow indicating the basal cell; m. anticlinal divisions formed a multicellular apical portion. (a-a'. macroscopy images; b. diaphanized leaf; c-d. SEM analyses; a-d. front view; e-m. cross sections).

Beyond ecological importance (Pacini *et al.* 2007), the diversity of shapes and location of this structure, as with secretory trichomes, is considered taxonomically valuable (Bentley 1977; Koptur 1992). According to the classification proposed by Zimmermann (1932), ENs observed in the leaves of the analyzed *Ceiba* species are "hollow-type nectaries". Such nectaries are characterized by the presence of numerous multicellular secretory trichomes lodged at the bottom of a furrow, and for the order Malvales, they represent a striking diagnostic characteristic (Sawidis *et al.* 1987; Rocha & Machado 2009). These trichomes have prominent wall thickening in their basal cells, a characteristic normally associated with the control of the flow of material secreted through the protoplast, in addition to promoting the gradual isolation of these trichomes, which later suffer degeneration when the nectary ceases to be active (Fahn 1988; Schnepf 1969; Shimony *et al.* 1973; Rocha *et al.* 2011).

In Malvaceae, Rocha & Machado (2009) describe the extrafloral nectary of *Hibiscus pernambucensis* as having deep grooves with a protruding edge over the veins of the abaxial face of the leaf blade, consisting of numerous multicellular secretory trichomes, epidermal cells arranged in a palisade and non-vascularized parenchyma. This is a pattern similar to that described for *C. erianthos*, *C. jasminodora*, *C. pentandra*, and *C. speciosa* in the present study. Additionally, however, we found that ENs in the secretory phase are tumescent and located only in the midrib and in the region of the middle third of the leaf already expanded. It should also be noted that not all leaflets on the same leaf have active ENs and that such phenomenon leads to the emergence of these structures, thus requiring further investigation and analysis. Furthermore, ENs can vary widely in ontogeny, morphology, and structure (Fahn 1988; Rocha & Machado 2009).

As well defined in the literature, nectaries are specialized tissues that secrete sugar solution and are involved in plant-animal interactions. Nevertheless, the term does not indicate a uniform or well-defined structure (Fahn 1979; Pacini *et al.* 2003). In general, structured nectaries have an epidermis, with or without stomata and trichomes, through which nectar is released, and specialized parenchyma, which produces or stores nectar solutes (Fahn 1979; Pacini *et al.* 2003). Nectaries may also be supplied by vascular tissue or be directly in contact with the vascular system (Fahn 1979). In the *Ceiba* species analyzed here, a specialized vascular tissue connected to the nectary was not observed. However, when observed, such structures are located in the midrib. Therefore, it is suggested that the vascular system of the vein itself participates in the production of the secretion, which will be exuded by the secretory trichomes, after passing through the subepidermal collenchyma tissue. Further evidence for accepting this hypothesis is given by the plasmodesmata seen in the collenchyma tissue adjacent to the glandular trichomes from the extrafloral nectaries.

The results of the present study show that the types of idioblasts and trichomes identified in *Ceiba* are in line with the previous literature described for Malvaceae. However, ENs, as described in this study, represent the first morphoanatomical and ontogenetic record, both for Bombacoideae and for the genus *Ceiba*.

The gathered data are relevant to understanding the evolution of secretory structures in Malvaceae, especially in Bombacoideae. In the future, such data can be used as an additional resource for phylogenetic studies of the group, complementing the information available in the literature. Although morphoanatomy and histochemistry are considered important for distinguishing and identifying the secretory structures in the studied species, ontogeny proved to be conclusive and indispensable in the interpretation and final analysis of the results.

While the results found did not demonstrate significant differences among the analyzed species, we describe, for the first time, their secretory structures, data that may represent synapomorphies for the genus or tribes in Bombacoideae. It is anticipated that this study will stand as a methodological standard for the description of secretory structures in Bombacoideae and Malvaceae, serving to resolve the evolutionary relationships of this group.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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