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FLOWER EVOLUTION IN SPECIES OF *CROTON* L. (EUPHORBIACEAE): ONTOGENY AND GLOBAL PROFILE OF GENE EXPRESSION

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Flower evolution in species of *Croton* L. (Euphorbiaceae): ontogeny and global profile of gene expression

Evolução floral em espécies de *Croton* L. (Euphorbiaceae): ontogênese e perfil global da expressão gênica

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Orientação: Prof. Dr. Diego Demarco

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Prof. Dr. Diego Demarco

"A garden to walk in and immensity to dream in - what more could he ask? A few flowers at his feet and above him the stars."

— **Victor Hugo, Les Misérables**

Dedico

As pessoas que mais amo: meus pais, Deise e Luiz,

e meu esposo e companheiro,

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ABSTRACT

The Euphorbiaceae are notable for floral and inflorescence diversity and evolutionary complexity. *Croton* is the second largest genus in the family and exhibits particular diversity in its flowers, especially regarding perianth and number of stamens, besides the inflorescences, which are also very diverse. Considering *Croton's* great variability in the reproductive structures, the aim of this thesis was to study flowers and inflorescences with an evolutionary approach, including morphology, ontogeny, vasculature, auxin regulation and genetic expression. Flowers in several stages of development were analyzed using light microscopy and scanning electron microscopy. Inflorescences were analyzed in stereomicroscope and the traits were plotted on the most recent phylogeny of the genus. The genetic expression was tested using RNAseq. In the first chapter the flowers showed similarity in the initiation of sepals and the presence of filamentous, petaloid structures in *Croton lundianus* (Didr.) Müll. Arg., interpreted here as staminodes. In *Croton sphaerogynus* Baill., staminodes were described for the first time. The staminodes reported here could be interpreted as transitional structures that we considered as evolutionary reductions. In the second chapter, the staminate flowers showed polystemonous androecium and the delay in petals' initiation and the antesepalous nectaries development interfered in the development of the stamens, characterizing obdiplostemony. Vasculature corroborated obdiplostemony and revealed a central stamen in *C. fuscescens* with carpelar features, interpreted here as a homeosis case. Glandular staminodes were registered and interpreted as a heterotopy case. The obdiplostemony may be related to modulation of the free IAA concentrations during floral developmental steps and *Croton* flowers can be used as good models for obdiplostemony, homeosis and heterotopy. In the third and fourth chapter we studied *Croton* inflorescences, which showed 17 patterns with differences on the organization and distribution of pistillate flowers. The inflorescence traits analyzed were very homoplastic, most likely determined by convergent evolution in distantly related lineages distributed in similar habitats. The genetic expression of *C. fuscescens* was particularly analyzed and the transcriptome showed that the different zones have their development guided through the same transcripts set. Each zone has different expression level and these variations and gradient could be interpreted as the boundary between each inflorescence zone. The floral developmental novelties and evolutionary links identified here raise the importance of future floral studies with the genus, what would bring a better understanding on how the reproductive structures evolved in the history of the group.

Key words: auxin, flower development, nectary, reproductive structure, RNAseq, staminodes, vasculature

RESUMO

Euphorbiaceae é uma família que recebe destaque quanto à diversidade de flores e inflorescências, além de sua complexidade evolutiva. *Croton* L. é o segundo maior gênero da família e exibe particular diversidade floral, em especial quanto a o perianto e número de estames, além das inflorescências, que também se apresentam muito diversas. Considerando a grande variação nas estruturas reprodutivas de *Croton*, o objetivo desta tese foi estudar as flores e inflorescências com abordagem evolutiva, incluindo morfologia, ontogênese, vascularização, regulação hormonal e expressão gênica. Flores em diversos estágios de desenvolvimento foram analisadas em microscopia der luz e varredura. Inflorescências foram estudadas em estereomicroscópio e os caracteres observados foram analisados nas filogenias mais recentes do grupo. A expressão gênica foi analisada com a técnica RNAseq. No primeiro capítulo as flores apresentaram semelhanças na iniciação das sépalas e presença de filamentos, estruturas petaloides em *Croton lundianus* (Didr.) Müll. Arg., interpretadas como estaminódios. Em *Croton sphaerogynus* Baill., estaminódios foram descritos pela primeira vez. Estas estruturas podem ser interpretadas como estruturas de transição evolutiva e reduções florais. No segundo capítulo as flores estaminadas apresentaram androceu polistêmone e o retardo na iniciação das pétalas e o desenvolvimento antessépalo dos nectários foram considerados como fatores chave para o desenvolvimento do androceu como obdiplostêmone. A vascularização corroborou a obdiplostemonia e revelou um estame central com características carpelares em *C. fuscescens*, interpretado aqui como um caso de homeose. Nectários glandulares foram registrados e interpretados como uma mudança heterotópica. A obdiplostemonia pode estar relacionada com as diferentes concentrações de auxina ao longo das etapas de desenvolvimento e as flores de *Croton* podem ser consideradas como bons modelos de obdiplostemonia, homeose e heterotopia. No terceiro e quarto capítulo nós investigamos as inflorescências de *Croton*, que apresentaram 17 padrões com diferenças na organização e distribuição das flores pistiladas especialmente. Os caracteres das inflorescências se mostraram homoplásticos e provavelmente determinados por evolução convergente em linhagens distantes distribuídas em habitats semelhantes. A expressão gênica de *C. fuscescens* foi particularmente analisada e o transcriptoma demonstrou que o desenvolvimento das diferentes zonas é regulado pelo mesmo conjunto gênico. Cada zona, pistilada ou estaminada, apresenta níveis distintos de expressão diferencial e o gradiente na expressão pode ser o delimitador entre as zonas. Os novos relatos quanto ao desenvolvimento floral em *Croton* e os links evolutivos identificados nesta tese levanta a importância de estudos para uma melhor compreensão sobre a evolução das estruturas reprodutivas neste grupo tão importante.

Palavras chave: auxina, desenvolvimento floral, estaminódios, estruturas reprodutivas, nectário, RNAseq, vascularização

CONTENTS

General Introduction

1. Euphorbiaceae: what do we know about this family?

In the mid XVIII century, the botanist and zoologist Carolus Linnaeus observed for the first time the Tricoccae family, which had a few genera within the order Naturales (Bruyset 1787) and was furtherly named as Euphorbiaceae. The traditional Euphorbiaceae *sensu lato* was originally described by Antonii Laurentii De Jussieu in *Genera Plantarum,* 1789, in which the family was composed of about 30 genera divided in two big groups, one with flowers of an entire style and another with bifid style, both with unisexual flowers and placental obturator.

In XIX, Euphorbiaceae was classified according to the system of Baillon (1858) and in the same century more systems of classification appeared and new description and contributions were added to the family. These contributions were mainly in the monographies of the botanist Jean Müller Argoviensis and in the Prodomus Systematis Naturalis Regni Vegetalis (De Candolle, AP) of 1866. In the study of Müller, he classified the family based in the Müllerian system, the first one to organize Euphorbiaceae in subfamilies, tribes and subtribes.

Nevertheless, the systematics of Euphorbiaceae *s.l.* was greatly advanced by professor Grady Linder Webster, who extensively studied the family and gave countless contributions to the classifications of it, as well to the description of taxa in the family. He assumed Euphorbiaceae had 300 genera and five subfamilies (Phyllanthoideae, Oldfieldioideae, Acalyphoideae, Crotonoideae and Euphorbioideae) spread through the neotropics (Webster 1975). The morphological characters were crucial in the arrangement of these subfamilies, the main ones were the number of ovules per locule, the presence of laticifers and the pollen grain morphology.

Webster went further and proposed the first synopsis of the genera and suprageneric taxa of Euphorbiaceae, where he states the presence of 317 genera

organized in 49 tribes. In this paper, there are some detailed morphological descriptions that strengthened the classification of Euphorbiaceae *s.l.* as monophyletic, although other studies were already proposing another type of circumscription (Webster 1994 a,b).

With the advent of the phylogenetic analyses, Euphorbiaceae *s.l.* were first shown to be potentially polyphyletic by Chase et al. (1993). Since then, various publications have corroborated and refined these results (Fay et al. 1997; Litt & Chase 1999; Savolainen et al. 2000b; Chase et al. 2002, Wurdack *et al*. 2005, Tokuoka and Tobe 2006), so the separation of Euphorbiaceae *s.l.* into five families was accomplished. The subfamilies Phyllanthoideae and Oldfieldoideae, both biovulated comprised the families Phyllanthaceae, Picrodendraceae and Putranjivaceae (part of Phyllanthoid in Euphorbiaceae *s.l.*), and the uniovulated genus were arranged within Pandaceae (tribe Galearieae in Euphorbiaceae *s.l.*) and Euphorbiaceae (Euphorbioideae, Crotonoideae and Acalyphoideae).

Further studies supported the monophyly of these new families (Wurdack et al. 2005), despite the various questions about the Euphorbiaceae *s.s.* monophyly, which demonstrate a great morphological variation between its species, such as the features of the ovules development that have an exotegmic palisade layer in species of the whole family, except for Peroideae (Tokuoka 2007). Wurdack and Davis (2009) in their phylogenetic studies have suggested the presence of other modifications beyond the ovules that elevated Peroideae to the family Peraceae.

According to APGIV (2016), Euphorbiaceae *s.s.* is currently composed of 6.745 species and 218 genera grouped in 4 subfamilies (Cheilosoideae, Acalyphoideae, Crotonoideae and Euphorbioideae) with pantropical distribution.

