FOLIAR SECRETORY STRUCTURES IN CROTONEAE (EUPHORBIACEAE): DIVERSITY, ANATOMY, AND EVOLUTIONARY SIGNIFICANCE[1]

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- *Premise of the study:* Phylogenetic and morphological studies have helped clarify the systematics of large and complex groups such as the tribe Crotoneae (Euphorbiaceae). However, very little is known about the diversity, structure, and function of anatomical features in this tribe. Crotoneae comprises the species-rich pantropical genus *Croton* and six small neotropical genera. Here we characterized the anatomy of leaf secretory structures in members of this tribe and explored their function and evolutionary significance.
- *Methods:* Young and mature leaves of 26 species were studied using standard anatomical light microscopy techniques. Three sections of *Croton* and one representative of *Brasiliocroton* and *Astraea* were sampled.
- *Key results:* We identified five types of secretory structures: laticifers, colleters, extrafloral nectaries, idioblasts, and secretory trichomes. Laticifers were present in all species studied except *Croton alabamensis*, which instead presented secretory parenchyma cells. Articulated laticifers are reported in Crotoneae for the first time. Colleters of the standard type were observed in the majority of the sampled taxa. Extrafloral nectaries were present in section *Cleodora* and in *B. mamoninha*, but absent in section *Lamprocroton* and *Astraea lobata*. Idioblasts were spread throughout the palisade and/or spongy parenchyma in most of the studied species. Secretory trichomes were restricted to *Lamprocroton* except for *C. imbricatus*.
- *Conclusions:* This study revealed a high diversity of secretory structures, including novel ones, in one of the largest clades of Euphorbiaceae. Our results are promising for investigations on the anatomical and ecophysiological bases of species diversification within Euphorbiaceae.

Key words: anatomy; *Astraea*; *Brasiliocroton*; colleters; *Croton*; Euphorbiaceae; extrafloral nectaries; laticifers; secretory idioblasts; secretory trichomes.

Among the angiosperms, lineages within Euphorbiaceae show conspicuous examples of the diversification of secretory elements. In particular, the tribe Crotoneae stands out as a group for which several types of secretory structures have been reported (Froembling, 1896; Metcalfe and Chalk, 1950; Ganeshaiah and Shaanker, 1988; Rudall, 1989, 1994; Webster et al., 1996; Freitas et al., 2001; de Sá-Haiad et al., 2009; Wiedenhoeft et al., 2009; Narbona and Dirzo, 2010; Machado et al., 2015), although detailed studies of the morphology, distribution, and nature of these features spanning the taxonomic breadth of this group have not been yet conducted. Secretory structures could be the sites of synthesis and/or accumulation of metabolites with high phytochemical and pharmacological value (Fahn, 1979). Such structures can also be a source of characters for systematics, and they are likely key players in many plant–animal ecological interactions. The ecological role of secretory structures in the case of *Croton*, the largest genus of Crotoneae, could be related to its evolutionary success, as these structures are involved in the attraction of pollinators and seed dispersers, and their secretions may also protect the plant against herbivores and pathogens at different stages of development. Additionally, mutualistic relationships appear to be common in species that are rich in secretory structures (Fahn, 1979).

The tribe Crotoneae of Euphorbiaceae consists of the species-rich genus *Croton* L. (ca. 1300 spp.) and six small genera (*Acidocroton* Griseb., *Astraea* Klotzsch, *Brasiliocroton* P.E.

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Berry & Cordeiro, *Ophellantha* Standl., *Sagotia* Baill., and *Sandwithia* Lanj.) that together have no more than 30 species (Berry et al., 2005; Wurdack et al., 2005; Riina et al., 2014). Species in this tribe range from herbs to trees and occasionally lianas and are found in a wide range of habitats. They are distributed in tropics and subtropics worldwide (Berry et al., 2005), but the largest concentration of species is in the neotropics with important centers of diversity in Brazil, the Antilles, and Mexico (Burger and Huft, 1995; van Ee et al., 2011). Many species in this tribe, especially in the genus *Croton* (Salatino et al., 2007), are used in folk medicine, and some have been the focus of phytochemistry and pharmacological studies.

Molecular phylogenetics has been essential for clarifying the relationships among *Croton* and other Crotoneae lineages (Berry et al., 2005; van Ee et al., 2011; Riina et al., 2014). Because *Croton* contains the largest number of species in the tribe, its systematics and taxonomy have been studied in more detail (e.g., Lima and Pirani, 2008; Riina et al., 2010; van Ee and Berry, 2010; Caruzo et al., 2011; van Ee and Berry, 2011; Caruzo and Cordeiro, 2013). However, this effort has not been paralleled by a thorough search of morphological and anatomical traits that would help to interpret clade diversification within this tribe. Less than 2% of the species of Crotoneae have been included in anatomical surveys (De-Paula and Sajo, 2011; De-Paula et al., 2011; de Sá-Haiad et al., 2009; Machado et al., 2015). The role played by different types of secretory elements (e.g., laticifers, secretory ducts) in the evolution of angiosperms remains an open question (Pickard, 2008).

Floral and extrafloral nectaries are probably the best-known secretory structures in Crotoneae (Metcalfe and Chalk, 1950; Fahn, 1979; Elias, 1983; Freitas et al., 2001), particularly in *Croton*. They were found to be related to mutualistic interactions, like the reward for pollinators and recruitment of plant-protective insects. For example, in *Croton suberosus* Kunth, wasps and bees promote pollination while they feed on floral nectar (Narbona and Dirzo, 2010). These authors also found that the wasp *Polistes instabilis* Saussure (Vespidae) contributes to defending *C. suberosus* against herbivores. Some *Croton* species possess postpollination nectaries, also known as postfloral nectaries (Freitas et al., 2001), which are specialized structures that produce nectar following anthesis and during fruit development (Faegri and van der Pijl, 1979; Keeler, 1981; Ganeshaiah and Shaanker, 1988; Gracie, 1991). Postpollination nectaries seem to be responsible for a close mutualistic relationship between *Croton bonplandianus* Baill. and the ants that disperse its seeds (Ganeshaiah and Shaanker, 1988).

Secretory trichomes (Webster et al., 1996) and idioblasts containing lipophilic compounds have been reported for some *Croton* species (Froembling, 1896; Metcalfe and Chalk, 1950; Webster et al., 1996; de Sá-Haiad et al., 2009); however, no specific role has been suggested for these structures. Most recently, resin-secreting colleters have been described on the inflorescence axis of *C. glandulosus* L. (Machado et al., 2015). These authors suggested that these colleters may protect flower tissue against desiccation, herbivores, and pathogens.

Another typical characteristic of Crotoneae, is the presence of latex, particularly in many lineages within *Croton* (Metcalfe and Chalk, 1983; Rudall, 1989, 1994; Farías et al., 2009; Wiedenhoeft et al., 2009). In some clades like *Croton* section *Cyclostigma* Griseb. (dragon's blood trees), the usually abundant red latex produced by these species is used in folk medicine throughout the neotropics (Riina et al., 2009). The question remains whether laticifers have been lost in some Crotoneae lineages or that latex is just not noticeable in species for which it has been reported as absent (from field observations).

The presence, position, and type of secretory structures have been used to support or clarify phylogenetic relationships among several angiosperm groups as well as to understand evolutionary patterns of lineage diversification (Dickson, 2000; Tilney and van Wyk, 2004; Marazzi et al., 2006; Delgado et al., 2009; Araújo et al., 2010; De-Paula et al., 2011, Coutinho et al., 2012, 2013; Pečnikar et al., 2012; Weber and Keeler, 2013; Angulo and Dematteis, 2014). Likewise, secretory structures have been relevant in the taxonomy and phylogenetics of groups within Crotonoideae and Crotoneae (Caruzo et al., 2011; De-Paula et al., 2011; van Ee et al., 2011; Riina et al., 2014). For instance, the presence of floral nectaries in crotonoid genera (sensu Wurdack et al., 2005) is a diagnostic character for the CI clade. Likewise, vascularized floral nectaries may be a diagnostic character for *Croton*, while in *Astraea* these structures are not vascularized (De-Paula et al., 2011).

The distribution and type of secretory structures in Crotoneae might be important characters for elucidating the evolutionary history of the group, especially regarding clades whose species occur in contrasting environments, such as in sections *Cleodora* (Klotzsch) Baill. and *Lamprocroton* (Müll. Arg.) Pax, which are sampled in this study. Secretory structures could be also a source of new characters useful for the circumscription of taxa and to support phylogenetic hypotheses in Crotoneae.

In this study we investigate the diversity, morphology, distribution patterns, development, and function of leaf secretory structures of species belonging to three sections of *Croton* (*Alabamenses* B.W. van Ee, *Cleodora*, *Lamprocroton*) and two other Crotoneae genera (*Brasiliocroton* and *Astraea*) (Fig. 1). We discuss our findings under the current Crotoneae phylogenetic framework. This work contributes toward the goal of understanding the evolution of secretory structures and other anatomical features and their role in shaping patterns of species diversification within Crotoneae.

