











Original Article

Trichomes in the megadiverse genus *Croton* (Euphorbiaceae): a revised classification, identification parameters and standardized terminology

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ABSTRACT

Trichomes are key functional traits that protect plants against abiotic stresses, herbivores and pathogens. In addition to their ecological roles, trichomes show high morphological variability in many lineages, providing useful characters for species characterization and taxon delimitation. Here, we focus on trichomes in *Croton*, a megadiverse genus (c. 1200 spp.) of Euphorbiaceae, in which these structures are ubiquitous and exhibit significant variation in form, location on the plant body, density and function. Owing to their functional and taxonomic relevance and the current need for further standardization of their terminology and classification, we revised previous trichome classifications used in the genus. We also provide new trichome data by expanding taxon sampling and using different methodological approaches in plant anatomy to improve understanding of the structural patterns of the trichome types present in the genus. On the basis of our results and literature review, we propose a new classification for trichomes in *Croton* based on well-defined parameters; this clarifies existing redundancies, is consistent with anatomical evidence and can be easily updated as new types are revealed in future surveys. This contribution will improve communication among taxonomists and promote more meaningful morphological comparability across systematics studies, including those focusing on morphological evolution and functional traits.

Keywords: anatomy; Crotonaeae; hairs; systematics; taxonomy; terminology; traits

INTRODUCTION

Trichomes are epidermal appendages that occur on different surfaces of the plant body and exhibit a wide variety of forms and functions (Radford *et al.*, 1974; Evert, 2006; Wang *et al.*, 2021). Plant trichomes play major roles in protection against extreme temperature, dehydration, herbivores and pathogens (Dickison, 2000; Ballego-Campos *et al.*, 2020; Wang *et al.*, 2021). In addition to their protective functions, some trichomes have been shown to have the capacity for atmospheric water absorption, as reported for several groups of angiosperms, including *Croton* L. (Euphorbiaceae) (Burrows *et al.*, 2013; Fernández *et al.*, 2014; Kowalski *et al.*, 2016; Vitarelli *et al.*, 2016, 2021; Berry *et al.*, 2018).

The great morphological diversity of trichomes has allowed botanists to use their morphology, position and density for taxonomic purposes across many plant families (Wurdack, 1986; Harley *et al.*, 2004; Krak and Mráz, 2008; Nogueira *et al.*, 2013; Webster, 2014; Weigend *et al.*, 2016; Carvalho *et al.*, 2017; Arabameri *et al.*, 2020; Santos *et al.*, 2020). A common and primary division in trichome classification is the distinction between glandular (secretory) and non-glandular trichomes, and each of these in turn may be divided into unicellular or multicellular structures and further subtypes (Radford *et al.*, 1974; Webster *et al.*, 1996; Appezzato-da-Glória and Carmello-Guerreiro, 2006). However, comprehensive and standardized classifications of trichomes are lacking for most plant groups in which these structures are widespread and diverse (Nogueira *et al.*, 2013). This deficiency generates confusion and redundancy in terminology, making communication and meaningful comparisons across taxa problematic.

Croton, with c. 1200 species, is the second most diverse genus of Euphorbiaceae (Berry *et al.*, 2005), occurring in the tropics and subtropics worldwide. The genus is remarkable because of the widespread occurrence of trichomes across all its species (Webster *et al.*, 1996; Van Ee *et al.*, 2011; Santos *et al.*, 2017; Sodr e *et al.*, 2019a, 2019b). Under the current classification (Van Ee *et al.*, 2021), *Croton* is divided into four monophyletic subgenera and numerous sections. *Croton* subgenus *Croton* includes all the Palaeotropical species (c. 450 species), whereas the other three subgenera are restricted to the Neotropics (c. 712 species): subgenus *Adenophylli* (Griseb.) Riina, B.W.Van Ee & P.E.Berry (two sections); subgenus *Geiseleria* A.Gray (20 sections) and subgenus *Quadrilobi* (Müll.Arg.) Pax (nine sections).

In *Croton*, trichomes may be present all over the plant body, and their morphology, distribution and density patterns are widely used in taxonomic studies at the species level (Aplet *et al.*, 1994; González Ramírez and Poveda Álvarez, 2003; Caruzo *et al.*, 2016; Feio *et al.*, 2018b; Sodr e *et al.*, 2019a, 2019b; Mateo-Ramírez and Riina, 2020) and, in combination with other characters, for the circumscription of sections and subsections (Webster *et al.*, 1993; Van Ee *et al.*, 2008; Lima and Pirani, 2008; Van Ee and Berry, 2009; Van Ee *et al.*, 2011; Caruzo and Cordeiro, 2013; Gomes *et al.*, 2018).

Baillon (1864, 1958) and Müller (1866, 1973) were the first botanists to explore trichome morphology in the genus for taxonomic purposes. However, they limited their scope to two basic types: the stellate trichome, formed by free rays, and the lepidote trichome, with rays united in various degrees. A few decades later, Froembling published the first comprehensive

study of trichomes of the tribe Crotonae and other groups of Euphorbiaceae *s.l.*, including 132 *Croton* spp. (Froembling, 1896) and proposed a classification recognizing five basic trichome types: lepidote (*schilghaare*); stellate (*sternhaare*); fasciculate or rosulate (*buschelhaare*); fasciculate-stipitate (*morgensternförmig Haare*) and dendritic (*candelaberhaare*).

A century later, Webster *et al.* (1996) revisited and revised Froembling's work. They surveyed trichome morphology from 120 *Croton* spp. and proposed a new classification that included seven basic types of trichomes: lepidote, stellate, fasciculate, multiradiate/rosulate, dendritic, papillate and glandular. Since its publication, Webster's classification has served as the main reference for trichome typology and terminology, not only in taxonomic and anatomical studies focusing on *Croton* (Van Ee *et al.*, 2011; Caruzo and Cordeiro, 2013; Vitarelli *et al.*, 2015, 2016; Feio, *et al.*, 2018a; Feio *et al.*, 2018b; Riina *et al.*, 2021), but also in other groups of Euphorbiaceae (Cervantes *et al.*, 2009; Bigio and Secco, 2012; Riina *et al.*, 2014; Silva and Cordeiro, 2020) and other plant families (Clark *et al.*, 2010; Tan *et al.*, 2012; Wurdack, 2017; Ganesan *et al.*, 2021).