Similar to Webster (1975, 1994a,b) description for Euphorbiaceae *s.l.*, in this new circumscription the family is known for species of different habits, monoecious and dioecious plants which may have latex or not. The leaves are alternate or opposed, rarely verticillate, simple or composed, usually digitate with free or united stipules, which are sometimes reduced to glands or absent and usually have varied indumentum, since stellate to lepidote trichomes.

About reproductive structures, Euphorbiaceae *s.s.* is characterized by terminal or axillary inflorescences, basically cymes, sometimes fascicles, panicles, dichasium, racemes, spikes and also pseudanthia, which is described as a key morphological character of *Dalechampia* and *Euphorbia*, also known as cyathium in this late genus.

Flowers are unisexual, actinomorphic, monochlamideous, dichlamideous or achlamydeous, with varied number of stamens, free or united styles, rimose anthers and tricolpate pollen grains (Judd et al. 2009). The gynoecium shows key morphological characters for the family, such as tricarpelar and trilocular ovary with one ovule per locule and axillar placentation. The styles can be ramified and the fruit is usually a tricoque capsule with exotegmic seeds (Webster 1994a).

Most of Euphorbiaceae pollinators are attracted by the nectar and they are usually insects, including bees, wasps, flies and butterflies, besides bigger animals, such as birds, bats and mammals. Although nectar is a reward for different pollinators, some species are pollinated by the air, such as in some *Acalypha*, *Alchornea* and *Ricinus* (Judd et al., 2009).

2. Croton **L.: a giant genus with great diversity of reproductive structures**

Croton L. is a giant and diverse genus of Euphorbiaceae (Webster 1994a). It is a monophyletic genus (Berry et al. 2005; Van Ee et al. 2011, Van Ee et al. 2015, Arevalo

et al. 2017) and the second largest in the family with almost 1300 species. *Croton* species have cosmopolitan distribution (Govaerts et al. 2000, Berry et al. 2005) though most of them are found in tropical regions, especially in Brazil, the West Indies, and Mexico (Burger & Huft 1995).

Croton was firstly described with a sectional approach by Muller (1866) in the Euphorbiaceae monography for De Candolle Prodromus. This same author included all the known species by the time, though with missing identification keys, which were furtherly included in *Flora brasiliensis* (Müller, 1873). Baillon (1858, 1864) also contributed to the description of *Croton* and suggested new sections to compose the genus.

Later, Webster (1993) used Baillon (1858, 1864) and Grisebach (1864) studies to integrate new sections to the genus, thereby totalizing 40 sections, which were grouped based on indumentum, phyllotaxis, presence of extrafloral nectaries, presence of bisexual basal cymes in the inflorescences, the occurrence of petals in both staminate and pistillate flowers, number of stamens and ramification of styles.

Webster (1993) proposed a great contribution to the genus, classifying it as belonging to the Tribe *Crotoneae*. Since then, several phylogenetic studies have clarified the relationships in the genus, such as Berry et al. (2005), Riina et al. (2009, 2010), Caruzo et al. (2011), Van Ee et al. (2011), Van Ee et al. (2015) and Arevalo et al. (2017).

Apart from the phylogenetic studies, *Croton* is a well characterized genus when it comes to general morphology. The genus shows great morphological diversity, with plant habit ranging from herbs to trees and a wide range of inflorescence architectures and floral morphology (Caruzo 2013). The staminate flowers have a calyx (4) 5 (6)-lobed, free petals, 3-400 free stamens, filaments usually inflexed in the bud, extrorse anthers, globose, inaperturate pollen, the receptacle is often densely lanate, with 5 nectaries alternate to the petals and without pistilodium. The pistillate flowers have a calyx (4) 5-7 (-10)-lobed, entire, pinatissected, imbricate or with conduplicate-valvar

lobes, sometimes acrescent in the fruit, petals almost reduced to filaments structures or absent, sometimes with staminodes, a receptacle usually with an annular nectary, rarely segmented, an ovary (2) 3-carpelar; styles 3, free or bifid,; with one anatropous ovule in each locule (Webster 1967, 1993; Cordeiro 1989; Caruzo & Cordeiro 2006, 2013).

The usual inflorescence type in *Croton* is thyrse and flowers are frequently arranged as pistillate cymules in proximal position and staminate cymules distally located (Webster 1967, Webster 1993). However, solitary pistillate flowers may also compose the proximal inflorescence region, besides bisexual cymes (Webster 1967, 1993, Cordeiro 1989, Caruzo and Cordeiro 2013).

Studies with flower morphology, ontogeny and genetic expression are essential to understanding the origin of different floral shapes and inflorescence architecture in a group. Considering *Croton's* great variability in the reproductive structures, the aim of this thesis was to study flowers and inflorescences with an evolutionary approach, and thus highlight key answers to this great diversity.

3. General structure of the thesis

This thesis is composed by this general introduction, followed by four chapters in format of manuscripts, and final conclusions. The layout of each chapter is according to the journal that we intend to submit. The sequence of the chapters was organized as follows:

I. **Flower development in species of *Croton* (Euphorbiaceae) and its implications for floral morphological diversity in the genus.** This manuscript was published in *Australian Journal of Botany* and proposes the study of the ontogeny and structure of *Croton* flowers focusing on the development of the perianth and intermediate structures. With the results

obtained we related the characters to the current floral development and phylogenetic knowledge for the group and stablished a new interpretation for the petaloid structures present in pistillate *Croton* flowers.

II. **Androecium and nectaries in *Croton* flowers: cases of obdiplostemony, heterotopy and homeosis.** This manuscript consists on the study of staminate *Croton* flowers. With the flower development, vasculature and analysis of auxin concentrations in different development stages, we aimed to understand the ontogenetic steps of flowers with different androecium merism and the role of nectaries in the androecium configuration. We propose here the first description of obdiplostemony for *Croton* and examples of heterotopy and homeosis. We intend to submit this manuscript to *American Journal of Botany*.

III. **Morphology and evolution of inflorescences in the large genus *Croton* L. (Euphorbiaceae).** This manuscript consists on a detailed morphological analysis of inflorescences of all *Croton* sections. Our aim was to determine and describe the inflorescence patterns and features of the genus and cross our data with the most recent phylogeny in order to understand the characters in *Croton* evolutionary history. With over 200 species analyzed we proposed 17 inflorescence patterns and inferred associations with habitat and evolution. We intend to submit this manuscript to *Annals of Botany*.

IV. **Inflorescence transcriptome of *Croton fuscescens* Spreng (Euphorbiaceae): an overview and first insights.** This chapter consists on the study of *Croton fuscescens* Spreng inflorescence transcriptome with RNAseq. The aim of this study was to comprehend the genetic atlas of this *Croton* inflorescence and verify the changes in gene expression in the pistillate and staminate regions.

We answered our main question, though further gene annotation will be done to conclude the study. We intend to submit this study to *Genomics*.

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Chapter 1 - Flower development in species of Croton (Euphorbiaceae) and its implications for floral morphological diversity in the genus

Abstract

The Euphorbiaceae are notable for floral diversity and evolutionary complexity. *Croton* is the second largest genus in the family and exhibits particular diversity in its flowers. The aim of this study was to investigate the floral ontogeny and structure of three *Croton* species with distinct morphologies, with a focus on testing the hypothesis that the filaments of female flowers, which have received different interpretations in the literature and are currently described as reduced petals, are staminodes and part of a vestigial androecium. With the ontogenetic study we can understand the origin of the organs and associate these with flower evolution in the genus. Flowers in several stages of development were analyzed using light microscopy and scanning electron microscopy. In the early stage of development, the sepals are the first structures to be formed, although they do not continue to grow in female *Croton fuscescens* Spreng. flowers. Petals are absent in female flowers, with filamentous, petaloid structures, interpreted here as staminodes, alternating with the sepals in *Croton lundianus* (Didr.) Müll. Arg. In *Croton sphaerogynus* Baill., the staminodes are located between the nectary lobes. The stamens exhibit centripetal development in the flower bud stage, and the carpels are post-genitally connate, with differences in style branching. Besides the ontogenetic interpretation for the filamentous structures, the genus shows transitional structures that we consider evolutionary reductions. Our results can explain how developmental alterations have influenced the suppression and modification of floral organs in the genus.

Key words: Crotonoideae - evolution - ontogeny - perianth - petals - staminodes.

Introduction

Among the angiosperms, families such as the Euphorbiaceae are notable for their floral and inflorescence diversity. Many types of cymose inflorescences are found in this family, and some of them are unique and morphologically different from other groups, such as the pseudanthia of *Dalechampia* and *Euphorbia*.

Croton, the second largest genus of Euphorbiaceae, includes between 1200 and 1300 species of herbs, shrubs, trees, and lianas; it is pantropical, with about 300 species in Brazil (Berry *et al*. 2005; Cordeiro *et al*. 2016). Its inflorescences are terminal thyrses that have proximal cymules with either female or both female and male flowers, and distal cymules usually with only male flowers.

Croton has female and male flowers with particular differences, especially concerning the presence or absence of perianth whorls (Webster 1967). *Croton* female flowers have the combination of lobed, entire, pinatissected calyx, usually imbricate or valvate and petals are usually reduced or absent; filaments are usually common, though their interpretation is debatable, sometimes described as petals or glands (De-Paula *et al*. 2011, Caruzo and Cordeiro 2013). The male flowers have valvate calyx, as many petals as there are sepals and stamens with no filaments (Webster 1967).