MATERIALS AND METHODS

***Studied taxa*—**We selected the taxa for this study using geographical and phylogenetic criteria, i.e., sampling clades centered in Brazil. Most *Crotoneae* genera are well represented in Brazil with the exception of *Acidocroton* and *Ophellantha*, which occur farther north in the neotropics. Among the *Croton* clades, sections *Cleodora* (Caruzo et al., 2011) and *Lamprocroton* (van Ee and Berry, 2011) stand out due to the high number of species distributed in Brazilian ecosystems. *Cleodora* species are shrubs or trees occurring mainly in the Atlantic Forest domain and the Amazon region (Caruzo et al., 2011; Caruzo and Cordeiro, 2013), while *Lamprocroton* species are shrubs or subshrubs predominantly distributed in rocky fields and high-altitude grasslands within the Atlantic Forest domain, but also in open vegetation areas such as the Cerrado and the Caatinga (Lima and Pirani, 2008; van Ee and Berry, 2011).

A total of 26 species were analyzed: 24 *Croton* species including 12 from section *Cleodora* (of the 18 total), 11 from section *Lamprocroton* (of the 37 total), and the only known species of section *Alabamenses* (*Croton alabamensis* E.A. Sm. ex Chapm.); one species of *Astraea* [*A. lobata* (L.) Klotzsch], and one of the two known species of *Brasiliocroton* (*B. mamoninha* P.E. Berry & Cordeiro) (Table 1, Fig. 1, Appendix 1). In the case of section *Lamprocroton*, we focused on the species occurring in the Brazilian campos rupestres and campos de altitude, above 1000 m a.s.l. These species are all members of section *Lamprocroton* subsection *Lamprocroton* (Müll. Arg.) van Ee & P.E. Berry, whereas *Croton gnaphalii* Baill. was the only species sampled from subsection *Argentini* van Ee & P.E. Berry. *Croton alabamensis*, from North America, was studied because it was a former member of section *Lamprocroton* sensu Webster (1993), which was recovered as an independent and monotypic lineage

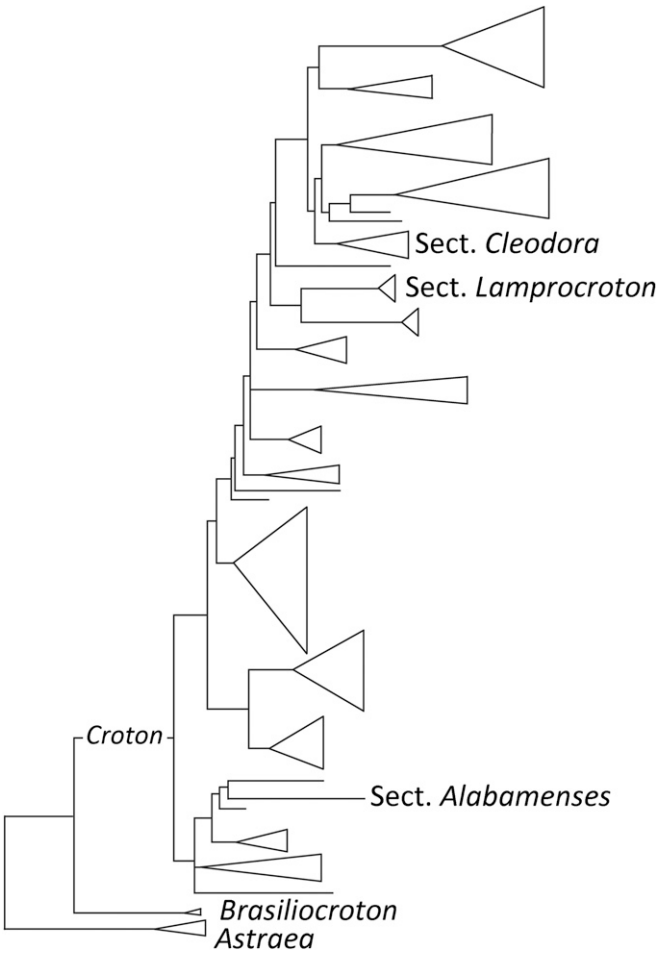


Fig. 1. Schematic phylogeny of *Croton* and two additional representatives (*Brasiliocroton* and *Astraea*) of tribe Crotoneae indicating the clades sampled in this study (modified from van Ee et al., 2011).

(section *Alabamenses*) in molecular phylogenetic analyses (Berry et al., 2005; van Ee et al., 2011).

Leaf samples were obtained from natural populations either during field expeditions or from herbarium material (Appendix 1). Vouchers of newly collected plant material were deposited in VIC. Herbaria acronyms follow those of Thiers (2014), with the exception of the HUEMG herbarium (Minas Gerais State University, Carangola campus, Minas Gerais State, Brazil).

***Tissue sampling*—**Samples of leaves at different developmental stages (leaf primordia, young and mature leaves) were obtained from each species, and whenever possible, three specimens per species were used as replicates. Sections were obtained from the leaf blade comprising the apical, median, and basal portions (including midrib, margin, and the region between them) and from the apical and basal portions of the petiole.

***Light microscopy*—**Plant material was processed at the Laboratory of Plant Anatomy of the Federal University of Viçosa. Samples acquired from herbarium specimens were subjected to the herborization reversion process (Smith and Smith, 1942), dehydrated in an ethanol series, stored in 70% ethanol, and embedded in methacrylate for sectioning (Meira and Martins, 2003). Materials from field collections were fixed either in FAA (formaldehyde–glacial acetic acid–50% ethanol, 1:1:18 v/v) (Johansen, 1940) or NBF (neutral buffered formalin) for general characterization and histochemical tests (Clark, 1973). All materials were held in a vacuum desiccator throughout the fixation process (48 h) and then stored in 70% ethanol. Part of the leaf tissue was dehydrated in an ethanol series and embedded in methacrylate (Historesin, Leica Microsystems, prepared according to manufacturer instructions). Samples were cross- and longitudinally sectioned (5 μm thick) with an automatic rotary microtome (model RM2155, Leica Microsystems) using disposable glass knives.

Sections were stained with toluidine blue pH 4.0 (O'Brien and McCully, 1981). For the description of superficial characters, entire leaves were cleared with a solution of 10% sodium hydroxide and 20% sodium hypochlorite, interspersed with successive washes in distilled water (Johansen, 1940, modified). The materials were stained with safranin (1% alcoholic solution) and mounted in glycerinated gelatin. The classification and terminology used for colleters according to their morphology followed that of Thomas (1991). For the histochemical tests, freehand sections obtained from either fresh or fixed samples stained with the following reagents: Sudan IV and Sudan red (Pearse, 1980) and with neutral red under fluorescence (Kirk, 1970) for the detection of total lipids; Nile blue sulfate for acid/neutral lipids (Cain, 1947); Nadi reagent for essential oils and resin oils (David and Carde, 1964). For the identification of total polysaccharides, sections recovered from historesin-embedded samples were treated with periodic acid Schiff's reagent (PAS) (McManus, 1948).

Sections were observed and images captured using a light microscope (model AX70TRF, Olympus Optical, Tokyo, Japan) equipped with an image capture system and coupled digital camera (AxioCam HRc; Zeiss, Göttingen, Germany). For fluorescence microscopy, an epifluorescence HBO 50W mercury vapor lamp and filter block A (exciter filter BP 340–380, dichroic mirror 450, barrier filter LP-430) were used.

RESULTS

Five types of secretory structures were identified with varied distribution and morphology (Table 1): laticifers (Fig. 2A–E), colleters (Fig. 3A–Q), extrafloral nectaries (Fig. 4A–M), secretory idioblasts (Fig. 5A–C, E–J), and secretory trichomes (Figs. 5D, J; 6A–C).

***Laticifers*—**Laticifers were observed in all but one of the species studied, namely *Croton alabamensis* (Table 1). They are associated with phloem in vascular bundles (Fig. 2D) and are easily observed due to the intense staining of the latex in stems and leaves (Fig. 2A–E). They are particularly abundant in the bundles branching toward the extrafloral nectaries (Fig. 4H, I). Laticifers also can be observed close to the vasculature in colleters (Fig. 3J). Although laticifers were not observed in *Croton alabamensis*, parenchymatous cells with densely stained content were visualized near the phloem, coinciding with the position of laticifers in other species.

From a developmental perspective, laticifers differentiate very early, already being fully formed on the young leaves (Figs. 2A–E; 3G). Laticifer cells are elongated and organized in rows that are separated by the terminal cell walls, which are thinner than the lateral ones (Fig. 2B, E), thus constituting articulated laticifers (Fig. 2A–E). Articulated laticifers do not anastomose in any species from section *Lamprocroton* (Fig. 2A, B), or in *C. hemiargyreus* Müll. Arg., *C. sphaerogynus* Baill. and *C. heterocalyx* Baill. from section *Cleodora*, or in *Brasiliocroton mamoninha*. Laticifers are articulated and anastomosed in the remaining species from section *Cleodora* and in *Astraea lobata* (Fig. 2C–E).

***Colleters*—**Colleters (Fig. 3, F–Q) occur on the leaves of all species of section *Cleodora*, in most species of section *Lamprocroton* (except for *Croton muellerianus* L.R. Lima and *C. gnaphalii* Baill.), in *C. alabamensis*, *Astraea lobata*, and *Brasiliocroton mamoninha* (Table 1). Colleters were found predominantly on leaf margins near vein terminations (Fig. 3H, I); however, variations in the position of these structures were observed. In *Astraea lobata*, they are located along the leaf margins (marginal), on the basal portion of the leaf blade (basilaminar),

TABLE 1. List of the studied species with type and position of the secretory structures observed.