Despite the wide use of the classification of Webster *et al.* (1996), inconsistencies in the identification and designation of trichomes in the taxonomic literature for *Croton* still remain. These are mostly due to the lack of clear and standardized parameters applied in the context of the different methods used to examine these structures. Common problems on the use of the trichome types proposed by Webster *et al.* (1996) include the distinction between stellate and lepidote types, and among the fasciculate, rosulate and multiradiate types (e.g. Lucena & Sales, 2006; Sá-Haiad *et al.*, 2009; Senakun & Chantaranothai, 2010; Caruzo *et al.*, 2011). Webster *et al.* (1996) also introduced a subdivision in the lepidote trichome type based on the degree (%) of webbing among rays. However, these subtypes have not been widely adopted in taxonomic studies of *Croton*, except for the 'stellate-lepidote' (30–50% of webbing) subtype (Secco, 2005; Riina *et al.*, 2010, 2018; Kainulainen *et al.*, 2017; Rossine *et al.*, 2020; Pereira *et al.*, 2022).

Having a standardized terminology is essential in comparative biology, taxonomy, trait-based approaches in ecology and studies focusing on morphological evolution (Kljuykov *et al.*, 2004; Vogt *et al.*, 2010; Nogueira *et al.*, 2013). Because of the persistent confusion and lack of consistency in trichome identification and terminology in previous studies of *Croton*, we aimed to revise and update the trichome classification of Webster *et al.* (1996). The overall goal of our study is to establish a new, parameter-guided classification of *Croton* trichomes that could be universally adopted by systematists and by biologists in other fields. Specifically, our objectives are: (1) to review the classification of trichomes proposed by Webster *et al.* (1996) in the light of new anatomical and morphological data; (2) to propose an updated classification and more accurate terminology for *Croton* trichomes based on a comprehensive taxon sampling and (3) to apply this new classification to verify previous designation of trichomes in studies of *Croton*, including detailed images of these structures.

MATERIAL AND METHODS

Survey of trichomes and new data

First, we conducted a detailed revision of the Webster *et al.* (1996) paper on *Croton* trichomes. Second, we surveyed the taxonomic

and anatomical literature of *Croton* literature for informative images of trichomes [i.e. Scanning Electron Microscopy (SEM) images, light microscopy photographs, detailed line drawings]. Then we conducted new analyses of trichome morphology for 152 specimens corresponding to 120 *Croton* spp. to complement the information obtained from Webster *et al.* (1996) and the literature survey. Overall, our sampling covered 195 species in 27 *Croton* sections or clades (Supporting Information, Appendix S1). Clades and sections follow the most current phylogenetic framework of Van Ee *et al.* (2011) and recent updates (Riina *et al.*, 2018, 2021; Van Ee and Berry, 2021). There are a few groups in *Croton* not yet recognized formally as sections, for which we used informal clade names. Appendix S1 in the Supporting Information includes voucher information for all the specimens analysed here for the first time and for those revised from previous publications.

Diaphanization and Light Microscopy (LM)

We used entire leaves (for species with small leaves) or sampled fragments of the median region of leaves including portions of the central vein and leaf margin in the case of species with large leaves. Even though trichomes in *Croton* can occur all over the plant, from our assessment of the taxonomic literature the trichome types found on leaves are also present in other organs of the plant, including reproductive structures. Compared to reproductive organs, leaves are also easier to sample and manipulate in the anatomy laboratory, and they are more readily available in herbarium material or in the field.

Samples from preserved herbarium specimens were subjected to the reversion process (Smith and Smith, 1942) and stored in 70% ethanol. Subsequently they were diaphanized with 10% sodium hydroxide, washed with distilled water and submitted to an alcoholic series. The samples were then stained with 50% alcoholic fuchsin (Shobe and Lersten, 1967; Vasco *et al.*, 2014, modified) and mounted in glycerine (Kaiser, 1880) in the Microscopy Laboratory of the Museu Paraense Emílio Goeldi (MPEG) or in the Plant Anatomy Laboratory of the Universidade Federal de Viçosa (UFV).

Longitudinal and transverse sections 5mm thick were obtained with a fully automated rotary microtome (Leica RM226), stained with toluidine blue pH 4.7 (O'Brien and McCully, 1981) and mounted with synthetic resin (Permount; Fisher, Fair Lawn, NJ, USA) in the Plant Anatomy Laboratory at UFV. Longitudinal and transverse sections were used to gather data about the number and disposition of cells on selected species representing each of the trichome types we described (*C. allemii* G.L.Webster, *C. argyrophyllus* Kunth, *C. calonervosus* G.L.Webster, *C. cupreatus* Croizat, *C. fuscescens* Spreng., *C. gnaphalii* Baill., *C. huberi* Steyererm. and *C. vulnerarius* Baill.).

Images were captured using a Leica DM6 B light microscope (Wetzlar, Germany) coupled with a digital camera (Leica Application Suite v.4.12) in the Microscopy Laboratory at MPEG or with an Olympus AX70TRG (Tokyo, Japan), or a Zeiss Stemi 2000-C light microscope (Göttingen, Germany) equipped with a U-Photo digital camera (AxioCam HRC, Zeiss, Göttingen, Germany) in the Plant Anatomy Laboratory at UFV.

Scanning Electron Microscopy (SEM)

Leaf samples c. 3 mm² were extracted from preserved herbarium specimens, transferred to stubs and then metalized with gold

(Silveira, 1998). These samples were observed using a Philips XL 20 Series S/W v.5.21 (Amsterdam, The Netherlands) electronic microscope in the Núcleo de Pesquisa em Anatomia of the Instituto de Botânica (São Paulo, Brazil) or using a Zeiss DSM940 (Göttingen-Germany) electronic microscope in the Centro de Aquisição de Imagens e Microscopia of the Instituto de Biociências from Universidade de São Paulo (São Paulo, Brazil).

Parameters used for the classification of non-glandular trichomes

We defined a set of diagnostic parameters for the classification of *Croton* trichomes based on relevant literature on plant anatomy (Metcalfe and Chalk, 1957, 1979; Evert, 2006) and the examination of studies including trichome micromorphology of *Croton* spp. (Webster *et al.*, 1996; Gordillo and Matías, 2005; Sá-Haiad *et al.*, 2009; Secco, 2012; Barros and Soares, 2013; Caruzo and Cordeiro, 2013; Liu *et al.*, 2013; Sodré and Silva, 2015; Vitarelli *et al.*, 2015, 2016, 2021; Feio *et al.*, 2018a). Types and subtypes of non-glandular trichomes were proposed based on those parameters.

RESULTS

This study covered 341 trichome observations from 209 *Croton* spp. representing 27 sections or clades, including 31 observations belonging to the large Old World clade (*Croton* subgenus *Croton*) and five observations from two informal clades (Supporting Information, Appendix S1). Of the 31 sections of New World *Croton* established by Van Ee *et al.* (2011), only seven small sections are not represented in our sampling: *Argyranthemis* B.W.Van Ee (two species); *Crotonopsis* (one species); *Drepanadenium* (Raf.) Müll.Arg. (six species); *Moacroton* (Croizat) B.W.Van Ee and P.E.Berry (eight species); *Nubigeni* B.W.Van Ee (one species) and *Olivacei* P.E.Berry (one species). Out of the 341 trichome observations in our dataset, 152 were newly generated in this study and 188 were obtained from the revision of 30 publications, including Webster *et al.* (1996) (Supporting Information, Appendix S1).