Caruzo *et al.* (2011) studied the evolution of characters and the phylogeny of *Croton* section *Cleodora* (Klotzsch) Baill., a group widely spread through America but which has not been revised recently. Caruzo *et al.* (2011) described the pistillate flowers of this group as generally apetalous, or with greatly reduced petals and rarely conspicuous petals. They argued that when filaments or glandular structures are present in the position of petals, these structures should be referred to as reduced petals. They also noted that the presence of filaments is shared by a majority of New World *Croton* species, where 2/3 of the species occur (Van Ee *et al*. 2011).

De-Paula *et al*. (2011) analyzed the flowers of two genera of the tribe Crotoneae, *Croton* and *Astraea* and interpreted the male flowers as having five sepals and five petals and the female ones as having five sepals and five filamentous structures, which are unvascularised and interpreted as reduced petals. Nectaries were interpreted as staminodial nectaries due to their external position in relation to the first staminal whorl.

The identification of petals in these flowers requires a deep study on the origin of the organs. In particular, the term "petals" is imprecise and has been applied to a diverse range of showy, non-homologous structures in the second whorl of a perianth. Several degrees and different forms of perianth allied to the numerous cases of perianth organs loss and gain could result in the misinterpretation of perianth parts (De Craene and Brockington 2013). These represent cases where morphological structures derived from different whorls may develop different functions, a phenomenon generally called heterotopy or transference of function (Baum and Donoghue 2002).

Considering the floral variations described for *Croton*, especially with respect to sepals, petals and filaments, the study of flower development allied to evolutionary perspectives is important to understand heterotopy and transference of function. Ontogenetic studies also help understand differences in floral shape, particularly explaining the differences in primordia development and meristem activity (cell division, expansion, and differentiation patterns).

The aim of this study is to test the hypothesis that the filaments of female flowers currently described as reduced petals are staminodes, and like the nectaries considered staminodes by De Paula *et al*. (2011), these filaments are also part of a vestigial androecium. In order to test the hypothesis above we chose three species: *Croton sphaerogynus* (*Croton* sect. *Cleodora* (Klotzsch) Baill.), *Croton fuscescens* (*Croton* sect. *Julocroton* (Mart.) G.L. Webster) and *Croton lundianus* (*Croton* sect.

Geiseleria (A. Gray) Baill.). These species were chosen because they show contrasting flower morphologies, especially concerning the number of whorls of the perianth, the shape of the sepals, petals and filaments, as well as the presence or absence of these structures. The chosen species represent much of the floral morphological variation found in *Croton*, they belong to different sections and were comparatively investigated in relation to the development and structure of the flowers, correlating our data with the available phylogeny in an evolutionary context.

Material and Methods

Inflorescences and flowers in several stages of development were collected in São Paulo at the Instituto de Botânica and in the city of Itanhaém. Voucher specimens were deposited in the Herbarium of the Universidade de São Paulo: *Croton fuscescens* Spreng. (*Gagliardi and Demarco 9* [SPF]), *Croton sphaerogynus* Baill. (*Gagliardi and Demarco 10* [SPF]), *Croton lundianus* (Didr.) Müll. Arg. (*Gagliardi and Demarco 11* [SPF]).

Flower meristems, flower buds, and pre-anthetic, anthetic, and post-anthetic flowers were isolated, fixed under vacuum in FAA (formalin, acetic acid, 50% ethyl alcohol) for 24 h (Johansen 1940) and in NBF (neutral buffered formalin) for 48 h (Lillie 1965), and then stored in 70% ethyl alcohol.

The material was dehydrated in a butyl series (Johansen 1940), embedded in Paraplast, and transversely and longitudinally sectioned every 10–12 µm in a rotary microtome (Microm HM340E). The sections were stained with astra blue and safranin (Gerlach 1984) and the blades mounted in synthetic resin. Photomicrographs were taken using a light microscope (Leica DMLB).

For the ontogenetic study, micromorphological analyses were performed using the material fixed in FAA. After isolation of the floral parts, the material was dehydrated in an ethanol series, critical point dried with CO_2 (Balzers CPD 030), mounted on aluminum stubs, and sputter coated with gold (Balzers SCD 050). Observations were then made and images taken using a scanning electron microscope (Zeiss DSM 940) with a digital camera attachment.

Results

Morphology of the inflorescences and flowers

The inflorescences of all three studied species are thyrses, with cymules of female and male flowers differently arranged (Fig 1A-C). In *Croton sphaerogynus*, the flowers are continuously distributed along the axis of the thyrse, and the proximal cymules have both female and male flowers and the distal ones have exclusively male flowers (Fig. 1A). In *Croton fuscescens*, the flowers are also continuous, with proximal cymules containing only female flowers and distal ones male flowers (Fig. 1B). In *Croton lundianus*, the cymules containing female flowers are separated from the ones containing male flowers through a sterile area on the main axis of the thyrse (Fig. 1C).

The female flowers are apparently monochlamydeous in the three species (Fig. 1E, 1F), but *C. lundianus* presents an extra whorl composed of petaloid, white, and slightly expanded filamentous structures alternating with the sepals (Fig. 1F). The female flowers are hexamerous in *C. lundianus* and pentamerous in *C. sphaerogynus* and *C. fuscescens*.

Fig 1: Inflorescences and flowers of *Croton* L. **(a).** Inflorescences of *Croton sphaerogynus* Baill. (**b).** Inflorescence of *Croton fuscescens* Spreng. (**c).** Inflorescence of *Croton lundianus* (Didr.) Müll. Arg. (**d).** Staminate flower of *C. fuscescens.* (**e).** Proximal region of the inflorescence of *C. fuscescens*. Detail of the pistillate flower. (**f).** Pistillate flower of *C. lundianus*. Note the petaloid structures (*arrow*).
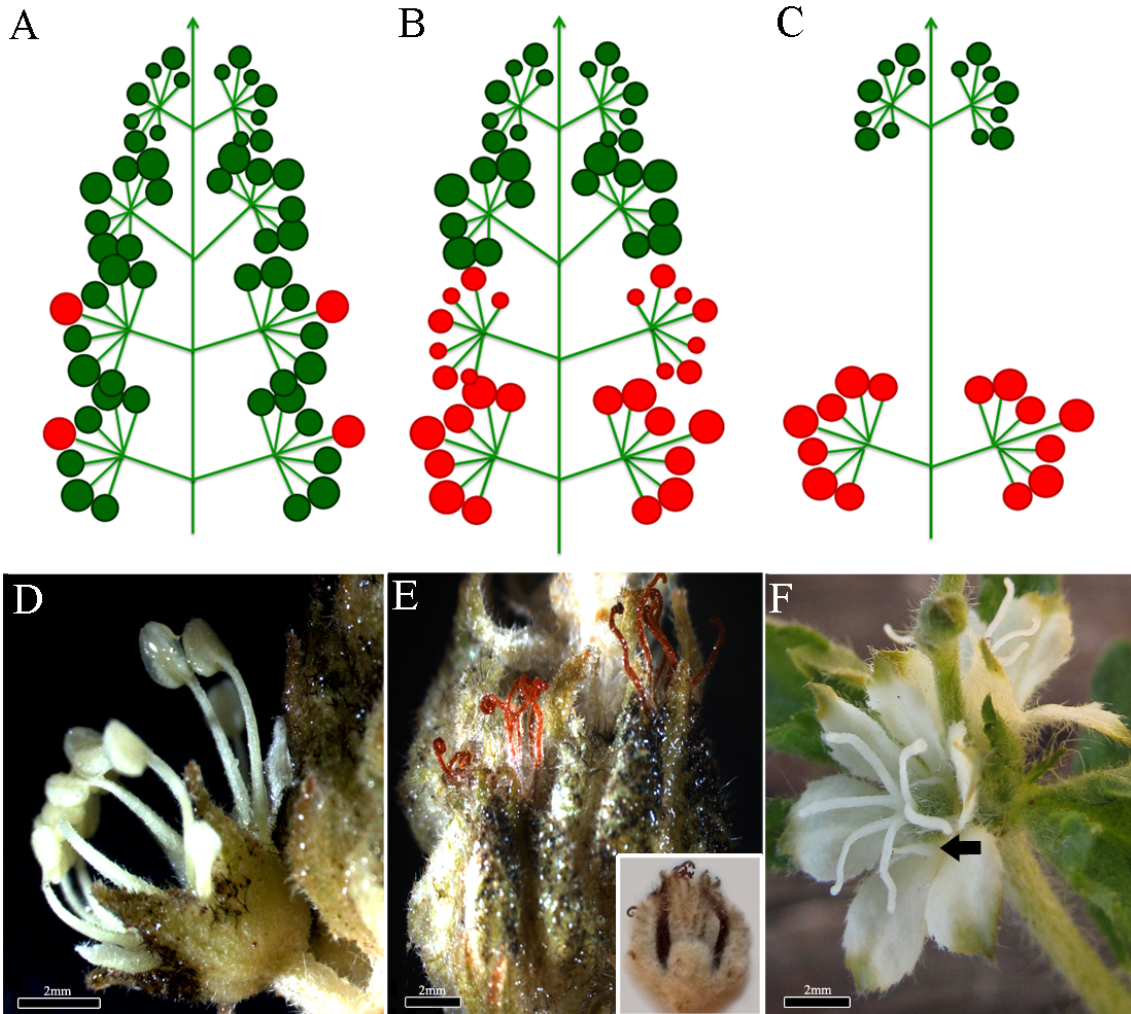
The male flowers (Fig. 1D) are all pentamerous, but the androecium varies in the number of stamens: eleven stamens in *C. fuscescens*, fifteen in *C. sphaerogynus*, and ten in *C. lundianus*.