Taxon		*Croton* species	Secretory structures				
			Laticifer type	Colleters position	EFN position	Secretory idioblast positions	Secretory trichomes
Sect. *Alabamenses*		*C. alabamensis*[1]	– ; PC	AP	—	—	+
Sect. *Lamprocroton*	Subsect. *Lamprocroton*	*C. ceanothifolius*	NA	M	—	ADA	+
		C. dichrous	NA	M	—	ADA, CB	+
		C. erythroxyloides	NA	M	—	ADA, CB	+
		C. imbricatus	NA	M	AB	ADA/ABA	–
		C. muellerianus	NA	—	—	ADA	+
		C. myrianthus	NA	M	—	ADA	+
		C. pallidulus	NA	M	—	ADA, CB	+
		C. pseudoadipatus	NA	M	—	ADA, CB	+
		C. pygmaeus	NA	M	—	—	+
		C. splendidus	NA	M	—	—	+
	Subsect. *Argentini*	*C. gnaphalii*	NA	—	—	ADA	+
Sect. *Cleodora*	Subsect.*Sphaerogyni*	*C. cajucara*	AA	M	AB	ADA/ABA	–
		C. campanulatus	AA	M	AB	ADA/ABA	–
		C. hemiargyreus	NA	M	AB	ABA	–
		C. heterocalyx	NA	M	AB	ABA	–
		C. organensis	AA	M	AB	ADA/ABA	–
		C. salutaris	AA	M	AB	ABA	–
		C. sphaerogynus	NA	M	AB	ADA/ABA, CB	–
	Subsect. *Spruceani*	*C. billbergianus*	AA	M	AB	ABA	–
		C. fragrans	AA	M	AB	ABA	–
		C. orinocensis	AA	M	AB, M	ABA	–
		C. rottlerifolius	AA	M	AB	ABA	–
		C. spruceanus	AA	M	AB	ABA	–
Astraea lobata			AA	M, AP, BL, BP	—	ADA, CB	–
Brasiliocroton mamoninha			NA	M ADA	AB	ABA, HB	–

Notes: AA, articulated anastomosed laticifers; AB, acropetiolar/basilaminar; ABA, abaxial epidermal leaf surface; ADA, adaxial epidermal leaf surface; AP, acropetiolar; BL, basilaminar; BP, basipetiolar; CB, secretory idioblasts at base of colleters; EFN, extrafloral nectaries; HB, secretory idioblasts at base of hairs; M, marginal; NA, articulated nonanastomosed laticifers; PC, parenchymatous cells with dense content; +, present; –, absent.

and on the apical (acropetiolar) and basal (basipetiolar) portions of the petiole (Fig. 3P, Q). In *Croton alabamensis*, colleters were restricted to the base of the leaf blade.

The structure of all colleters observed in this study followed the standard type, composed of a short stalk, a parenchymatous axis with or without vascularization, and elongated, vacuolated,

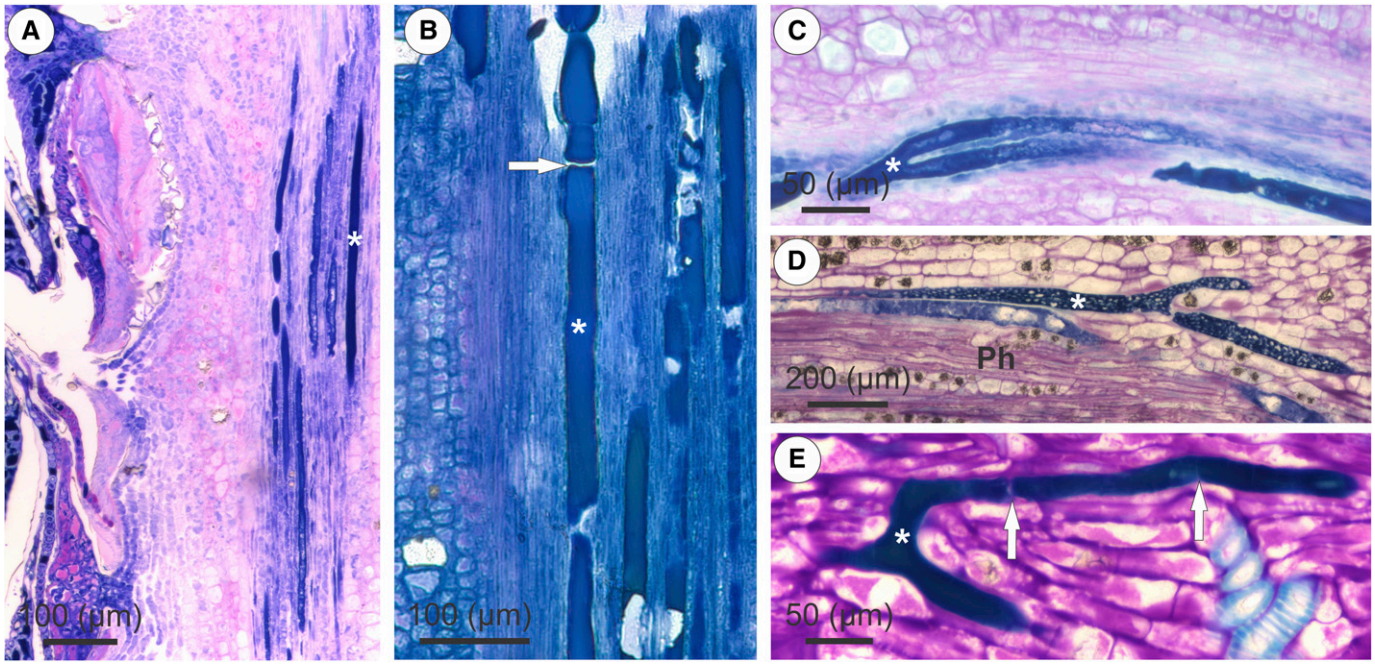


Fig. 2. Laticifers in some of the studied *Croton*. (A–E) Longitudinal sections of young leaves showing nonanastomosed articulated laticifers in (A) *C. muellerianus* and (B) *C. imbricatus*, articulated anastomosed laticifers in (C) *C. campanulatus*, (D) *C. rottlerifolius*, associated with phloem, and in (E) *C. orinocensis*. White asterisks, laticifers; white arrows, laticifer terminal walls; Ph, phloem.

thin-walled cells with cellulosic walls, some of which contained druses, and covered by a palisade secretory epidermis with a thick cuticle (Fig. 3F, J–N). In a few *Croton* species (Table 1), secretory idioblasts were observed at the base of the colleter stalk (Fig. 3K).

Ontogenetically, *Croton* colleters originate from the activity of the protoderm and ground meristem (Fig. 3A), but in some cases, procambium can also be involved. They can be observed in early stages of young leaves (Fig. 3G, O), developing precociously and asynchronously. On shoot apices, even at the margins of the earliest stage of young leaves, colleters are already fully formed and active (Fig. 3F, H–Q). In initial stages of colleter development, a group of protodermal initial cells becomes conspicuous due to their larger volume, dense cytoplasm, and prominent nuclei. These initial cells soon undergo a series of anticlinal divisions, generating a layer of tall cells that are projected above the level of the protodermal surface (Fig. 3A). Concurrently, ground meristem cells undergo periclinal division, producing a short stalk that elevates the structure, which then develops a globular shape (Fig. 3A–C). Developing trichomes or fully developed ones commonly occur adjacent to these developing colleters (Fig. 3A, D). The next step is characterized by the continued divisions and expansion of colleter cells (Fig. 3D, E). At final stages of colleter differentiation, the protoderm originates the palisade secretory epidermis and the nonsecretory epidermis of the stalk, while the ground meristem develops into the stalk inner portion and the parenchymatous cells of the central axis (Fig. 3E, F), where idioblasts containing druses are common (Fig. 3F, K, P). In a few species, the procambium produces the vasculature that reaches the base of the central axis. Laticifers accompanied the vasculature in *Croton ceanothifolius* Baill. (section *Lamprocroton*) (Fig. 3J).

In mature colleters, the secretion produced by the secretory epidermis accumulates in the subcuticular space, promoting cuticle detachment. The cuticle then distends (Fig. 3F, L) and eventually ruptures, releasing the exudate to the external environment. Upon release of the exudate, all the secretion is retained in the stem apical meristem where leaf primordia are developing (Fig. 3O). Histochemical tests confirmed the polysaccharide nature of the colleter secretion, as evidenced by the positive PAS reaction (Fig. 3M–O). Secretion accumulation in the subcuticular space was more commonly observed on early stages of young leaves (Fig. 3F, L). In some species, colleters fall off from mature leaves, being observed only on young leaves, as in in *Croton pygmaeus* L.R. Lima and *Brasiliocroton mamoninha*.