Non-glandular trichomes were classified and identified using a set of parameters established here for the first time (Table 1). Glandular trichomes were represented in our dataset (Supporting Information, Appendix S1) by a single type (knob-like), and non-glandular trichomes included six main types: dendritic, multiradiate, lepidote, simple, pin-cushion and stellate. These basic types and their structural variations denoted by different attributes are listed in Table 2 with all the alternative denominations previously used in the revised literature (see also Supporting Information, Appendix S1).

Overall, the most common observation of trichome type is the stellate trichome type with 45% of the 341 observations, followed by the lepidote type with 28%. The least frequent types were the pin-cushion, glandular and dendritic trichome types (Fig. 1; Supporting Information, Appendix S1). Stellate and lepidote trichomes were also the most widespread among *Croton* clades: they were present in 20 and 19 of the sections, respectively, and the rest of trichome types were found in four to ten clades (Fig. 2; Table 3; Supporting Information, Appendix S1). Some of the most-species rich *Croton* clades or sections (*Croton*

Table 1. Set of parameters (characters and their states) used in this study for the classification and identification of non-glandular trichomes in the genus *Croton*

Character	Character states	Comments
Ray cells orientation	horizontal, ascending	Horizontal rays are perpendicular to the longitudinal axis of the trichome. Ascending rays are in an oblique angle.
Fusion of ray cells	free, partially fused, completely fused	
Number of levels of ray cells insertion	1, 2, > 2	
Central region	unicellular, multicellular (disc)	Stellate trichomes have a single central cell, which can also be elongated and thick, forming a porrect ray. A disc is a multicellular structure only present in lepidote trichomes.
Stipe	present, absent	The stipe is a multicellular structure that elevates the set of ray cells from the underneath surface. Trichomes can be stipitate or sessile.
Stipe shape	regular (filiform), cushion-shaped	A regular stipe is a cylindrical stalk with a diameter slightly bigger than that of a ray cell. A cushion-shaped stipe is an enlarged, usually globose structure, which gives the entire trichome a pin-cushion aspect.
Stipe length	short, long	Short: shorter than the longest ray/s; long: longer than the longest ray/s.

subgenus *Croton* and sections *Adenophylli* Griseb., *Geiseleira* and *Cyclostigma* Griseb.) showed the highest diversity of trichome types (Table 3).

Revised definitions of trichome types in croton

Based on the proposed parameters (Table 1) and our trichome survey (Supporting Information, Appendix S1, S2), we provide revised definitions for the trichome types previously described in *Croton* and new definitions to accommodate novel types identified in this study. Type 1 trichomes are glandular (Fig. 3A–C) and types 2–7 are non-glandular (Fig. 3D–U).

Type 1: knob-like glandular trichomes

All glandular trichomes examined so far in *Croton* are formed by a single cell with a globular distal portion (Fig. 3A, B) oriented outwards and a cylindrical basal region, narrower than the distal portion and inserted in the epidermis (Fig. 3C). We propose the inclusion of the descriptor knob-like for a more precise description of this structure. Only five clades in our sampling had knob-like glandular trichomes [*Croton* sections *Geiseleria* A.Gray, *Barhamia* (Klotzsch) Baill., *Lamprocroton* (Müll. Arg.) Pax, *Lasiogyne* (Klotzsch) Baill. and subgenus *Croton*; Supporting Information, Appendix S1].

Type 2: simple trichomes

These can be uni- or multicellular (Fig. 3A, C), uniseriate, unbranched, with a filiform shape, generally erect and with thick cell walls (Fig. 3F). Eleven of the 27 clades examined presented simple trichomes (Supporting Information, Appendix S1).

Type 3: stellate trichomes

These are multicellular and their main body is constituted by radially oriented cells or rays (Fig. 3G). Rays are free, originating from the central region (Figs 3H, 4A), and can be on a single plane parallel to the epidermis or ascending (oblique) (Figs 3H, 4A). Stellate trichomes may be sessile (Fig. 4B; Feio et al., 2018a, b, their fig. 1k) or bear a short/long stipe (Figs 3I, 4C).

They present a single (rarely several) central erect cell (porrect ray) that is usually longer and with thicker cell walls than the other rays (Figs 3G, 4D). Twenty-one of the 27 clades sampled present stellate trichomes (Fig. 2; Supporting Information, Appendix S1).

Type 4: pin-cushion trichomes

These are multicellular structures with free rays that are ascending and coming out from cushion-like stipe (Fig. 3J, K); a central porrect ray is usually present (Figs 3J, L, 4E). Associated secretory cells (idioblasts), similar to the ones found on the leaf blade, may occur on the cell wall of the stipe. This trichome type was recorded in seven species belonging to six different lineages (*Croton* sections *Adenophylli*, *Geiseleria* and *Cyclostigma* and the pulegioidorus and unknown clades: Supporting Information, Appendix S1).

Type 5: multiradiate trichomes

These have free rays that are inserted at more than one point on the main trichome axis, but without spacing among their insertion points (Figs 3M–O, 4F, G). They may be sessile (Fig. 4A; Rosa et al., 2021, their fig. 2a, b) or stipitate (Figs 3M–O, 4G). A central, vertical ray cell (porrect ray) may be present (Fig. 4G). Multiradiate trichomes were present only in eight of the *Croton* clades sampled (Fig. 2; Supporting Information, Appendix S1).

Type 6: dendritic trichomes

These are multicellular; ray cells are free and inserted along a pluristratified central axis at different points, spaced and with different angles (Fig. 3P–R). These trichomes may present an erect central cell (porrect ray) (Figs 3R, 4H), which is a novelty found in this study. Dendritic trichomes seem to be uncommon in the genus, being found so far only in six clades [*Croton* subgenus *Croton* (Old World clade) and sections *Adenophylli*, *Barhamia*, *Cleodora* (Klotzsch) Baill., *Cyclostigma* and *Sampatik* (G.L.Webster) Riina (Fig. 2; Supporting Information, Appendix S1)].

Table 2. Standardized denominations of trichomes occurring in the genus *Croton* and their correspondences in the published taxonomic and anatomic studies surveyed here (see [Supporting Information, Appendix S1](#)). In the first column, the first term denotes the type of trichome (e.g. lepidote) as proposed in this work; additional attributes (e.g. stipitate, porrect) follow, separated by commas.