Development of the inflorescences and cymules

The cymules containing female flowers are the first to initiate development in the inflorescences of all three species, and the terminal flower is the first to initiate differentiation in each cymule. This characterizes the development as basipetal for the cymule and acropetal for the main axis (Fig. 2A).

Development of female flowers

Calyx

The first whorl to start differentiation is the calyx (Fig. 2B–C), and the sepals develop in an anticlockwise direction (Fig. 2B). The species have sepals with similar size and shape, except for *C. fuscescens,* which has larger, anterior sepals firstly differentiated, followed by small sepals (Fig. 2C).

When the elongation is almost complete, it can be observed in *C. sphaerogynus* and *C. lundianus* that the sepals develop continuously and all at the same rate (Fig. 2D), different from *C. fuscescens* (Fig. 2E)*.* During the development of the calyx in *C. fuscescens,* the different rate of sepal elongation is notable and there is a delay in the elongation of the two posterior sepals allied to a laciniation of the three anterior ones, resulting in a calyx with two reduced sepals and three that are longer and laciniate (Fig. 2E, F).

Fig 2: Ontogeny of inflorescences and female flowers. **(a-c, e-f, j, l, m-o).** Young inflorescence of *Croton fuscescens* Spreng. (**b**). Reproductive bud with sepals' primordia (*arrow*). **(c).** Reproductive bud with sepals in development, long sepals and reduced sepals. **(d, k).** Flower bud of *Croton sphaerogynus* Baill. with elongated sepals. **(e).** Flower bud with sepals in different rhythm of elongation. (**f**). Detail of the abaxial long sepals and reduced sepals (*arrow*). **(g-I, p).** Flower bud of *Croton lundianus* (Didr.) Müll. Arg. with sepals removed (*asterisk*). Note the petaloid structures (*arrow*). (**h**). Anthetic flower with sepals removed (*asterisk*) and petaloid structures in elongation process (*arrow*). (**i**). Mature flower with sepals removed (*asterisk*), elongated petaloid structures (*arrow*) and nectary (highlighted) associated with the removed sepal. **(j).** Gynoecium with nectary (*asterisk*) in the basis of the ovary. **(k).** Gynoecium with nectary (*asterisk*) in the basis of the ovary and filament structure (*highlighted and in detail*). **(l).** Detail of the horse-shaped nectary around the ovary, which was removed (*asterisk*). **(m).** Flower buds with carpels in early stage of development. **(n).** Detail of the gynoecium with carpels almost fused (*arrow*) and ovule primordia (*asterisk*). (**o**). Anthetic flower with elongation of styles (*arrow*) and ramification of stigmatic region. **(p).** Mature flower with elongated styles. (Ls = long sepal; Rs= reduced sepal).

Filamentous-petaloid whorl

The development of filamentous, petaloid structures alternating with the sepals was observed in *C. sphaerogynus* and in *C. lundianus* (Fig. 2G, K). Following the elongation of the calyx, these filamentous are alternate with the sepals (Fig. 2G), and in *C. lundianus* the petaloid structures become more elongated and exhibit two distinct regions: a long basal portion, and a slightly flattened apical region (Fig. 2H). With the elongation of the sepals, the petaloid structures become spatulate, with a thin elongation at the apex in *C. lundianus* (Fig. 2I), but short and pointed in *C. sphaerogynus* (Fig 2K).

Nectaries

During the elongation of the organs in the three species studied, the adaxial-basal meristematic cells adjacent to the sepals start differentiating into nectaries (Fig. 2I-L). The development of these glands around the ovary is followed by elongation. In *C. sphaerogynus*, the nectaries become lobed and develop around the ovary with filamentous structures between the lobes (Fig. 2K); in *C. lundianus*, the nectaries are segmented into small, rounded structures, associated with the sepals (Fig. 2I). *C. fuscescens*, by contrast, exhibits deeply 3-lobed nectaries that develop only opposite and adjacent to the three large sepals, thereby surrounding part of the ovary (Fig. 2J, L). When the flowers are completely developed, the nectaries become larger and secretory (Fig. 2I–K-L).

Gynoecium

After the elongation of the calyx, the meristematic cells internal to the calyx start developing into the carpels. These are initially separate and then become fused during the course of development (post-genital fusion) in all three species (Fig. 2M-N), with

evident ovule primordia inside the young ovaries (Fig. 2N). The elongation of the carpels is followed by the formation of the styles (Fig. 2J-K, O-P). A complete gynoecium is observed at this stage, with a hairy stigma in all the species, glandular and nonglandular trichomes (Fig. 2O); the styles are in division and are quite elongated in *C. fuscescens* and in *C. lundianus* (Fig. 2O-P), and also curved in the latter species (Fig. 2P), with a globose, hairy ovary. With elongation and complete development of the gynoecium, the styles become long and hairy, bifid in *C. lundianus* and *C. fuscescens* (Fig. 2O) and multifid in *C. sphaerogynus*. They have further curved branches in *C. fuscescens* and *C. sphaerogynus*, in contrast to *C. lundianus*, in which the stigmas become more elongate and less curved (Fig. 2P).

Anatomy of female flowers

Calyx

The sepals present a uniseriate epidermis composed of round-shaped cells in all the studied species (Fig. 3A) and slightly elongated cells in *C. sphaerogynus* and in the small sepals of *C. fuscescens* (Fig. 3B). In some regions of the abaxial epidermis of *C. sphaerogynus* there are large and round crystal idioblasts and mucilage cells (Fig. 3B). The mesophyll is homogeneous, with chlorophyllous parenchyma, and the vasculature is formed by one to three collateral vascular bundles or even more, as in *C. fuscescens* (Fig. 3A).

Petaloid whorl and filamentous structures

The petaloid structures of *C. lundianus* and the filamentous structures of *C. sphaerogynus* are anatomically similar to the sepals, although the mesophyll is thinner, with fundamental parenchyma and no vasculature (Fig. 3C-D).

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Fig 3: Anatomy of pistillate flowers. **(a, e, g-h).** Transverse section of *Croton fuscescens* Spreng. Sepal with vascular traits (*arrows*). **(b, d, f, i).** Transverse section of *Croton sphaerogynus* Baill. sepal with mucilage cells (asterisk). **(c, j-k).** Longitudinal section of petaloid structure of *Croton lundianus* (Didr.) Müll. Arg. (*asterisk*). (**d**). Longitudinal section of filament structure of *C. sphaerogynus* (*asterisk*). (**e**). Longitudinal section of style and stigma. **(f).** Longitudinal section of the curved and ramifies styles. **(g)** Transverse section of style. Note the stylar canal (*asterisk*) and vasculature of the ramified stylar branches (*arrows*). **(h)** Division process (*arrow*) of the styles/stigmas. **(i).** Transverse section of the ovary. **(j).** Details of the ovary and ovule. Note the ovary wall (*bar*) and vasculature (*arrow*). **(k)**. Ovule with nucellar beak (*arrow*) and placentary obturator (*asterisk*) (Ov = ovule).

Gynoecium

The gynoecium has long stigmas with a uniseriate and papillose epidermis in all three species (Fig. 3E); stigmas are slightly elongated in *C. sphaerogynus* (Fig. 3F) and more pronounced in *C. fuscescens* and *C. lundianus* (Fig. 3E). The style branches are hollow in all three species and consist of a uniseriate epidermis of small, cubic cells on both faces, and a central region with a stylar canal composed of secretory palisade cells (Fig. 3G-H) that are present through the whole style branch, from the stigma to the ovary. In the apical region of the ovary, the styles are united in a short extension that divides into three in the larger distal portion (Fig. 3H); each one of these parts divides again into two stigmata, creating a total of six branches. In *C. sphaerogynus* the styles are more curved than those in the other species and crystal idioblasts and mucilage cells are widely present in the style cells (Fig. 3F).

The ovary of all three species is tricarpellate and trilocular, with one ovule in each locule (Fig. 3I). It presents a uniseriate outer epidermis composed of small, isodiametric cells in *C. fuscescens,* a biseriate epidermis with cubic cells in *C. lundianus* (Fig. 3J), and a uniseriate epidermis with slightly elongated cells in *C. sphaerogynus* (Fig. 3I). Nonglandular trichomes are found in all three species, and crystal idioblasts are present in *C. sphaerogynus* (Fig. 3I). The mesophyll is homogeneous, with dorsal and ventral collateral vascular bundles (Fig. 3J). The inner epidermis is composed of elongated cells, uniseriate in *C. sphaerogynus* and *C. lundianus,* and biseriate in *C. fuscescens* (Fig. 3I-J).

The ovules are bitegmic (Fig. 3J) and anatropous, with a thicker (3–5 cell layers) outer integument and a thinner (2–3) inner integument. The micropyle is composed of both integuments; there is also a placentary obturator and nucellar beak present in all three species (Fig. 3K).

Development of male flowers

The cymules containing male flowers show later development compared to the ones with female flowers (Fig. 2A and 4A).

<u>Calyx</u>

The sepals are the first structures to be formed, with five protuberances in anticlockwise direction (Fig. 4B). After differentiation and the beginning of elongation, the sepals undergo post-genital fusion (Fig. 4C). The elongation of the sepals is followed by the formation of trichomes on their surface, covering the stamens (Fig. 4C).