***Extrafloral nectaries*—**One pair of extrafloral nectaries at the junction of the petiole with the leaf blade, the acropetiolar/basilaminar nectaries, constitutes a diagnostic character of section *Cleodora* (Fig. 4A–C). On the other hand, the absence of this type of nectaries is characteristic of section *Lamprocroton*, with the exception of *Croton imbricatus* L.R. Lima & Pirani for which we detected such nectaries. Acropetiolar/basilaminar nectaries are also present in *Brasiliocroton mamoninha* (Fig. 4F), while *Astraea lobata* lacks such structures (Table 1).

In addition to the acropetiolar/basilaminar nectaries, *Croton orinocensis* Müll. Arg. (section *Cleodora* subsection *Spruceani* Caruzo) bears extrafloral nectaries interspersed with colleters along the leaf blade margin. Among all the studied species, *Croton fragrans* Kunth (section *Cleodora* subsection *Spruceani*) is the only species possessing two pairs of acropetiolar/basilaminar nectaries.

The morphology of extrafloral nectaries is variable among species from section *Cleodora* (Table 2). They can be either sessile (Fig. 4B) or stalked. Using an arbitrary criterion, we characterized the stalk as long (Fig. 4D, E) when its length extends three times the height of the secretory region or as short (Fig. 4F, G) for any stalk shorter. The secretory portion is located at the distal region of the nectaries and may be either concave (Fig. 4E, H) or convex (Fig. 4G, I) surface.

As expected from its secretory nature, extrafloral nectaries are intensely vascularized by branches from the petiolar vascular system (Fig. 4A, G); xylem and phloem (the latter in a higher proportion) reach the nectary parenchyma, which occupies a subepidermal position. Laticifers accompany the vasculature of extrafloral nectaries (Fig. 4H, I), and idioblasts containing druse-type crystals are concentrated at xylem and phloem ends, although they can also occur in the ground tissue of the stalk (Fig. 4H–J, L). The nectary parenchyma is composed of one to five layers of thin-walled cells with dense cytoplasm (Fig. 4H–L). The secretory surface of the nectaries is dilated and composed of a palisade secretory epidermis with a thick cuticle (Fig. 4H, I), and thick-walled cells at the margin. The central portion of the secretory surface is the site of nectar secretion, where the epidermal cells possess thinner walls and subcuticular spaces are commonly full of secretion (Fig. 4I–L); nectar is exuded by cuticle rupture (Fig. 4M). Trichomes and secretory idioblasts may occur along the epidermis, at the margins of extrafloral nectaries as well as on the stalk (Fig. 4H, I).

***Secretory idioblasts*—**Large cells containing a secretion that occupies virtually the entire protoplasm (Fig. 5A–C) and that stain strongly with toluidine blue (Fig. 5E, G, H) were identified as secretory idioblasts (Fig. 5A–C, E–J). The secretions of idioblasts stained red in Sudan IV or Sudan red tests, indicating that the secretions have a lipidic nature. (Fig. 5C). Idioblasts may occur on the leaf surface (Fig. 5A, B), throughout palisade parenchyma cells (Fig. 5E–G) and/or spongy parenchyma cells (Fig. 5G–I). They may be projected up to the leaf surface (Fig. 5C, E–G) or even above it (Fig. 5I, J). In some species, idioblasts may extend from one leaf surface to the other, occupying the entire mesophyll. Due to the rapid development of secretory idioblasts, it was impossible to identify from which foliar meristem they originated, even when analyzing serial sections from the apical meristems.

Secretory idioblasts are common in *Croton* species, but their locations may vary among species. In section *Cleodora*, they can be located on both leaf surfaces (Fig. 5G) or only on the abaxial surface (Fig. 5H, I). In the mesophyll of species from subsection *Spruceani*, idioblasts occur only on the abaxial leaf surface (Fig. 5I), while in subsection *Sphaerogyni* Caruzo their position varies among species. They can occur only on the abaxial leaf side (Fig. 5H; Table 1) or on either leaf side (Fig. 5G; Table 1). In section *Lamprocroton* subsection *Lamprocroton*, secretory idioblasts are absent only in *Croton splendidus* Mart. and *C. pygmaeus*. In *Lamprocroton* species, secretory idioblasts occur only on the adaxial side of the leaf (Fig. 5J), except for *C. imbricatus*, where these structures are located throughout the leaf (abaxially and adaxially) (Fig. 5F; Table 1). In *C. gnaphalii* (section *Lamprocroton* subsection *Argentini*) the secretory idioblasts follow the general distribution pattern verified in subsection *Lamprocroton*, i.e., positioned on the adaxial

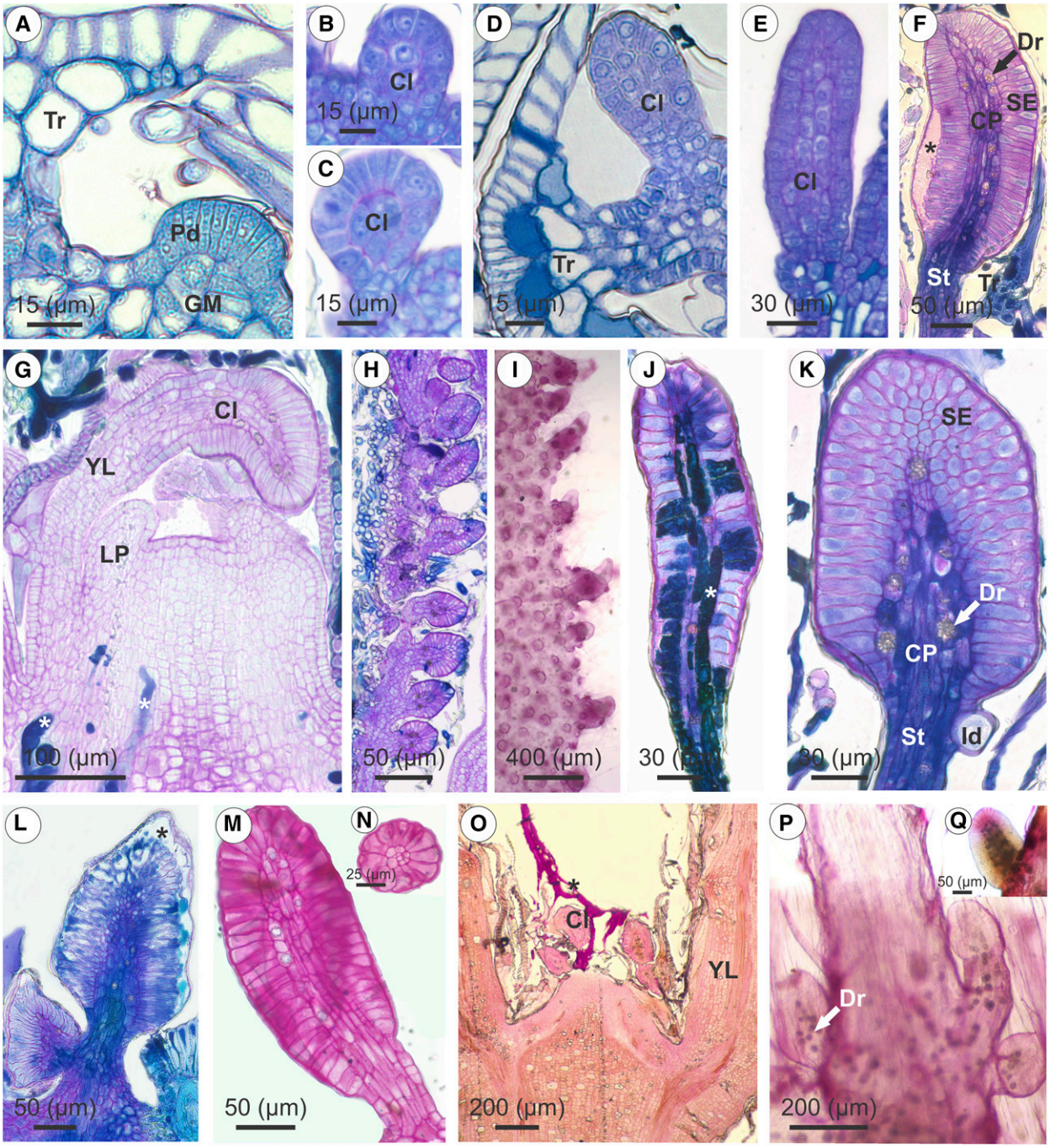


Fig. 3. (A–F) Transverse sections of leaf primordia and young leaves of *Croton* species and *Astraea lobata* showing colleters and their ontogeny. (A) Early colleter development on early stage of young leaf of *Croton erythroxyloides*; observe the dome-shaped epidermal protuberance produced by anticlinal divisions of protodermal cells and ground meristem. (B–D) Developing colleters on young leaf at the third node; note that protodermal cells maintain their anticlinal division, and the ground meristematic cells start periclinal divisions in (B, C) *C. erythroxyloides* and (D) *C. splendidus*. (E) Colleter stretching in late stage of their development in *C. erythroxyloides*. (F) Active, fully formed colleter in *C. pallidulus*; note the secretion in the subcuticular space. (G) Fully formed colleter associated with young leaf of *C. campanulatus*. (H) Marginal colleters on young leaf of *C. cajucara*. (I) Marginal colleters on diaphanized mature leaves of *C. heterocalyx*. (J) Vascularized marginal colleter in *C. ceanothifolius*; intensely stained structures on the central parenchyma correspond to laticifers. (K) Marginal colleter in *C. pallidulus* showing the vasculature reaching the base of the central parenchyma, and the presence of secretory idioblasts at the base of the structure. (L) Active colleters on young leaf of *C. imbricatus*. (M, N) Positive PAS reaction on young leaf colleters of *C. erythroxyloides* in (M) longitudinal and (N) transverse sections. (O) Longitudinal section of shoot apex of *C. imbricatus*, with positive PAS reaction on the colleter secretion covering the leaf primordia and early stages of young leaves. (P, Q) Basipetiolar colleters on diaphanized petiole of *Astraea lobata*. White asterisks indicate laticifers; black asterisks, colleter secretion; Cl, colleter; central parenchyma; Dr, druse; GM, ground meristem; Id, secretory idioblast; Pd, protodermal cells; SE, palisade secretory epidermis; St, stalk; YL, young leaf; LP, leaf primordium; Tr, trichomes.

leaf side. The same pattern was observed in *Astraea lobata*, while in *Brasiliocroton mamoninha* the idioblasts occur on the abaxial leaf side and have a unique arrangement so that they are located in pairs at the base of trichomes (Table 1).