Trichome type, attributes (this study)	Denominations in previous studies (references in Appendix S1)
Glandular	Glandular • Secretory trichome
Dendritic	Dendritic
Dendritic, porrect	Multiradiate
Dendritic, stipitate, porrect	Dendritic stipitate
Lepidote	Lepidote
Lepidote	Dentate-lepidote • Lepidote • Lepidote dentate • Lepidote subentire • Stellate-lepidote • Typical lepidote
Lepidote, porrect	Lepidote porrect
Sublepidote	Appressed stellate • Lepidote dentate • Lepidote stellate • Lepidote subentire • Pseudolepidote rosulate (pin-cushion) • Stellate • Stellate-lepidote • Stellate rotate • Transitional stellate-lepidote • Transitional from appressed stellate to rosulate and multiradiate
Sublepidote, porrect	Appressed-stellate porrect • Dendritic • Dendritic porrect • Multiradiate • Multiradiate porrect • Stellate-lepidote • Stellate-lepidote porrect • Stellate porrect • Stellate rotate • Transitions from stellate-lepidote to lepidote
Sublepidote, stipitate	Stellate rotate with stipe
Multiradiate	Multiradiate • Rosulate
Multiradiate, sessile	Appressed rosulate • Non-glandular, pluricellular • Rosulate • Stellate • Stellate porrect • Transitional from appressed stellate to rosulate
Multiradiate, stipitate	Multiradiate • Rosulate • Transitional from multiradiate porrect to dendritic
Multiradiate, stipitate, porrect	Multiradiate
Pin-cushion	Fasciculate • Fasciculate cushion-shaped • Multiradiate-cushion • Porrect multiradiate • Stellate porrect on pin-cushion
Simple	Simple • Single-celled trichome • Simple confused with colleter
Stellate	Dendritic • Stellate
Stellate, porrect	Fasciculate • Multiradiate • Simple • Stellate • Stellate porrect • Stellate rotate • Stipitate-stellate porrect • Transitional from simple to 2–3-radiate porrect • 2–6-radiate trichomes • 2–7-radiate trichomes
Stellate, sessile	Multiradiate or stellate • Stellate multiradiate • Stellate rotate
Stellate, sessile, porrect	Appressed-stellate porrect • Appressed stellate • Appressed-stellate porrect • Appressed stellate with porrect radius • Multiradiate or stellate • Stellate porrect • Stellate rotate porrect • Stellate porrect, dendritic
Stellate, stipitate	Fasciculate • Fasciculate with stipe • Long stipitate trichome • Rosulate • Transitional types from multiradiate porrect to dendritic
Stellate, stipitate, porrect	Appressed-stellate porrect • Dendritic • Fasciculate • Multiradiate • Multiradiate porrect • Porrect, stipitate, appressed stellate • Rosulate with pedestal • Stellate • Stellate porrect • Stellate rotate, porrect • Stellate rotate • Stipitate multiradiate • Stipitate-stellate • Stipitate-stellate porrect • 2–4-radiate

Type 7: lepidote trichomes

These are shield- or scale-like structures that cover the epidermis ([Fig. 3S–U](#)). They consist of two main regions: the central region that can be multicellular (disc) or unicellular; and the ray region, formed by fused lateral ray cells. The central region has a thick wall that usually bears punctuations ([Figs 3S, 4L–K](#)); sometimes this trichome can have one or even three blunt rays or one to two porrect rays ([Fig. 4J](#); [Webster et al., 1996](#), their [fig. 14](#)). There can be different degrees of fusion among the anticlinal cell walls of the lateral rays. Punctuations are also common on the cell walls of lateral rays, especially on the anticlinal ones.

In lepidote trichomes with high level of fusion among lateral rays (i.e. forming an entire shield), sometimes there may be a third region: the subradial disc ([Vitarelli et al., 2016](#), their [fig.](#)

[5j](#)). The subradial disc is located underneath the shield and is constituted by radially elongated cells that surround the central axis of the trichome. These cells have a conspicuously sinuose margin, thick cell walls and a relatively high number of punctuations.

We subdivided lepidote trichomes, regarding the lateral fusion of rays, into two subtypes: (1) lepidote trichomes (i.e. entirely lepidote), in which the cell rays are fused their entire length ([Figs 3U, 4I, J](#)) and (2) sublepidote trichomes (i.e. partially lepidote) ([Figs 3S, 4K, L](#)) in which cell rays are partially fused (any level fusion except entirely fused). In both subtypes, the shield- or scale-like structure can be sessile or supported by a short, rarely long, stipe. Both subtypes may have one (rarely more than one) porrect ray ([Fig. 4J](#)).

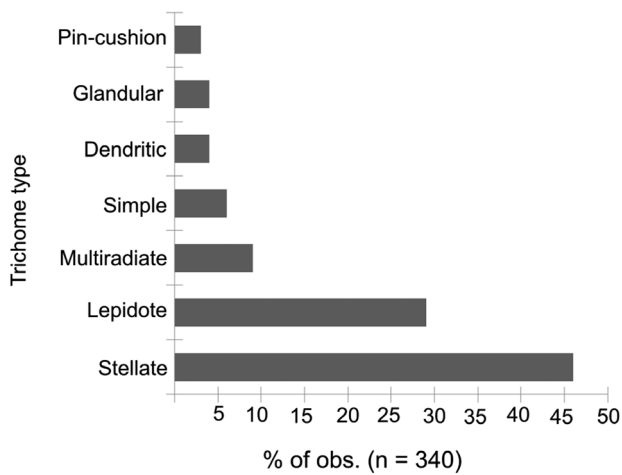


Figure 1. Percentage of observations per trichome type in our dataset of trichomes of *Croton* (see [Supporting Information, Appendix S1](#)). Note that the lepidote and sublepidote subtypes are included under the lepidote type.

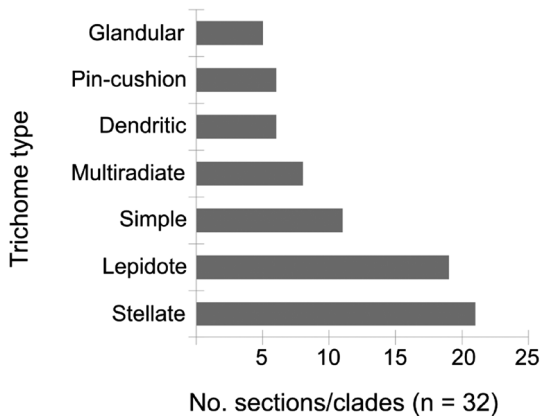


Figure 2. Trichome types and number of *Croton* sections or clades where each type was recorded in our dataset (see [Supporting Information, Appendix S1](#)). Note that in this graph the lepidote and sublepidote subtypes are included under the lepidote type.

Lepidote trichomes were relatively widespread across our sampled clades (19 out of 27; [Fig. 2](#)). They were most common in *Croton* sections *Barhamia*, *Cleodora*, *Cupreati* (G.L.Webster) Riina and P.E.Berry, *Eluteria* Griseb. and *Lasiogyne* ([Supporting Information, Appendix S1](#)).