<u>Corolla</u>

Following the development of the sepals and alternating with them, the free primordia of petals begin their differentiation in all three species (Fig. 4D). The petals exhibit slow and delayed elongation (Fig. 4D-E), and while the calyx is quite elongated, the corolla is still reduced. With the elongation of the perianth, the petals reach their final shape and size.

<u>Androecium</u>

Internal to the calyx and corolla primordia, a group of meristematic cells begins to differentiate and some stamen primordia may be observed in a centripetal formation in the marginal, outer region of the receptacle (Fig. 4E-F). Followed by cell divisions, in bud stage, the primordia of the stamens show elongation of the filaments and formation of the anthers, which are rounded prior to thecal differentiation. The anthers are quite immature with early differentiation of their walls (Fig. 4F).

Fig 4: Ontogeny of staminate flowers. **(a-f, h, j).** Young inflorescence of *Croton fuscescens* Spreng. **(b).** Flower buds with sepals (*thick arrow*) and petals formation (*middle arrow*). **(c).** Flower bud with sepals' elongation and initial trichomes (*arrow*). **(d).** Flower bud with sepals removed (*asterisk*) and petal in early stage of development (*arrow*). **(e).** Flower bud with petals starting elongation (*arrow*) and stamens initiating development (*asterisk*). **(f)**. Longitudinal section of flower bud with stamen filaments in elongation process (*arrow*). (**g, k).** Longitudinal section of *Croton sphaerogynus* Baill. flower with the stamens and anthers in differentiation. Note the nectary (highlighted). **(h).** Detail of the elongated and curved stamen **(i)**. Detail of the long connective (*asterisk*) which involves the anthers. **(j).** Anthetic-flower with long and developed whorls. **(k).** Post-anthetic flower with the maximum elongation of the whorls and mature stamens. Note the developed nectaries (highlighted). **(l).** Detail of a developed anther with pollen grains of *Croton lundianus* (Didr.) Müll. Arg.
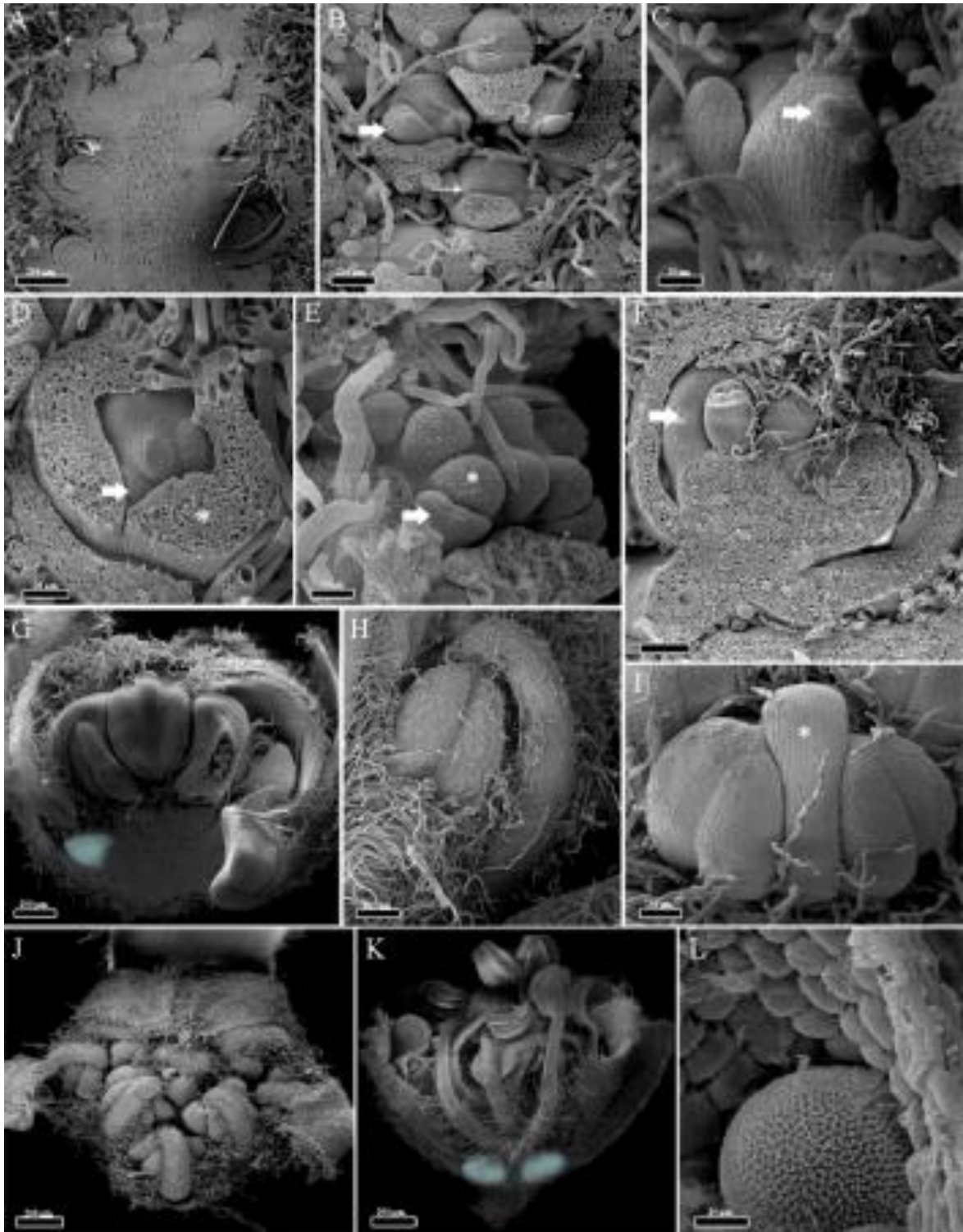
As they elongate, from bud stage to pre-anthesis, the filaments begin to curve and the anthers become completely formed, with pollen grains that are initiating their own development (Fig. 4G). With elongation, the stamens become more curved, with longer connectives that expand over the anthers (Fig. 4H-I). The anthers present only two layers of cells in their walls (epidermis and endothecium), with the secretory tapetum consumed for the production of the pollen grains, which are now completely formed (Fig. 4L). In the post-anthetic stage, the stamens show longer filaments, and the anthers are characterized by flattening of the thecae (Fig. 4J-K).

Nectaries

During the elongation of the perianth, the nectaries around the androecium differentiate (Fig. 4G). These glands go through the elongation process and develop as five small and cubic structures closely associated and almost adnate to the stamens (Fig. 4G). When the flowers are completely developed, the nectaries become fully differentiated and secretory (Fig. 4K).

Anatomy of male flowers

Calyx and corolla

The sepals and petals of the male flowers in all three species present a uniseriate epidermis, composed of round-shaped cells with abundant nonglandular trichomes only on the abaxial surface (Fig. 5A). The mesophyll is homogeneous with phenolic idioblasts and crystalliferous idioblasts containing druses. The vasculature is formed by collateral vascular bundles, with one medium vascular bundle and 1 or 2 lateral bundles in the sepals (Fig. 5A).

Fig 5: Anatomy of staminate flowers. **(a).** Sepal of *Croton fuscescens* Spreng. **(b, d, e).** Petal of *Croton sphaerogynus* Baill. Note the mucilage cells (*asterisk*). **(c, f).** Anthetic-flower of *Croton lundianus* (Didr.) Müll. Arg. with developed stamens, though sepals and petals still united by trichomes (*arrow*). **(d).** Developed stamen with long and curved filament. **(e).** Detail of developed anther. Note the connective with idioblast cells (*asterisk*). **(f).** Detail of the trichomes on the base of petals and stamens (*arrow*).
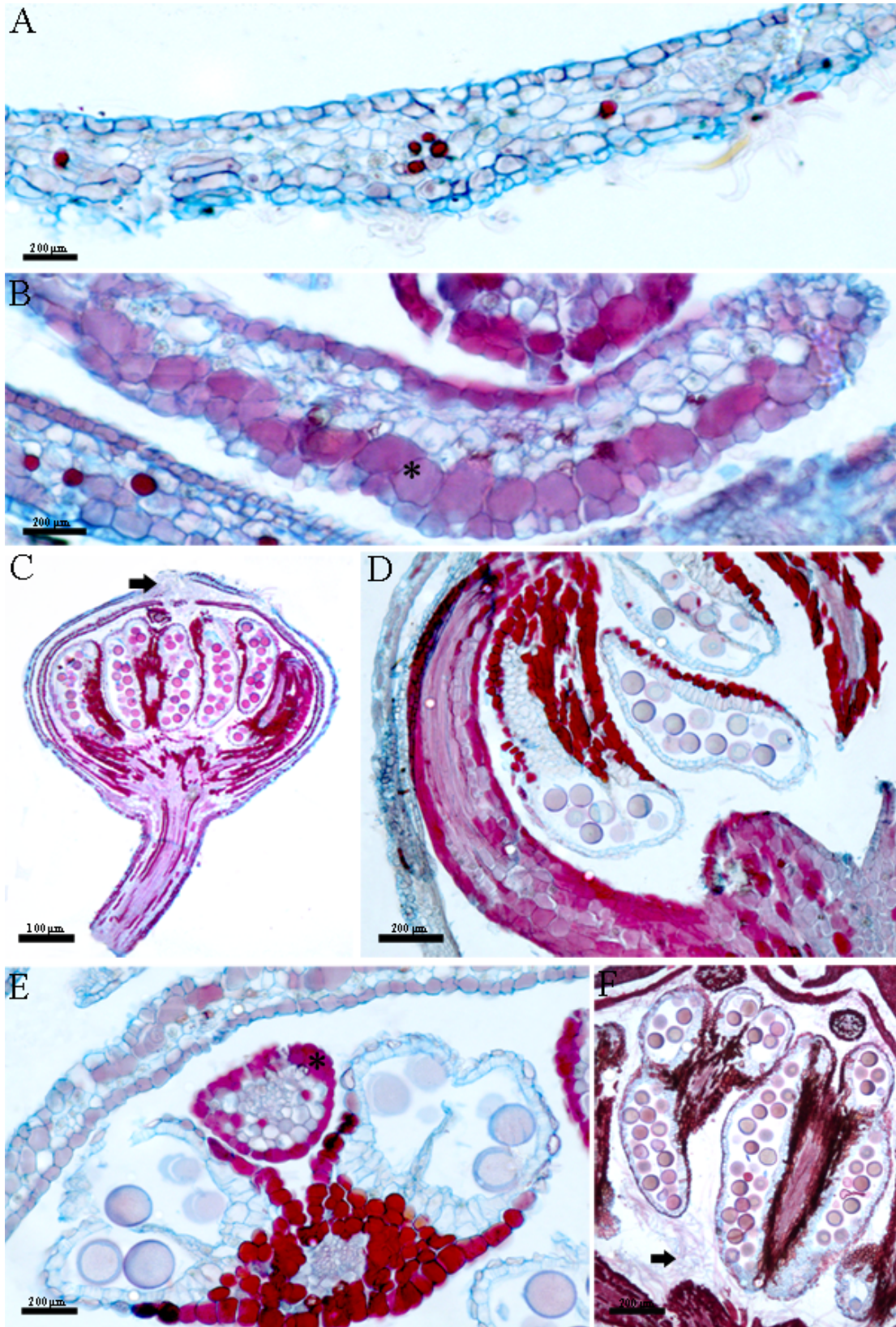
The petals have a similar anatomical structure, with one collateral vascular bundle; the abaxial epidermis of *C. sphaerogynus* is exceptionally composed of large, rounded, crystal idioblasts and mucilage cells (Fig. 5B). On the apex of the sepals and petals of all three species, there are nonglandular trichomes (Fig. 5C) and in *C. fuscescens* there are long nonglandular trichomes at the base of the petals also (Fig. 5F). These trichomes intercalate to each other and keep the calyx and corolla closely attached and associated with each other.