***Secretory trichomes*—**The secretory trichomes are unicellular, constituted by a narrow basal portion that forms a short stalk and a dilated distal portion where the secretion accumulates (Figs. 5D, J; 6B, C). The secretion reacted positively to Sudan IV (Fig. 5D), Sudan red, and Nile blue sulfate in visible light and to neutral red in UV light, revealing its lipidic nature. The Nadi reagent stained the material a purplish blue, which typically indicates an oil-resin secretion.

The protodermal cell that gives rise to the secretory trichome can be recognized by its larger volume, prominent nucleus, and dense cytoplasm (Fig. 6A). The external portion of this cell dilates and expands (Fig. 6B), and as a consequence of these expansions, the nucleus is dislocated toward the basal portion (Fig. 6B, C). Trichomes have a more intense activity on younger leaves, i.e., from the first to third node, and as leaves develop, these trichomes become senescent.

Among the studied species, secretory trichomes were found only in species from section *Lamprocroton* (except *C. imbricatus*) and were only detected on the abaxial side of the leaves (Fig. 5J). In the other species studied, secretory trichomes are absent, and secretory idioblasts occur on the abaxial leaf surface. Secretory trichomes and secretory idioblasts on the abaxial leaf side appear to be mutually exclusive. Structures in members from section *Cleodora* subsection *Spruceani* are intermediate between secretory idioblasts and secretory trichomes: the secretion accumulates in a cell that extends from the mesophyll and projects itself outward, surpassing the epidermal surface (Fig. 5I).

DISCUSSION

***The significance of laticifers in tribe* Crotoneae—**Articulated laticifers were observed in all studied species, except for *Croton alabamensis* (section *Alabamenses*) suggesting that they have been lost at least once in the evolution of this tribe. Laticifers might be present in many other *Croton* sections and *Crotoneae* genera, and they eventually might be recognized as a common feature for the entire tribe. An in-depth analysis including representatives from all *Croton* sections and other *Crotoneae* genera is needed to confirm this hypothesis. The presence and type of laticifers was one of the characters used by Webster (1975, 1994) to delimitate the five Euphorbiaceae subfamilies (Acalyphoideae, Crotonoideae, Euphorbiodeae, Oldfieldioideae, and Phyllantoideae). Chase et al. (2002) promoted Oldfieldioideae and Phyllantoideae to family status based on molecular data, but maintained Euphorbiaceae s.s. subdivided in Acalyphoideae, Crotonoideae, and Euphorbioideae. Species of the subfamilies Crotonoideae and Euphorbioideae are latescent, while in the Acalyphoideae laticifers have been reported only in *Omphalea* L. and *Macaranga* Thouars (Rudall, 1994).

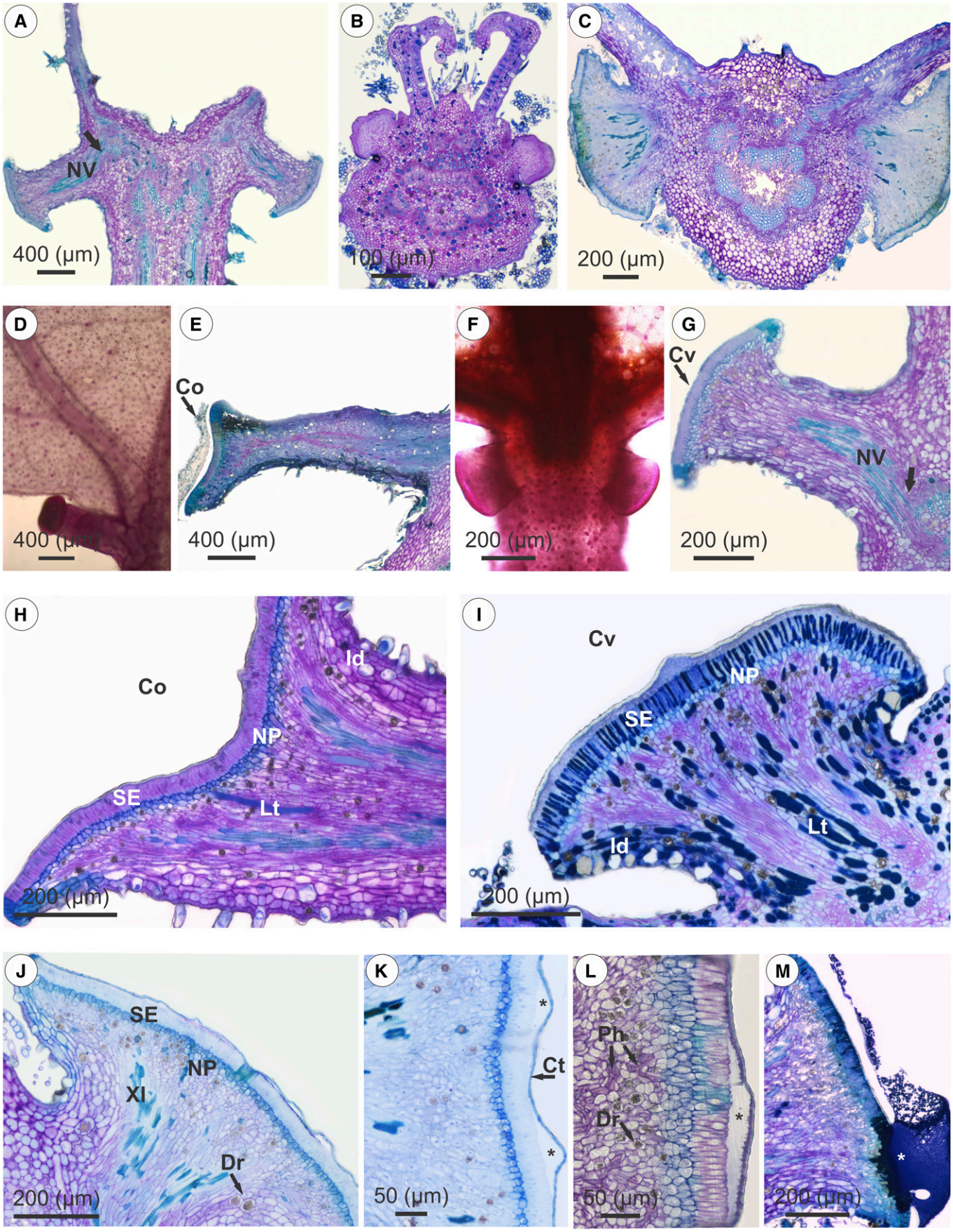
Our results suggest that articulated laticifers might be widespread in tribe Crotoneae as well as in subfamily Crotonoideae. Articulated laticifers have also been described in *Manihot* Mill. and *Hevea* Aubl. (Rudall, 1994), both genera from subfamily Crotonoideae. On the other hand, nonarticulated laticifers had been found in other Crotonoideae species as *Astraea comosa* (Müll. Arg.) B.W. van Ee, in 11 *Croton* species (*C. antisyphiliticus* Mart. ex Müll. Arg., *C. conduplicatus* Kunth., *C. heteropleurus* Urb., *C. megalobotrys* Müll. Arg., *C. occidentalis* Müll. Arg., *C. sagreanus* Müll. Arg., *C. sylvaticus* Hochst., and four unidentified species) and in other taxa of the subfamily Euphorbioideae, i.e., *Euphorbia lathyris* L. and *Synadenium grantii* Hook. (Rudall, 1994). Nonarticulated laticifers are the most common type among the eudicots, and articulated laticifers may have evolved from nonarticulated ones in the Euphorbiaceae (Rudall, 1994). An alternative hypothesis is that articulated laticifers constitute a plesiomorphic character for Euphorbiaceae and Malpighiaceae, which would have shared a common latescent ancestor (Vega et al., 2002), in which case, articulated laticifers gave rise to nonarticulated laticifers independently within both families.