DISCUSSION

The high diversity of trichomes present in *Croton* revealed by our comprehensive survey confirms and expands the findings of previous accounts ([Froembling, 1896](#); [Webster *et al.*, 1996](#); [Lucena and Sales, 2006](#); [Sá-Haiad *et al.*, 2009](#); [Senakun and Chantaranothai, 2010](#); [Liu *et al.*, 2013](#); [Feio *et al.*, 2018a](#); [Rosa *et al.*, 2021](#)). In addition, we clarify conflicting denominations and provide precise parameters to facilitate the interpretation of trichome typologies in the genus. This set of parameters can be also applied, with the necessary adjustments or expansion, in the classification and designation of trichomes in other plant families.

Trichome types present on the leaves of *Croton* species are representative of the morphotypes occurring in other organs of the plant, including those found on reproductive structures such as flowers and fruits ([Caruzo *et al.*, 2013](#); [Barbosa *et al.*, 2018](#); [Sodré *et al.*, 2019b](#); [Thaowetsuwan *et al.*, 2020](#)). In fact, both [Froembling \(1896\)](#) and [Webster *et al.*, \(1996\)](#) conducted their trichome surveys of the genus focusing mainly on leaves as we did here.

There is only one type of glandular trichome in *Croton*, and although [Webster *et al.* \(1996\)](#) already reported this type for species in *Croton* sections *Adenophylli* and *Barhamia*, they did not include specific parameters to describe and identify it. Glandular trichomes have been often confused in the taxonomic literature on *Croton* with other secretory structures of similar shape. According to [Vitarelli *et al.* \(2015\)](#), taxonomists, including [Webster *et al.* \(1996\)](#), may have confused glandular trichomes with colleters. See, [Vitarelli *et al.* \(2015\)](#) for a detailed discussion of Webster's report of the presence of glandular trichomes in section *Adenophylli* (as section *Adenophyllus*). These putative glandular trichomes are referred as 'small inconspicuous (0.1 mm or less) spherical marginal [leaf margin] glands' in [Webster *et al.* \(1996\)](#), but [Vitarelli *et al.* \(2015\)](#) argued that they are most likely colleters based on their assessment of these structures across *Croton* and tribe Crotonaeae. A more recent example illustrating this confusion is a report of glandular trichomes on the pedicels of *C. hirtus* L'Hér. ([Nichodemus and Ekeke, 2020](#), their fig. 7h, i). Although we found glandular trichomes in members of section *Geiseleria* (in which *C. hirtus* belongs), the structures illustrated by [Nichodemus and Ekeke \(2020\)](#) are probably colleters, as have been described for the inflorescence of *C. glandulosus* L., a close relative of *C. hirtus* ([Machado *et al.*, 2015](#)). We define the glandular trichome in *Croton* as a unicellular secretory structure, which so far shows only one morphology (knob-like shape). In addition, glandular trichomes are usually smaller than colleters, which can be spotted at simple sight or with a 10 × magnification lens ([Barros and Soares, 2013](#); [Vitarelli *et al.*, 2015, 2021](#); [Feio *et al.*, 2016](#); [Gancedo *et al.*, 2022](#)).

In addition to the glandular trichomes, [Webster *et al.* \(1996\)](#) also included 'papillate trichomes' as one of their seven trichome typologies. However, they did not provide data or images for these structures, so we could not assess their nature. From the brief description provided by [Webster *et al.* \(1996\)](#), it is possible that they were referring to idioblasts, which seem to be widespread in *Croton* ([Sá-Haiad *et al.*, 2009](#); [Vitarelli *et al.*, 2015, 2021](#); [Feio *et al.*, 2016, 2018a](#)). [Rosa *et al.* \(2021\)](#) reported the presence of a papillate epidermis in *C. gracilipes* Baill., but [Webster *et al.* \(1996\)](#) were not referring to structures that covered the entire leaf surface but rather to dispersed (not hair-like) papillae on the leaf surface usually interspaced with trichomes, epidermal cells and stomata. The latter is a common pattern of the distribution of idioblasts ([Vitarelli *et al.*, 2015, 2021](#); [Feio *et al.*, 2016, 2018a](#)).

[Webster *et al.* \(1996\)](#) regarded the simple trichome in *Croton* as an intermediate form in an evolutionary context, resulting from a reduction from stellate or multiradiate trichomes, in which the lateral rays are strongly suppressed. Similarly, [Froembling \(1896\)](#) interpreted simple trichomes as a reduction from the stellate or fasciculate types. Our analysis indicates that some stellate trichomes may in fact have a reduced number of rays, but this is probably due to the process of cellular differentiation, corresponding

Table 3. Trichome types identified in surveyed species of *Croton* (see [Supporting Information, Appendix S1](#)) arranged by section or clade. Note this is not an exhaustive inventory so additional trichome types may occur in a given section clade