<u>Androecium</u>

The filaments exhibit a uniseriate epidermis and are composed of parenchyma cells (Fig. 5D). The connective is long and presents an apical region composed of cells with a secretory aspect (Fig. 5D-E). The anther is tetrasporangiate with longitudinal dehiscence of the pollen grains (Fig. 5E). The anther walls present epidermal cells that are tangentially elongated, an endothecium with palisade cells and wall thickenings forming trabeculae; the secretory tapetum is totally consumed during the production of the pollen grains, which are organized in monads (Fig. 5E–F).

Discussion

Perianth, petaloid whorl, and transitional structures

The results obtained in this study for the development of *Croton* flowers corroborate our hypothesis that the filaments in the female flowers of *C. lundianus* are staminodes, thus part of a vestigial androecium, such as the nectaries studied by De Paula *et al*. (2011).

Croton flowers have similarities in the initiation developmental step and differences in origin of the whorls and in elongation. In the initiation, the first structures

to develop in all the species were sepals, followed by the petals and androecium in male flowers, staminodes and gynoecium in the female flowers. The differences are focused on the elongation developmental step, which we assume to be the responsible for the high morphological floral diversity in *Croton,* especially the elongation of the sepals and filaments of the female flowers. In *C. sphaerogynus* and *C. lundianus* the five sepals elongate in the same way and rhythm, while in *C. fuscescens* the three exposed sepals become thicker faster and irregularly elongated, resulting in a deeply laciniate calyx, with large three sepals and two smallest ones. In the other species, the sepals are not as thick and large as in *C. fuscescens*, although they are present around the whole flower, thereby providing protection to the fertile whorls.

Croton male flowers have a whorl of petals that alternate with the sepals. The female flowers are all monochlamydeous but sometimes show an extra whorl of filaments or petaloid structures, which were initially, described as reduced petals (De-Paula *et al*. 2011). Although, the elongation of the petaloid structures of *C. lundianus* is different from a petal development and characterizes a stamen with two different regions, one basal and more elongated, such as a stamen filament and an apical portion slightly flattened, such as an anther. The flattening in the apical portion characterizes these structures as staminodes that did not complete anther development. In the mature stage, they exhibit an elongated and thin connective region and a more flattened antheroid.

Staminodes bearing antheroids are relatively uncommon, but widely distributed taxonomically (Walker-Larsen and Harder, 2000). Rijpkema *et al*. (2006) studied the ontogeny of *Petunia* flowers and suggested that the modification of petals into staminodes could be associated with a mutation of BLIND-BL gene, as *bl* mutants displayed a homeotic conversion of the corolla into antheroid structures in the second

whorl. The development of *Croton* staminodes observed here corroborates our hypothesis that petals in the core eudicots have a staminodial nature as previously suggested by De Craene (2007).

In taxonomical studies, *C. lundianus* has been described as a monochlamydeous flower with reduced petals (Lima and Pirani 2003; Caruzo and Cordeiro 2007; Silva *et al.* 2010), but according to our ontogeny results, *C. lundianus* should be interpreted as a monochlamydeous flower with a whorl of staminodes. In *C. sphaerogynus* filamentous structures were also observed between the nectary lobes, and based on their position (opposite to the sepals) these structures are described and interpreted here for the first time as staminodes. The same interpretation was used for De Paula *et al.* (2011), who have described nectaries as staminodes based on the position of these structures opposite to the sepals.

Besides the elongation developmental step which explains the high morphological diversity in *Croton,* the presence of staminodes in female flowers can be interpreted as transference of function and heterochrony cases. The staminodes of *C. lundianus* provide an example of transference of function, in which the staminodes assumed the role of petals. This is similar to the case of the colored staminodes of *Jacquinia macrocarpa* (Theophrastaceae), which are morphologically very similar to the petals, but represent an aborted stamen whorl (Caris and Smets, 2004). Ronse De Craene (2003) studied Papaveraceae flowers and concluded that the organ identity can switch at the boundary of petals and stamens, thereby culminating in the transition of petals into stamens and vice versa.

Heterochrony is observed in the development of *C. sphaerogynus* staminodes, a process defined by Baum and Donoghue (2002) as a temporal developmental change (a phenotypic modification of preexisting structures). This phenotypic modification is the

loss of function of filamentous structures, observed through the initial development of the stamens filament, which showed an early and premature maturation of the tissues, resulting in no further development of the stamens, thereby anthers and pollen grains were not developed.

Contrary to our conclusion, De-Paula *et al.* (2011) suggested that these filamentous/petaloid structures of *Croton* may be interpreted as reduced and transformed petals; further ontogenetic and vasculature analysis is necessary to draw firm conclusions on the origin of these structures.

Based on our observations and the available literature, Crotonoideae flowers exhibit sterile whorls and fertile whorls in the composition of their flowers, though with different patterns of development including transitional structures, such as the staminodes, that give rise to different floral morphologies. Staminodes are considered transitory structures, and according to Walker-Larsen and Harder (2000) and Ronse de Craene and Smets (2001), these structures point to an evolutionary change, either the loss or modification of a whole whorl of petals, such as in *C. lundianus* in which the petals were modified in staminodes, though keeping the attraction role. *C. sphaerogynus* presented the partial reduction of stamens within a whorl, which was also observed in other angiosperms, such as in Geraniaceae, Primulaceae, Myrtaceae, Scrophulariaceae and Verbenaceae (Ronse de Craene and Smets, 2001).

Evolutionary interpretation of the floral developmental patterns

The species analyzed belong to different sections of *Croton*. *Croton fuscescens* belongs to *Croton* sect. *Julocroton* (Mart.) G.L. Webster; *C. sphaerogynus* is included in *Croton* sect. *Cleodora* (Klotzsch) Baill.; and *C. lundianus* belongs to *Croton* sect. *Geiseleria* (A. Gray) Baill. (van Ee *et al.* 2011). *Croton* sect. *Julocroton* is the most

recent section when compared to *Cleodora* and *Geiseleria*, and based on the results discussed above we could assume that the irregular sepal development in the female flowers of *C. fuscescens* explains the zygomorphic morphology of the flower, which results from the different sized, laciniate sepals. The zygomorphy and the laciniate calyx would be considered an apomorphic character.

Croton sphaerogynus belongs to *Croton* sect. *Cleodora* (Klotzsch) Baill, a phylogenetically oldest lineage section when compared to *Geiseleria* and *Julocroton* (van Ee *et al*. 2011). *Croton* sect. *Geiseleria* (A. Gray) Baill. (van Ee *et al*. 2011), in which *C. lundianus* is included, is an intermediate lineage section when compared to *Cleodora* and *Julocroton*. The petaloid structures, interpreted here as staminodes, and the filamentous structures in *C. sphaerogynus*, also interpreted as staminodes, are assumed to be transitional structures in the evolution of *Croton* flowers. These staminodes are absent in *C. fuscescens* (sect. *Julocroton*), which represents the most recent lineage species here, and thus the presence of staminodes could be expected to be a conserved characteristic in *C. lundianus* sect. *Geiseleria* and in *C. sphaerogynus* sect. *Cleodora.*

Other species of Euphorbiaceae show perianth initiation similar to what we observed in *C. sphaerogynus* and *C. lundianus.* Liu *et al.* (2008) analyzed the ontogeny of *Jatropha* flowers, which also develop simultaneous and continuous sepals and petals. In other species of *Croton* and *Astraea*, such as *Croton glandulosus* Müll. Arg., *Croton piptocalyx* Müll. Arg., *Croton urucurana* Baill.*,* and *Astraea lobata* (L.) Klotzsch, the pattern of sepal initiation is similar, although in *Croton triqueter* Lam. the sepals show unidirectional development (De-Paula *et al*. 2011), similar to the pattern we observed in *C. fuscescens* and both species belong to *Croton* sect. *Julocroton* (Mart.) G.L. Webster.