As for the origin of laticifers (articulated and nonarticulated), it is believed that these structures may have arisen independently from parenchymatous cells that began to secrete latex (Scott, 1885; Rudall, 1994). The presence of such cells exclusively in *C. alabamensis* gives additional support to the monotypic nature of section *Alabamenses* (Berry et al., 2005; van Ee et al., 2011), a clade well separated from section *Lamprocroton* in the *Croton* phylogeny (Fig. 1); *C. alabamensis* was originally placed in section *Lamprocroton* based on morphology (Webster, 1994).

We did not identify any strong pattern regarding the occurrence of anastomosed and nonanastomosed laticifers. In subsection *Lamprocroton*, articulated laticifers are exclusively nonanastomosed, while in section *Cleodora* the predominant type is the articulated anastomosed one, except for *Croton hemiargyreus*, *C. sphaerogynus*, and *C. heterocalyx*. *Astraea lobata* has anastomosed articulated laticifers and *Brasiliocroton mamoninha* nonanastomosed articulated ones. Broader taxon sampling may better clarify the evolutionary pattern of laticifer types in Crotoneae.

***Colleters are widespread secretory structures in* Crotoneae—**The colleters we observed correspond to emergences because they originate from the protoderm and ground meristem and, when vascularized, from the procambium. Colleters are active in early stages of leaf ontogeny, where they produce abundant polysaccharidic secretions. This result agrees with their function of producing secretions that protect leaf primordia and young leaves during their development (Horner and Lersten, 1968; Lersten, 1974; Fahn, 1979) and/or that promote the lubrication of the shoot meristem to minimize the attrition of the developing leaf tissues and to avoid their desiccation (Fahn, 1979; Thomas, 1991; Mayer et al., 2011, 2013; Silva et al., 2012; Chin et al., 2013). The function of the laticifers observed in colleters of *C. ceanothifolius* remains obscure, but a plausible hypothesis is that latex provides compounds for the presecretion, as has been suggested in the case of resin-secreting colleters (Thomas, 1991).

The mode of secretion of colleters in the studied species is similar to what has already been described for colleters in other angiosperm families, e.g., Asclepiadaceae (Kuriachen and Dave, 1989), Rhizophoraceae (Lersten and Curtis, 1974), and Caryocaraceae (Paiva and Machado, 2006). Within the order Malpighiales (APG II, 2003), the presence of colleters has been reported in Euphorbiaceae, Erythroxylaceae, Passifloraceae, Rhizophoraceae, Turneraceae (Thomas, 1991), and Caryocaraceae (Paiva and Machado, 2006). In all the species studied here, the colleters are of the standard type, which has been considered


A
NV
400 (μm)
B
100 (μm)
C
200 (μm)
D
400 (μm)
E
Co
400 (μm)
F
200 (μm)
G
Cv
NV
200 (μm)
H
Co
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SE
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as the most common type in angiosperms (Thomas, 1991). But the positioning of colleters at the leaf margins is a rare report in angiosperms, although the occurrence of colleters at stipule margins in Rubiaceae has been observed before (Lersten, 1974). In Rubiaceae, stipules commonly cover the shoot apices and contain numerous colleters that protect the developing leaves (Lersten, 1974; Fahn, 1979; Klein et al., 2004; Vitarelli and Santos, 2009). The developing leaves of *Croton* species are folded in such a way that only the dorsal leaf surface remains exposed to the external environment. The colleters are found along the leaf margins, and their secretion covers the entire marginal zone of the expanding leaves and insulates the adaxial surface from the external environment, protecting the developing leaves against desiccation. This function is complemented in the majority of *Croton* species by a dense indument of trichomes on the abaxial leaf surface. In contrast, the adaxial leaf surface has a considerably lower trichome density or may even be glabrous.

The presence of standard type colleters on the leaves of 21 of the 23 sampled *Croton* species indicates that these structures might be very common across *Croton.* It also represents a new record for the genus although a report of these structures on the inflorescence axis of *C. glandulosus* has been very recently published (Machado et al., 2015). When analyzing images of anatomical sections from flowers and leaves of *Croton* species from previous studies (Freitas et al., 2001; de Sá-Haiad et al., 2009; De-Paula et al., 2011), we identified features with great structural and positional resemblance with colleters. However, such colleter-like structures have been named as follows: "filamentous structures" on pistillate flowers of seven *Croton* species, which appeared to have originated from modified petals (De-Paula et al., 2011), "extrafloral nectaries" on the stipules and leaf margins of *C. sarcopetalus* Müll. Arg. (Freitas et al., 2001), "extrafloral nectaries" on leaf margins in species of *Croton* section *Cyclostigma*, *Brasiliocroton mamoninha*, *Astraea lobata*, *A. jatropha* Klotzsch, and *A. klotzschii* Didr. (de Sá-Haiad et al., 2009) and as "long cylindrical extrafloral nectaries with round apices" at the junction of blade and petiole in *Astraea jatropha*, *A. klotzschii*, *A. lobata*, and *A.* sp. (de Sá-Haiad et al., 2009).

Extrafloral nectaries are not uncommon on the leaf margins of *Croton* species, but they occur interspersed with other secretory structures with distinct morphologies and considerably reduced sizes. As demonstrated in the present work, such reduced structures correspond to standard type colleters. These results reinforce the report of Webster et al. (1996), in which the leaves of *Croton* are described as having specialized glands at the base of the leaf blade or at the petiole apex, and frequently along the margin. Such glands differ from the small marginal glandular hairs, being much more massive and morphologically more complex, and they are therefore unlikely to be homologous. Probably these "marginal glandular hairs" correspond, in most reports, if not in all of them, to the leaf marginal colleters described here.

The extrafloral nectaries and colleters observed here are morphologically and functionally distinct structures; however, these two structures originate from the same meristematic tissue (protoderm and ground meristem) and might be regarded as homologous. Indeed, in *Astraea lobata* and *Croton alabamensis*, basilaminar extrafloral nectaries do not occur, but in their place there are clusters of colleters. It seems plausible that during the evolutionary history of tribe Crotoneae, selective pressures for resource allocation favored the loss of basilaminar colleters and the appearance of more complex structures such as extrafloral nectaries, which may provide adaptive value to the species under different ecological conditions. Lersten (1975) observed that some genera of Rubiaceae lost the ability to produce colleters, and associated this evolutionary trend with investments in other specializations.

Defining certain secretory structures as trichomes, colleters, nectaries, hydathodes, or salt glands is not an easy task. Besides their morphology, the type of secretion must be considered (Fahn, 1979), as it may be crucial for elucidating the function of the structure. We also noticed that the periods of activity of colleters and extrafloral nectaries were different in the studied taxa. When colleters are already fully formed and active on young leaves, extrafloral nectaries are still undergoing early formation, becoming active only on mature leaves where colleters are no longer active or may have already fallen off.

Our results highlight the occurrence and role of colleters in Crotoneae, since the morphology, position, type of secretion and period of activity of these structures reflect a function of protecting leaf primordia against dehydration and the attack of microorganisms during early development. Freitas et al. (2001) and de Sá-Haiad et al. (2009) justified the classification of these structures as extrafloral nectaries rather than colleters due to the presence of sugars in their secretions, but they did not consider the timing of secretion activity during development. Moreover, sugars can also occur in colleter secretions (Thomas, 1991), such as rhamnose in the colleter secretion of *Aganosma* (Blume) G. Don (Dave et al., 1987), *Strophanthus* DC. (*Roupelia* in Thomas et al., 1989), *Alstonia* R. Br. (Thomas and Dave, 1989), and *Ixora* L. (Dave and Thomas, 1991).

The data reported herein suggest that the presence of colleters might be a widespread character in members of tribe Crotoneae. The absence of colleters in the leaves of *Croton muellerianus* (subsection *Lamprocroton*) and *C. gnaphalii* (subsection *Argentinii*) is unexpected. A possible explanation is that colleters are probably present in these species, but the particularly small size of both the leaves in *Lamprocroton* species (<2 cm long) and the colleters themselves might have hampered access to them during sectioning. To confirm the presence of

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Fig. 4. Extrafloral nectaries in *Croton* and *Brasiliocroton mamoninha* in longitudinal sections (A–C, E, G–M) and diaphanized leaves (D, F). (A) Pair of nectaries with short stalk in *C. heterocalyx*; note the vasculature from the ramification of petiole vascular bundles (arrow) giving rise to the nectary vasculature. (B) Pair of sessile nectaries in *C. cajucara*. (C) Pair of nectaries with short stalk in *C. spruceanus*. (D) Nectary with long stalk in *C. hemiargyreus*. (E) Nectary with long stalk and concave secretory surface in *C. salutaris*. (F) Nectary with short stalk in *Brasiliocroton mamoninha*. (G) Nectary with short stalk and convex secretory surface in *C. heterocalyx*; note the vasculature from the ramification of petiole vascular bundles (arrow) giving rise to the nectary vasculature. (H) Detail of the concave secretory surface in *C. fragrans*; note the laticifers accompanying nectary vasculature, and secretory idioblasts along the stalk. (I) Detail of the convex secretory surface in *C. sphaerogynus*; note the laticifers accompanying nectary vasculature, and secretory idioblasts along the stalk. (J) Nectary overview in *C. spruceanus*; note xylem, phloem, and laticifers reaching the nectary parenchyma. (K, L) Detail of secretory surface of nectary in *C. spruceanus* and *C. rottlerifolius*, respectively, showing palisade epidermis with nectar in the subcuticular space. (M) Detail of nectary secretory surface of *C. billbergianus*, with nectar exuded through a cuticle rupture. Asterisks indicate nectar location. Co, concave secretory surface; Ct, cuticle; Cv, convex secretory surface; Dr, druse; Ph, phloem; Id, secretory idioblast; Lt, laticifer; NP, nectary parenchyma; NV, nectary vasculature; SE, secretory epidermis; Xl, xylem.