Section/clade	Trichome types
<i>C. sect. Adenophylli</i>	Dendritic • Dendritic, porrect • Glandular • Multiradiate • Multiradiate, sessile • Multiradiate, stipitate • Multiradiate, stipitate, porrect • Pin-cushion • Simple • Stellate, sessile • Stellate, stipitate • Stellate, stipitate, porrect • Sublepidote • Sublepidote, porrect
<i>C. sect. Alabamenses</i>	Lepidote • Simple • Stellate • Sublepidote
<i>C. sect. Barhamia</i>	Dendritic, porrect • Dendritic, stipitate, porrect • Lepidote • Simple • Stellate, porrect • Stellate, sessile • Stellate, stipitate • Stellate, stipitate, porrect • Sublepidote
<i>C. sect. Cleodora</i>	Dendritic • Lepidote • Lepidote, porrect • Multiradiate • Simple • Stellate • Stellate, sessile • Stellate, stipitate, porrect • Sublepidote • Sublepidote, porrect
<i>C. sect. Cordifolii</i>	Simple • Stellate
<i>C. sect. Corinthii</i>	Stellate
<i>C. sect. Corylocroton</i>	Multiradiate, stipitate • Stellate, stipitate
<i>C. sect. Cuneati</i>	Lepidote • Sublepidote
<i>C. sect. Cupreati</i>	Lepidote
<i>C. sect. Cyclostigma</i>	Dendritic • Lepidote • Multiradiate, sessile • Multiradiate, stipitate • Pin-cushion • Simple • Stellate, sessile • Stellate, sessile, porrect • Stellate, stipitate • Stellate, stipitate, porrect • Sublepidote • Sublepidote, porrect
<i>C. sect. Eluteria</i>	Lepidote • Lepidote, porrect • Multiradiate, sessile • Simple • Stellate, stipitate, porrect • Sublepidote
<i>C. sect. Eutropia</i>	Lepidote • Sublepidote
<i>C. sect. Geiseleria</i>	Glandular • Lepidote • Multiradiate • Multiradiate, sessile • Multiradiate, stipitate • Pin-cushion • Simple • Stellate, porrect • Stellate, porrect (>1 porrect rays) • Stellate, sessile • Stellate, sessile, porrect • Stellate, stipitate • Stellate, stipitate, porrect • Sublepidote • Sublepidote, porrect
<i>C. sect. Heptallon</i>	Stellate, porrect • Stellate, stipitate
<i>C. sect. Julocroton</i>	Multiradiate • Simple • Stellate, stipitate • Stellate, stipitate, porrect • Sublepidote, porrect
<i>C. sect. Lamprocroton</i>	Glandular • Lepidote • Simple • Stellate, sessile • Stellate, stipitate, porrect • Sublepidote
<i>C. sect. Lasiogyne</i>	Glandular • Lepidote • Stellate • Stellate, stipitate • Stellate, stipitate, porrect • Sublepidote • Sublepidote, porrect
<i>C. sect. Luetzelburgiorum</i>	Stellate, stipitate
<i>C. sect. Luntia</i>	Lepidote • Lepidote, stipitate • Pin-cushion • Stellate, stipitate • Sublepidote, porrect • Sublepidote, stipitate
<i>C. sect. Pachypodi</i>	Lepidote
<i>C. sect. Pedicellati</i>	Stellate, porrect
<i>C. sect. Prisci</i>	Stellate, stipitate • Sublepidote
Clade <i>C. pulegioidorus</i>	Pin-cushion • Stellate, stipitate
<i>C. sect. Quadrilobi</i>	Stellate, sessile
<i>C. sect. Sampatik</i>	Dendritic • Sublepidote
<i>C. subg. Croton</i> (Old World)	Dendritic • Glandular • Lepidote • Lepidote • Multiradiate • Multiradiate, sessile • Multiradiate, stipitate • Simple • Stellate • Stellate, sessile • Stellate, sessile, porrect • Stellate, stipitate • Stellate, stipitate, porrect • Sublepidote • Sublepidote, porrect
Unknown (<i>C. scutatus</i>)	Lepidote • Pin-cushion • Sublepidote

to developmental stages and not to an evolutionary process of reduction giving place to a different type of trichome. Caducous lateral rays can also give the false impression of a simple trichome, particularly when these rays are much shorter than the central porrect one (see [Sodré et al., 2019a](#), their fig. 5e–g). Simple trichomes are present not only in *Croton*, but also in other genera of Crotonaeae, including *Acidocroton* Griseb., *Astraea* Klotzsch, *Sagotia* Baill. and *Sandwithia* Lanj. ([Berry et al., 2005](#); [Webster, 2014](#); [Secco et al., 2019](#); [Silva and Cordeiro, 2020](#)).

The stellate trichome is the most common type among the surveyed and reviewed species. From our analysis of the literature, previous descriptions of the stellate trichome indicating the

presence of ‘adpressed rays’ were the most problematic ([Van Ee and Berry, 2009](#); [Silva et al., 2010](#); [Santos et al., 2017](#); [Barbosa et al., 2018](#); [Sodré et al., 2022](#)). Stellate trichomes have been often confused with the lepidote type, even in anatomical studies ([Gordillo and Matías, 2005](#); [Lima and Pirani, 2008](#); [Silva et al., 2010](#); [Carneiro-Torres et al., 2011](#); [Sodré et al., 2019b](#)). This confusion is mainly due to the fact that the ‘stellate-adpressed trichome’ was treated by [Webster et al. \(1996\)](#) as an intermediate type between stellate and lepidote, with 0–30% lateral fusion of rays. Both [Froembling \(1896\)](#) and [Webster et al. \(1996\)](#) suggested the existence of numerous transition forms between the stellate and lepidote trichome types. The intervals of degree (%) of ray fusion