In contrast to our observations, some Euphorbiaceae flowers exhibit vestigial or even no perianth formation (Prenner and Rudall 2007; Narbona *et al*. 2008; Cacho *et al*. 2010; Prenner *et al.* 2011). Gagliardi (2014) studied the pseudanthia of Peraceae and Euphorbiaceae (Acalyphoideae and Euphorbioideae), and the Acalyphoideae inflorescences showed female and male flowers with developed sepals, whereas the perianth is entirely lacking in Euphorbioideae.

The loss of petals in female flowers, as documented here in *C. fuscescens* and *C. sphaerogynus,* and the modification of staminodes into petaloid structures, as in *C. lundianus*, have occurred numerous times in flower evolution and may characterize large groups, such as the Ranunculales, Fagales and Cyperaceae (Endress 2011*a*). The developmental study of *Croton* flowers can help us to understand floral evolution in the genus and in Euphorbiaceae, since *Croton* is an intermediate flower morphotype in the whorl reduction issue in comparison to many other Euphorbiaceae. In addition to the filamentous structures (staminodes) assuming the role of petals, *Croton* flowers maintain all the whorls present during ontogeny, unlike what has been described for *Calycopeplus, Euphorbia*, *Dalechampia* (Prenner and Rudall 2007; Gagliardi 2014).

With respect to the fertile whorls, the studied species are similar in the connation of the carpels, which has also been reported in other Crotonoideae species (De-Paula *et al*. 2011; Gagliardi 2014), and which may be considered a common ontogenetic characteristic for these phylogenetically associated flowers. The gynoecia of the species studied exhibit long and secretory stigmata, transmitting tissues, and anatropous ovules with nucellar beaks and placentary obturators. The long stigma is important to attract pollinators and maximize pollen grain deposition. All the characteristics above, except for the long stigma, have been frequently reported in the Euphorbiaceae (Tokuoka and

Tobe 1995, 1998, 2002, 2003, 2006; Tokuoka 2007; Souza *et al*. 2010; Gagliardi *et al*. 2012, 2013; Gagliardi 2014).

The development of the stamens in all the species studied here was found to be centripetal, and according to Rudall (2007), this may be considered a basal characteristic of the eudicot clade, one also described by Johri *et al*. (1992) when understanding the evolutionary history of angiosperms.

Anatomy of flowers

The sepals and the petals of the species studied show indumentum in the abaxial epidermis with a larger number of trichomes in the apex that closely associate the calyx and corolla together through their intertwining. According to Weberling (1989), this may be interpreted as a special type of gamosepaly, described as capillinection, in which a close intertwining of trichomes maintains the adhesion of the perianth. This mechanism keeps the fertile whorls protected.

Crystal druses and phenolic idioblasts occur in the epidermis and mesophyll of the sepals, petals and anthers, which is associated with a special mechanism of protection against herbivores, dehydration and calcium control (Fahn 1979, 2000).

The gynoecium is quite similar in all *Croton* flowers and its structure seems to be a pattern for other flowers in Euphorbiaceae (Haber 1925, Souza *et al*. 2010, De-Paula and Sajo 2011, Gagliardi 2014, Martins *et al*. 2016).

The ovule characteristics of *Croton* flowers, such as curvature (antitropous), number of integuments (bitegmic), the thickness of integuments (thicker outer integument and a thinner inner integument), and the presence of nucellus are common in apparently all Euphorbiaceae (Endress 2011*a*) and in some Malpighiaceae as well.

The androecium exhibits a tetrasporangiate anther with an epidermis and endothecium similar to those described for other *Croton* species (De-Paula and Sajo 2011). The curvature and elongation of the filaments and the position of the anther are also similar to other *Croton*. This is an important character to differentiate *Croton* from *Brasiliocroton,* which shows erect filaments when in flower bud (Berry *et al.* 2005).

The structural characteristics described here have not been previously reported for the studied species and may be able to play an important role in the classification of *Croton* species and in the clarification of the differences among them.

Conclusions

The flowers of *Croton* studied here represent the different morphological patterns found in the genus and we corroborated the two hypotheses which motivated this study. The morphologically different flowers of *Croton* result from different developmental steps, especially the first steps, which include sepals initiation and elongation, emphasized by the lacination process of *C. fuscescens*, considered an apomorphic character for the genus. Based on our ontogenetic analysis, the filaments of female flowers usually described as reduced petals are interpreted here as staminodes, firstly described for *C. sphaerogynus.* These structures represent cases of transference of function and heterochrony and are considered a conserved characteristic.

The flowers of *Croton* exhibit intermediate conditions with respect to whorl reduction when compared to many other Euphorbiaceae; this is due to the modifications in their floral developmental patterns. These evolutionary modifications gave rise to an extensive diversity of floral morphologies in the large genus *Croton,* though future floral development studies including different species are important to verify the application of the patterns described here or also report unknown developmental

alterations. Considering that *Croton* is one of the largest genus among angiosperms, studies concerning floral vasculature and molecular analysis are also essential to understand the floral evolution of the genus.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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Chapter 2 - Androecium and nectaries in Croton Flowers: cases of obdiplostemony, heterotopy and homeosis

ABSTRACT

PREMISE OF THE STUDY: Floral diversity is directly associated with merosity. Considering *Croton's* great variability in the androecium merism, the general aims of this study are to test the following hypothesis: 1) obdiplostemony is the predominant development pattern for staminate flowers; 2) nectaries are staminodes and part of the androecium. 3) different levels of auxin lead to the obdiplostemonal pattern of development.

METHODS: Flowers with different androecium merism and belonging to four sections were studied. Flowers in several stages of development were analyzed using light microscopy and scanning electron microscopy (ontogeny and vasculature study). Immunocytochemistry tests were performed to verify the IAA influence in the developmental stages.

KEY RESULTS: The studied flowers show polystemonous androecium, which differs in number and organization of the whorls. Sepals arise free and become fused early in the initiation and petals primordia show a delay in development. Antesepalous nectaries interfere in the development of the first stamen whorl in antepetalous direction, characterizing an obdiplostemonal development pattern. Vasculature corroborates obdiplostemony and reveals a central stamen in *C. fuscescens* with carpelar features. In one of the species, nectaries are calycinal but in three of the studied species, nectaries are glandular staminodes. This transference of function and distinct developmental programs may be related to modulation of the free IAA concentrations which were detected during floral development.

CONCLUSIONS: The studied flowers of *Croton* can be used as good models for obdiplostemony cases. The delay in petal development and the first androecium whorl transformed in staminodal nectaries explain the obdiplostemony in the genus, which is corroborated by vasculature study. The transference of nectaries from androecium to the calyx in one species may be interpreted as a heterotopy case and the vasculature study evidenced a homeosis case with the central stamen of *C. fuscescens*. The different auxin concentration during floral development may help understand these morphological alterations in *Croton* evolution.

KEY WORDS: flower development, flower vasculature, free IAA, nectary, stamens

INTRODUCTION

Floral diversity is directly associated with merosity, which by definition is the number of parts within whorls of floral organs, leaves, or stems (Ronse Decraene and Smets 1994, Ronse Decraene et al., 2003; Ronse De Craene, 2010, Ronse Decraene 2016).

Euphorbiaceae is a notable family regarding floral diversity, especially when it comes to *Croton* L., a giant genus and the second largest in the family comprising between 1200 to 1300 species (Govaerts et al., 2000, Berry et al., 2005, Cordeiro et al., 2015). *Croton* flowers are small and unisexual, usually pentamerous, with tricarpelar gynoecium and staminate flowers with a special wide range of stamens, varying from 10 – 100 (-350) (Caruzo and Cordeiro 2006, Van Ee. et al., 2011).

Besides the merism diversity of the androecium, *Croton* staminate flowers usually have five nectaries closely associated with the androecium. The position of these glands, as well as petals, has been recently studied and described as part of the androecium due to their location, thereby representing an outer stamen whorl transformed into secretory staminodes (De Paula et al., 2011; Gagliardi et al. 2017). This evolutionary change in which morphological structures may be shifted from one whorl to another, or a structure which alter its functions, is generally called heterotopy (Baum and Donoghue 2002).

Besides heterotopy, different merosities have important consequences in the flower arrangement, especially in the androecium (Ronse Decraene and Smets 1994). Flowers with a large number of stamens arranged in two or more whorls are usually associated with obdiplostemony, which is an androecial configuration consisting of two stamen whorls, where the arrangement of whorls is inversed, as the outer stamen whorl is inserted in front of the petals (Chatin 1855, Ronse De Craene and Bull-Hereñu 2016).