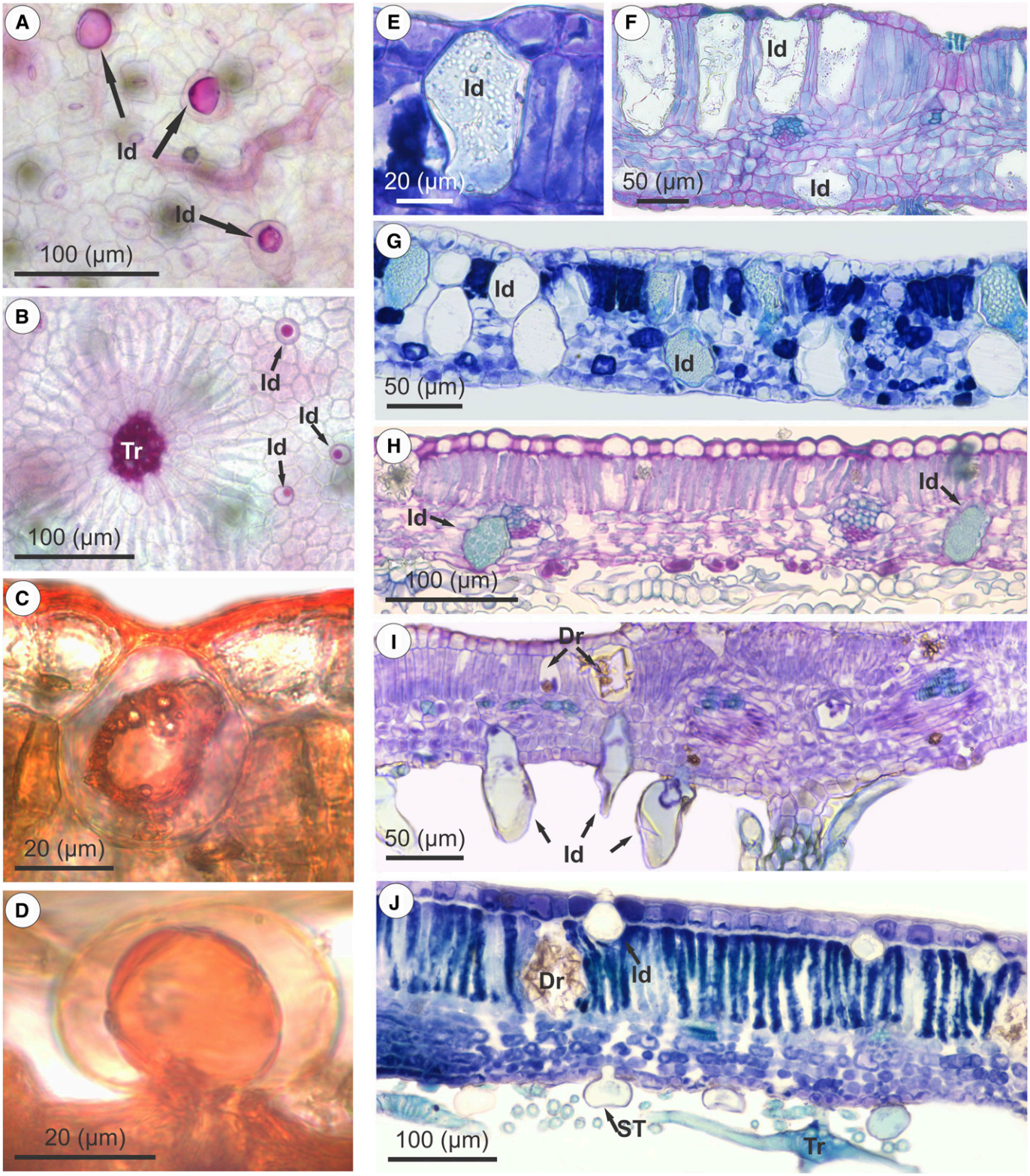

Fig. 5. Idioblasts and secretory trichomes in *Croton*. (A, B) Front view of secretory idioblasts at epidermal cell level on adaxial leaf surface in *C. myrianthus* and on abaxial one in *C. cajucara*. (C, D) Leaf transverse sections submitted to Sudan IV test; positive reaction on (C) secretory idioblasts in *C. dichrous* and on (D) secretory trichomes in *C. splendidus*. (E) Detail of secretory idioblast at interface between mesophyll and epidermis in *C. organensis*. (F, G) Idioblasts on both leaf sides in (F) *C. imbricatus* and (G) *C. sphaerogynus*. (H) Idioblasts only on abaxial leaf side in *C. hemiargyreus*. (I) Secretory idioblasts only on abaxial leaf side in *C. fragrans*; note how they emerge from the mesophyll toward the external environment, being placed above the epidermal cell level. (J) Secretory idioblasts only on adaxial leaf side with epidermal projections and secretory trichomes on abaxial leaf surface in *C. dichrous*. Dr, druse; Id, secretory idioblasts; ST, secretory trichome; Tr, trichomes (nonsecretory).

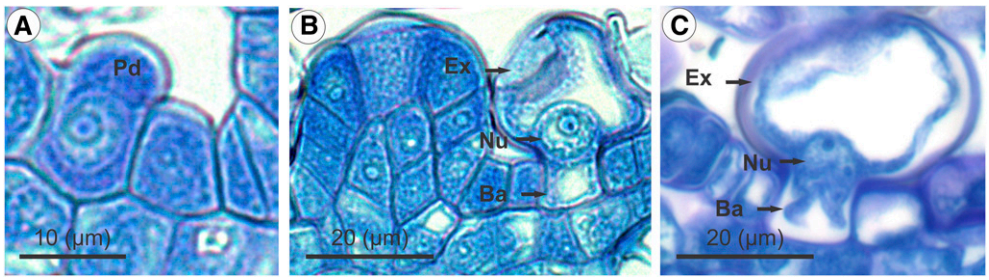

Fig. 6. Secretory trichome ontogeny in two species of *Croton* section *Lamprocroton*. (A) Protodermal cell that gives rise to the secretory trichome stands out by its larger volume, evident nucleus, and dense cytoplasm in *C. splendidus*. (B) The external portion of the prododermal cell dilates and expands, the nucleus is dislocated toward basal region in *C. splendidus*. (C) Mature secretory trichome with secretion accumulated in their external dilated region in *C. erythroxyloides*. Ba, basal region; Ex, external dilated region; Nu, nucleus; Pd, protodermal cell.

colleters in these species, future studies should include serial sections that cover the entire shoot apical meristem.

Colleters also exhibit other traits with taxonomic value. The occurrence of druses in the parenchyma of the colleter central axis in all studied species is a character with potential taxonomic value within Euphorbiaceae or even in Malpighiales. In fact, within the other families in this order, druses have been reported in the core parenchyma region of colleters in Rhizophoraceae (Sheue et al., 2012, 2013), whereas they are absent in the colleters of *Caryocar brasiliense* Cambess. in the Caryocaraceae (Paiva and Machado, 2006).

***Extrafloral nectaries and their relationships to other plant secretory structures*—**Extrafloral nectaries are active in the studied species in fully expanded leaves and could be detected by the accumulation of nectar in the subcuticular space and its subsequent exudation through cuticle rupture. This mechanism of nectar secretion through nectaries (floral and extrafloral ones) is common in species from several plant families (Fahn, 1952; Thadeo et al., 2008).

The extrafloral nectaries observed in *Croton* and *Brasiliocroton mamoninha* are vascularized, as are the floral nectaries observed by De-Paula et al. (2011). This character seems to be an important morphological marker within tribe Crotoneae, since De-Paula et al. (2011) showed that the floral nectaries of *Astraea* are nonvascularized and discussed that these data support the segregation of *Astraea* from *Croton* proposed by Berry et al. (2005). Our results demonstrate that the extrafloral nectaries of *Croton* and *Brasiliocroton* are structurally similar, which corroborates the phylogenetic proximity between these two genera (Berry et al., 2005; Riina et al., 2014). Extrafloral nectaries probably evolved in the common ancestor of *Brasiliocroton* and *Croton*; however, some *Croton* lineages have ultimately lost these structures, because they can be observed in clades such as sections *Eluteria* Griseb., *Julocroton* (Mart.) G.L. Webster, *Lamprocroton*, and *Lasiogyne* (Klotzsch) Baill. (van Ee et al., 2011).

The notable presence of laticifers accompanying the phloem on extrafloral nectaries suggests that the latex could be contributing to the process of sugar transfer from the phloem to nectary tissues. A similar situation has been reported in extrafloral nectaries of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (Fahn, 1979). On the other hand, the presence of laticifers in extrafloral nectaries of *Croton* species could also indicate that some latex constituents may be part of nectar composition.