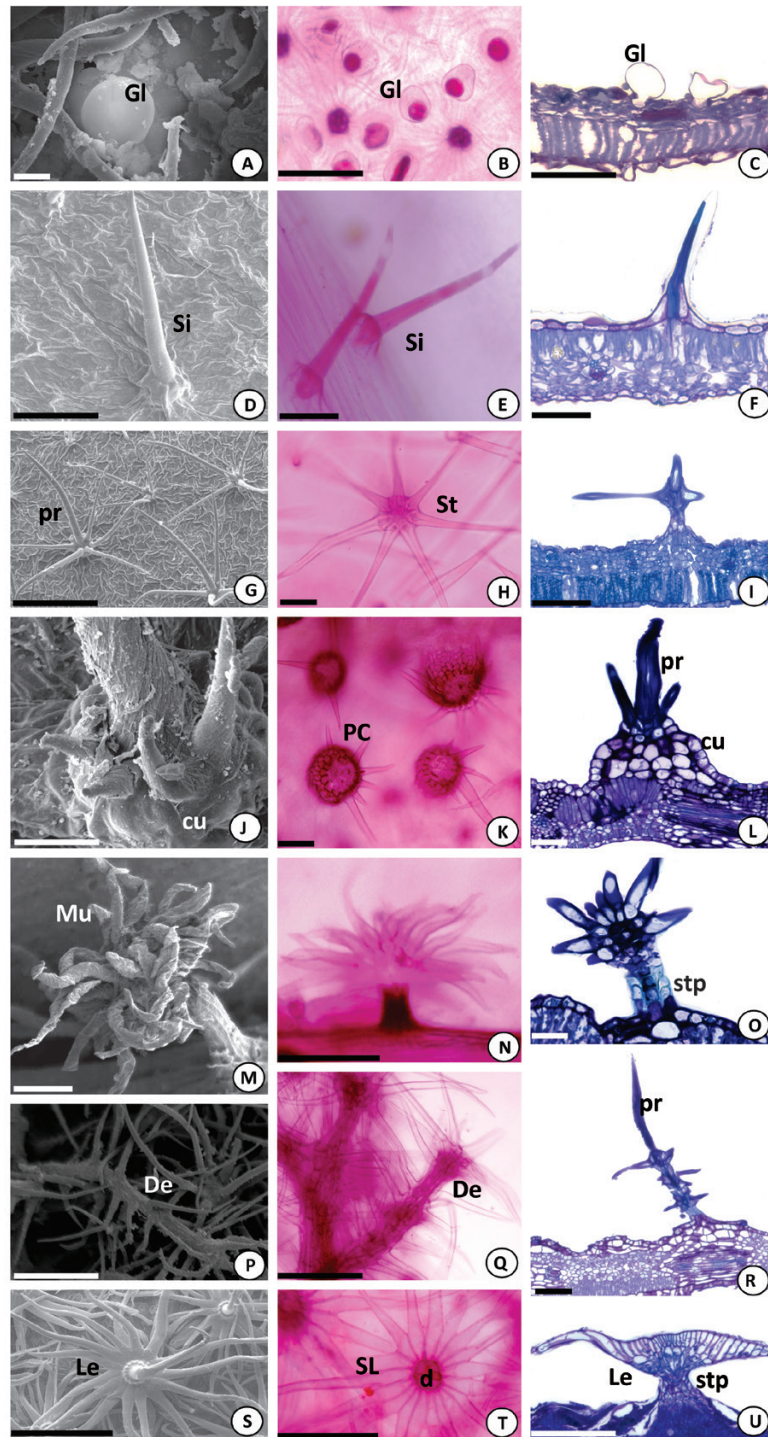


Figure 3. Types of leaf trichomes in representative *Croton* species newly analysed in this study using scanning electronic microscopy (SEM), diaphanization and anatomical sections. A, Glandular trichome, *C. gnaphalii* (Marchett 545, BHCB). B, Glandular trichome, *C. myrianthus* (Lima 296, SP). C, Glandular trichome, *C. sacaquinha* (Lima 296, SP). D, Simple trichome, *C. organensis* (Caruzo 72, SP). E, Simple trichome, *C. churutensis* (Cornejo 7590, WIS). F, Simple trichome, *C. allemii* (Gurtler, 75, MBML). G, Stellate trichome, *C. fragrans* (Caruzo 126, SP). H, Stellate trichome, *C. cerinodentatus* (Medeiros 372, R). I, Stellate trichome, *C. calonevovosus* (De Paula 229, BHCB). J, Pin-cushion trichome, *C. arenosus* (Miranda 613, HUEFS). K, Pin-cushion trichome, *C. huberi* (Riina 1276, WIS). L, Pin-cushion trichome, *C. huberi* (Riina 1276, WIS). M, Multiradiate trichome, *C. hibiscifolius* (Riina 1413, WIS). N, Multiradiate trichome, *C. floccosus* (Riina 1407, WIS). O, Multiradiate trichome, *C. huberi* (Riina 1276, WIS). P, Dendritic trichome, *C. cajucara* (Caruzo 98, SP). Q, Dendritic trichome, *C. vulnerarius* (Cordeiro 345, SP). R, Dendritic trichome, *C. huberi* (Riina 1276, WIS). S, Sublepidote trichome, *C. spruceanus* (Caruzo 101, SP). T, Lepidote trichome, *C. fenandezii* (Santos 695, MG). U, Lepidote trichome in profile view, *C. cupreatus* (Riina 1408, WIS). Scale bars: 30 μm (A, H, K, L, O, R), 50 μm (B, C, E, F, I, M), 100 μm (D, G, J, P, Q, S, U), 120 μm (N, T). Gl: glandular trichome; Si: simple trichome; PC: pin-cushion trichome; Mu: multiradiate trichome; De: dendritic trichome; Le: lepidote trichome; SL: sublepidote trichome; pr: porrect ray; d: central disc; stp: stipe; cu: cushion.

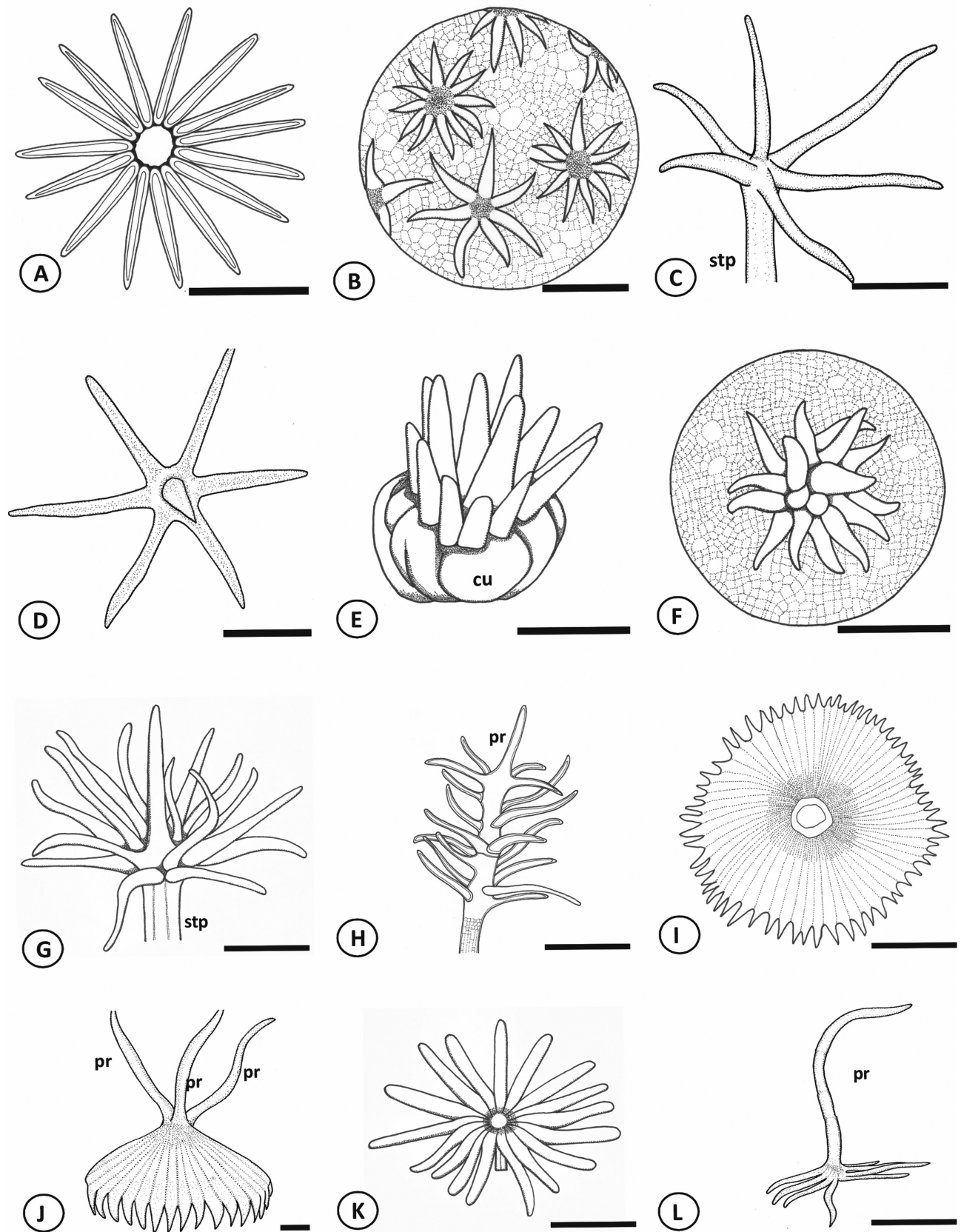


Figure 4. Schematic illustrations depicting the trichome types observed in *Croton* according to our new classification. A, Stellate trichome, upper view. B, Stellate trichome, sessile. C, Stellate trichome, stipitate. D, Stellate trichome, porrect. E, Pin-cushion trichome, porrect. F, Multiradiate trichome, sessile. G, Multiradiate trichome, stipitate. H, Dendritic trichome, porrect. I, Lepidote trichome. J, Lepidote trichome, porrect. K, Sublepidote trichome. L, Sublepidote trichome, porrect. Scale bars: 20 μm (J), 50 μm (B, D), 100 μm (A, C, E, F, G, H, I, K, L). Labels: cu: cushion, pr: porrect ray, stp: stipe.