The absence of a pair of acropetiolar/basilaminar nectaries is a diagnostic character for section *Lamprocroton*. The detection of such nectaries in *Croton imbricatus*, along with the other anatomical differences reported here, indicates that this species might be erroneously positioned within section *Lamprocroton*. The placement of *Croton imbricatus* within this section was based only on external morphology. However, acropetiolar nectaries were inadvertently overlooked probably because of their minute size and the dense layer of lepidote trichomes characteristic of this species (Lima and Pirani, 2008; van Ee and Berry, 2011).

Acropetiolar/basilaminar nectaries are present in all species of section *Cleodora*. We verified a correlation between the stalk

TABLE 2. Species possessing acropetiolar extrafloral nectaries, length of nectary stalk and shape of secretory surface.

Section	Subsection	Species	Stalk length	Secretory surface
Cleodora	*Spruceani*	*Croton rottlerifolius*	short	convex
		Croton orinocensis	short	convex
		Croton spruceanus	short	convex
		Croton billbergianus	short	convex
		Croton fragrans	long	concave
	Sphaerogyni	*Croton heterocalyx*	short	convex
		Croton sphaerogynus	short	convex
		Croton cajucara	sessile	convex
		Croton organensis	short	convex
		Croton salutaris	long	concave
		Croton campanulatus	long	concave
		Croton hemiargyreus	long	concave
Lamprocroton	*Lamprocroton*	*Croton imbricatus*	sessile	convex
Brasiliocroton		*Brasiliocroton mamoninha*	short	convex

length and the morphology of the nectary and its secretory surface. Sessile or short-stalked nectaries possess a convex secretory surface, while those with long stalk have a concave secretory surface. In subsection *Spruceani* (Caruzo et al., 2011), the nectaries possess a short stalk and convex secretory surface. *Croton fragrans* is the only exception in that subsection, and it also differs from the other species in the subsection in having more than one pair of acropetiolar/basilaminar nectaries. The particular morphology of the nectaries of *C. fragrans* agrees with its isolated phylogenetic position in the subsection *Spruceani* (Caruzo et al., 2011).

Within section *Cleodora* subsection *Sphaerogyni*, two distinct subclades are well supported (Caruzo et al., 2011), namely, subclade 1 (*C. organensis* Baill., *C. cajucara* Benth., *C. sphaerogynus*, and *C. heterocalyx*), and subclade 2 (*C. hemiargyreus*, *C. campanulatus* Caruzo & Cordeiro, and *C. salutaris* Casar.). Species of subclade 1 have short-stalked or sessile nectaries with a convex secretory surface, as do the species of subsection *Spruceani*, whereas the species of subclade 2 have long-stalked nectaries with a concave secretory surface.

We hypothesize that different strategies of animal-mediated, ecological interactions involving plant protection, especially ants with aggressive behavior, could have been selected for in *Croton*. In species possessing short-stalked or sessile nectaries with a convex secretory surface, nectar accumulation on the nectary surface does not occur, since the secretion flows and spreads over the leaf blade and petiole. Consequently, the area where visitors can find food is larger, but the amount of accumulated secretion is lower. In species in which the nectaries are long-stalked with a concave secretory surface, the exuded nectar accumulates in the concavity. Thus, the resource for visitors is concentrated in a reduced area. However, this rather speculative hypothesis needs to be evaluated in future ecological studies.

The role of secretory idioblasts and secretory trichomes and their evolutionary implications—The presence of secretory trichomes on the leaf abaxial surface is a common character for section *Lamprocroton* (except *Croton imbricatus*). Webster et al. (1996) reported the occurrence of "glandular trichomes" as a character restricted to a limited number of taxa in section *Barhamia* (Klotzsch) Baill. and to *C. ciliatoglandulifer* Ortega (section *Adenophylli* Griseb., sensu van Ee et al., 2011). However, the description of such glands does not correspond to the secretory trichomes observed in our study. The observation of oil-resin secretory trichomes in the leaves of section *Lamprocroton* is a new report for tribe Crotoneae, and we believe that these structures can be related to the distinctive scent produced by species in this section. Further anatomical investigations in *Croton* sections *Barhamia* and *Adenophylli*, as well as other *Croton* clades, are necessary to confirm whether secretory trichomes have evolved independently in other lineages within this genus. Secretory trichomes could be regarded as a synapomorphy for section *Lamprocroton* and possibly also for section *Barhamia* (Webster et al., 1996), although the evaluation of a larger number of species of the latter section would be necessary to confirm this hypothesis. On the other hand, the presence of secretory trichomes at the tribe level could be a homoplastic character because it has been reported in distantly related clades like sections *Lamprocroton*, *Barhamia*, and *C. ciliatoglandulifer* (section *Adenophylli*).

It is interesting to note that in Crotoneae, the presence of secretory idioblasts and secretory trichomes localized externally on the abaxial leaf surface are mutually exclusive. This unusual pattern of externalization of secretory idioblasts (Fig. 5I) shows that the storage site of secondary metabolites seems to have been transferred from internal secretory idioblasts to external ones in some *Croton* lineages.

The species of section *Cleodora* subsection *Spruceani* seem to be a key group for understanding the evolutionary history of secretory trichomes because all species from this subsection have secretory idioblasts on the abaxial leaf surface and intermediate idioblasts that extend from the mesophyll and project themselves outward, surpassing the epidermal surface (a transitory position between secretory idioblasts and secretory trichomes). Therefore, this subsection seems to illustrate a transitional stage in the evolution of external (trichomes) from internal (idioblasts). The study of the development of such idioblasts could confirm or reject the hypothesis formulated by Fahn (2002), who suggested that secretory tissues with protective function in vascular plants probably originate in the mesophyll (as verified in some pteridosperms) and have followed two different evolutionary pathways: one external, toward the epidermis and epidermal trichomes (as in many angiosperms) and a second internal one, toward the primary and secondary phloem (as in some conifers) and toward the secondary xylem.

Conclusions—The number and morphological diversity of secretory structures (laticifers, colleters, extrafloral nectaries, secretory idioblasts, and secretory trichomes) found in the leaves of the Crotoneae species, is in accord with the chemical richness of the tribe and highlights the bioprospecting potential of its species, particularly members of the large genus *Croton*. The presence of such structures also suggests that this group might be involved in numerous ecological interactions, which are still little explored and which could be relevant for its evolutionary success. The diverse secretory structures in Crotoneae constitute an important set of morphological markers, which can be useful for systematics. Further investigations expanding the taxonomic sampling within this tribe might provide additional insights into the morphological diversification and evolutionary success of this species-rich angiosperm lineage.

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Appendix 1. List of species and voucher information. Herbarium acronyms in parentheses follow Thiers (2014).

Species	Collector and number (herbarium)
Astraea lobata (L.) Klotzsch	Melo 1285 (SP)
Brasiliocroton mamoninha P.E. Berry & Cordeiro	Fiaschi 951 (SPF)
Croton alabamensis E.A. Smith ex Chapman	Keener 2472 (SP), Berry s.n. (VIC)
Croton billbergianus Müll. Arg.	Wiley 7 (RB)
Croton cajucara Benth.	Caruzo 95 (SP), Jangoux 1051 (RB)
Croton campanulatus Caruzo & Cordeiro	Lima 469 (SP), Monteiro 7964-I (RBR)
Croton ceanothifolius Baill.	Lima 325 (SP)
Croton dichrous Müll. Arg.	Alvim 46 (HUEMG), Vitarelli 012 (SP), Cordeiro 2771 (SP), Stehmann 2504 (SP)
Croton erythroxyloides Baill.	Vitarelli 002 (VIC), Vitarelli 005 (VIC), Vitarelli s.n. (VIC)
Croton fragrans Kunth	Caruzo et al., 125 (SP)
Croton gnaphalii Baill.	Marchett 545 (BHCB)
Croton hemiargyreus Müll. Arg.	Caruzo 114 (SP), Nascimento 473 (RB)
Croton heterocalyx Baill.	Caruzo 108 (SP)
Croton imbricatus L.R. Lima & Pirani	Laessor s.n. (SP), Furlan s.n. (SPF)
Croton muellerianus L.R. Lima	Ribas 926 (BHCB)
Croton myrianthus Müll. Arg.	Lima 296 (SP)
Croton organensis Baill.	Caruzo 90 (SP), Prado 366 (RB)
Croton orinocensis Müll. Arg.	Caruzo 124 (SP)
Croton pallidulus Baill.	Lima 319 (SP)
Croton pseudoadipatus Croizat	Monteiro et al., 009 (HUEMG), Monteiro et al., 017 (HUEMG)
Croton pygmaeus L.R. Lima	Falkenberg 6483 (BHCB), Marchett 549 (BHCB)
Croton rottlerifolius Baill.	Lima 139 (SPF)
Croton salutaris Casar.	Caruzo 89 (SP), Pardo 108 (RBR)
Croton sphaerogynus Baill.	Caruzo 88 (SP), Landrum 2052 (RB)
Croton splendidus Mart.	Alvim s.n. (HUEMG), Alvim 40 (HUEMG), Vitarelli s.n.* (VIC), Vitarelli s.n. (VIC)
Croton spruceanus Benth.	Ducke s.n. (RB 35702)