established by Webster *et al.* (1996) are probably the cause of misconceptions in later taxonomic publications on *Croton* including 'stellate-addressed trichomes' or similar designations (see next). In our classification scheme, any level of lateral fusion among the rays (> 0%) is enough to designate a trichome as lepidote. What Webster *et al.* (1996) and other authors described as 'stellate-addressed', 'stellate-lepidote' or 'lepidote stellate' correspond to the 'subentire lepidote trichome' in our classification. Following our parameters, subentire lepidote trichomes have been misclassified several times in the past (Supporting Information, Appendix S1; Webster *et al.*, 1996; Van Ee and Berry, 2009; Sá-Haiad *et al.*, 2009; Senakun and Chantaranonthai, 2010; Valduga *et al.*, 2021; Sodré *et al.*, 2022). For this reason, our classification avoids the use of the degree (%) of lateral fusion of rays as a parameter to identify trichomes in *Croton*, since it seems impractical [it has never been fully adopted after Webster *et al.* (1996) by taxonomists], and the few uses so far have just created wrong anatomical interpretations (e.g. 'stellate-addressed trichome', 'stellate-lepidote trichome'). The five subtypes of lepidote trichomes based on different levels (0–15, 15–30, 30–50, 50–80, 80–100%) of fusion among rays proposed by Webster *et al.* (1996) are replaced here by only two subtypes characterized as having partially (sublepidote trichome) or completely fused rays (lepidote trichome). One way to reduce uncertainty in the confusion between lepidote and stellate is to use at least one of the three anatomical approaches used in this work (light microscopy, diaphanization or SEM), in addition to the macro-morphological examination under a stereoscope.

The level of insertion of rays and the presence/absence of a multicellular disc in lepidote trichomes have not been considered in previous works as parameters that could be used in conjunction with other characters to classify and identify trichomes in *Croton*.

Webster *et al.* (1996) distinguished 'fasciculate trichomes' from 'stellate trichomes' because of the presence, in the former, of up to eight rays disposed in ascending angles. The number of rays appears to be a variable character within species (phenotypic plasticity), so we consider it poorly informative for taxonomic purposes. In fact, there are many incongruencies in previous studies using this character, mainly because of the overlapping intervals in number of rays between species (Lucena and Sales, 2006; Sá-Haiad *et al.*, 2009; Senakun and Chantaranonthai, 2010; Carneiro-Torres *et al.*, 2011; Liu *et al.*, 2013; Rosa *et al.*, 2021). Regarding the 'ascending rays', we include them as one of the states of the parameter 'orientation of rays' for the identification of stellate trichomes. Therefore, to avoid further confusion, we no longer use the denomination 'fasciculate trichome' as used in previous works (Lucena and Sales, 2006; Van Ee and Berry, 2009; Senakun and Chantaranonthai, 2010; Soares, 2013, unpublished work; Feio *et al.*, 2018a).

Feio *et al.* (2018a) described a 'fasciculate cushion-shaped trichome', whereas Webster *et al.* (1996) designated the same morphology as 'multiradiate-cushion trichome'. Here, both terms are synonymized under the 'pin-cushion trichome' type, which is treated as distinct from the stellate trichome type. This decision is supported by the consistent occurrence of pin-cushion trichomes in all the analysed specimens of the taxa in which they were present, and, more importantly, by its strong morphological divergence from the rest of trichome types in

Croton (Berry and Gaskin, 1998; Sá-Haiad *et al.*, 2009; Feio *et al.*, 2018a; Rosa *et al.*, 2021).

Previous designations of 'rosulate trichomes' are consistent among studies, but these trichomes have also been confused with our 'multiradiate trichome' type (Sá-Haiad *et al.*, 2009; Senakun and Chantaranonthai, 2010; Liu *et al.*, 2013; Feio *et al.*, 2018a). Webster *et al.* (1996) stated that 'rosulate trichomes' could be a subtype of the multiradiate type, from which they were only distinguished by their shorter rays. We could not identify unambiguous parameters distinguishing between multiradiate and rosulate trichomes. Therefore, we synonymize the 'rosulate type' under the 'multiradiate type'.

The identification of dendritic trichomes was the less problematic in the revised literature (Supporting Information, Appendix S1; Webster *et al.*, 1996; Liu *et al.*, 2013; Feio *et al.*, 2018a; Sodré and Silva, 2020). Nevertheless, we provide here specific diagnostic parameters for their identification in future works and a revised description of this structurally complex trichome type.

CONCLUSION

The new classification of *Croton* trichomes presented here with the proposed set of diagnostic parameters will allow the identification and use of this character more accurately and consistently across the genus and in different research contexts. Particularly, this update in trichome typology and terminology will be useful in systematic studies since taxonomic revisionary work is currently ongoing or pending in many lineages of *Croton* and in the description of new taxa. The main improvements from previous works on trichome classification in *Croton* are: (1) the recognition of the simple trichome as a distinct type and not as a transitional form between the stellate and lepidote types; (2) the exclusion of subtypes within the stellate and multiradiate trichomes types; (3) the synonymization of fasciculate trichomes with stellate trichomes; (4) the synonymization of the rosulate trichomes under multiradiate trichomes; (5) the new denomination 'pin-cushion trichome' that replaces the formerly 'fasciculate cushion trichome' type and (6) the reduction of the former five subtypes of the lepidote trichomes to just two subtypes: lepidote and sublepidote trichomes. The standardization of trichome terminology and classification is aimed at diminishing confusion and miscommunication in future taxonomic studies in the genus. It will also provide a stronger basis for the study of the evolutionary changes and the ecological relevance of these functional traits over the history of *Croton* and its lineages.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article on the publisher's website.

Appendix S1. Dataset of trichome observations ($n = 341$ obs.) from 209 *Croton* spp., including data newly generated in this work ($n = 152$ observations) and from a review of 30 publications ($n = 188$ observations).

Appendix S2. A selection of images of trichomes from species in different sections of *Croton*. These images complement those shown in the main text (Figs. 3, 4) and previously published images mentioned in Appendix S1. Images are ordered alphabetically by section and species name.

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DATA AVAILABILITY

The data underlying this study are available in the article and in its two online files included as supplementary material.

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