Obdiplostemony has been receiving different interpretations through the years, such as a puzzling and anomalous condition (Ronse Decraene & Smets, 1987), a disruption in the diplostemonous flower development with a regular alternation of whorls (Ronse De Craene and Bull-Hereñu 2016) and most specially has been considered to play a fundamental role in floral evolution as a possible transitional state of either diplostemony or haplostemony (Ronse De Craene and Smets 1998)

According to Ronse Decraene and Smets (1995), cases of obdiplostemony usually have antesepalous sterile stamens (staminodes) or the loss of petals and androecial configurations have generally been described as obdiplostemonous based on the study of mature flowers, without knowledge of flower ontogeny.

Changes in morphology are mainly induced by shifts in space, caused by the pressure of organs, alterations of the floral meristem size, genetic factors and hormonal control. Auxin is a central regulator in flower development and not only determines whether flower primordia are formed, but also plays an essential role in specifying floral organs and determining the pattern formation within a floral organ. (Cheng and Zhao 2007).

The flower ontogeny of species with different number of stamens in *Croton* is essential to understanding the developmental pattern that leads to different morphologies and merisms. Considering *Croton's* great variability in the androecium merism, the general aims of this study are: 1) study the flower development of the staminate flowers to test the hypothesis that obdiplostemony is the predominant development pattern. 2) Analyze the flower vasculature to test the hypothesis that nectaries are indeed staminodes and are part of the androecium. 3) Study the auxin regulation in flower development to test the hypothesis that different levels of auxin lead to the obdiplostemonal pattern of development.

MATERIAL AND METHODS

Studied species

In order to comprehend the androecium variations of part of the giant genus *Croton* L., we studied the species below, with the following information (Table1):

Species	Section	Stamens	Collection place	Herbarium voucher
Croton fuscescens Spreng	*Julocroton*	11	Instituto de Botânica, São Paulo, Brazil	Gagliardi and Demarco 9 (SPF)
Croton sphaerogynus Baill.	*Cleodora*	15	Itanhaém city, São Paulo state, Brazil	Gagliardi and Demarco 10 (SPF)
Croton lundianus (Didr.) Müll. Arg.	*Geiseleria*	10	Instituto de Botânica and Instituto de Biociências USP, São Paulo, Brazil	Gagliardi and Demarco 11 (SPF)
Croton heliotropiifolius Kunth	*Adenophylli*	20	Ilhéus city, Bahia state, Brazil	T. Vasconcelos 414 (K)

Table 1. Studied species information.

The studied species were selected due to their different phylogenetic position in *Croton* (Figure 1) and because they show distinct androecium morphology, i.e. stamens' number and display. Besides the androecium, the nectaries were also studied here (ontogeny and vasculature) to test the hypothesis that they are staminodes and thus belong to the androecium and interfere in the stamens developmental pattern.

Figure 1. *Croton* phylogeny highlighting the studied sections (modified from Van Ee et al. 2011).
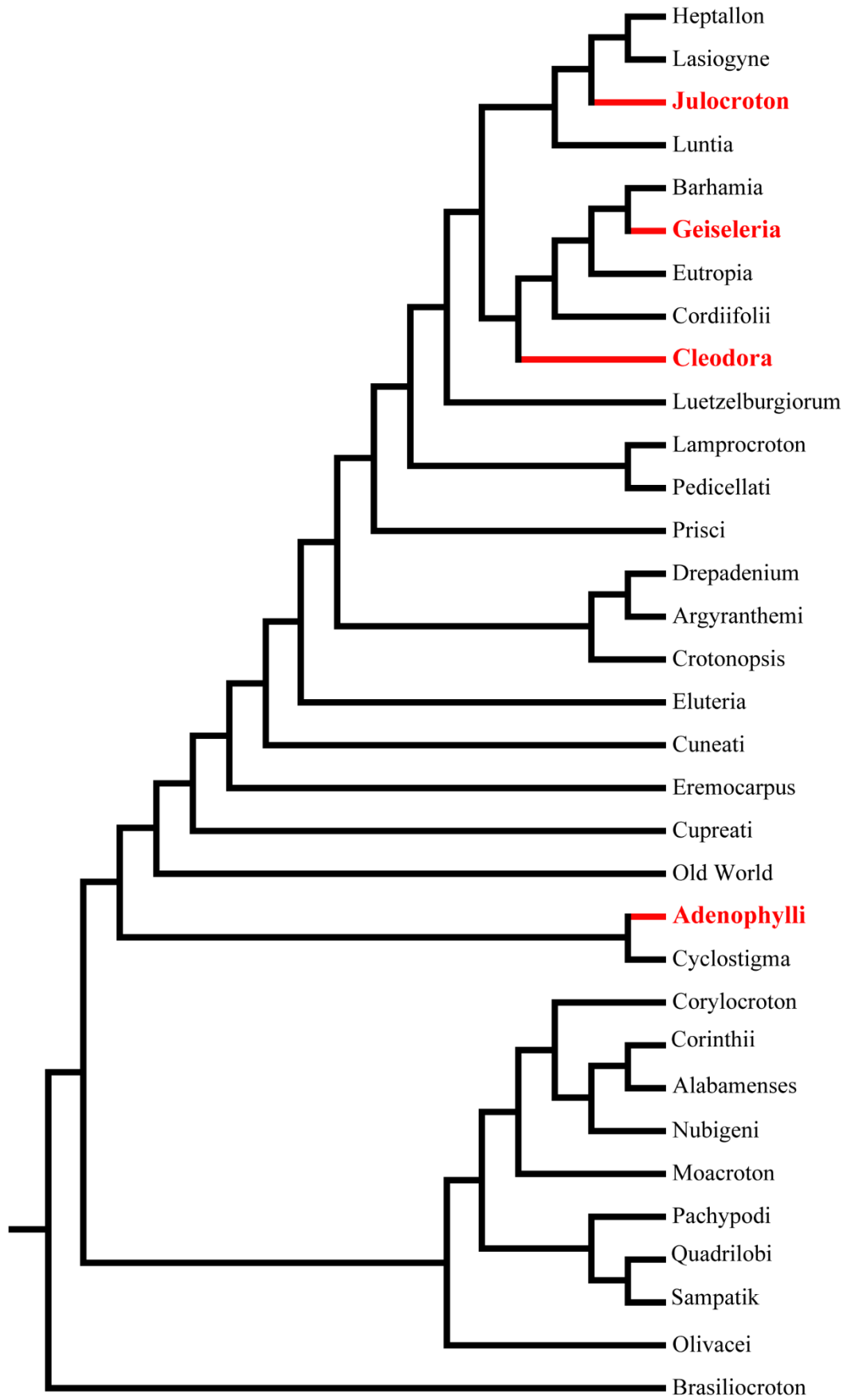
Androecium and nectaries ontogeny

For the ontogenetic study, staminate flower meristems, flower buds, pre-anthetic, anthetic, and post-anthetic flowers were isolated and fixed under vacuum in FAA (formalin, acetic acid, 50% ethyl alcohol) for 24 h (Johansen 1940). After isolation of the floral parts, the material was dehydrated in an ethanol series, critical point dried with CO_2 (Balzers CPD 030), mounted on aluminum stubs, and sputter coated with gold (Balzers SCD 050). Observations were then made and images taken using a scanning electron microscope (Zeiss DSM 940) with a digital camera attachment.

Vasculature

For the vasculature study, the same material above was fixed in BNF (buffered neutral formalin) for 48 h (Lillie 1965), and then stored in 70% ethyl alcohol.

The material was dehydrated in a butyl series (Johansen 1940), embedded in Paraplast, and transversely and longitudinally sectioned 10–12 µm in a rotary microtome (Microm HM340E). The sections were stained with astra blue and safranin (Gerlach 1984) and the slides mounted in synthetic resin. Photomicrographs were taken using a light microscope (Leica DMLB).

Immunocytochemistry

Staminate flower in different developmental stages were previously fixed in 1-Methyl-3- (3-dimethylaminopropyl) -N'-ethylcarbodiimide hydrochloride for two hours and afterwards fixed in FAA (formalin, acetic acid, 50% ethyl alcohol) for 24 hours. The material was dehydrated in an ethanol series, embedded in Paraplast, dewaxed in xylene and transversely/longitudinally sectioned (10–12) mm in a rotary microtome (Microm HM340E).

The slides were hydrated in an ethanol series and washed in PBS (Phosphate-Buffered Saline) buffer for the incubation step with the primary antibody against IAA. The sections were washed in TBS (Tris Buffered Saline) buffer and then, the secondary antibody anti-IGG Rabbit was treated overnight. After incubation, the slides were washed with PBS buffer and Milli-Q water, furtherly stained with Western Blue and mounted on glycerol. Photomicrographs were taken under blue light and UV using a fluorescence microscope (Leica DMLB).

RESULTS

Morphology of the staminate flowers

The studied *Croton* staminate flowers show similar morphology regarding symmetry and merism number. The flowers are actinomorphic and pentamerous with five fused sepals and five free petals composing the perianth, and five nectaries alternating with the petals (Fig 2A-D). The perianth differs in color and the sepals are white in *C. lundianus* and *C. heliotropiifolius* and green in *C. fuscescens* and *C. sphaerogynus*. The petals are white in *C. lundianus, C. sphaerogynus* and *C. heliotropiifolius* and green in *C. fuscescens* (Fig 2I-L).

The androecium is polystemonous and differs in number and organization of the whorls. In *C. lundianus* there are 10 stamens (2 whorls of 5 stamens) (Fig 2A, E), in *C. fuscescens* there are 11 stamens (2 whorls of 5 and one central stamen) (Fig 2B, F), in *C. sphaerogynus* there are 15 stamens (3 whorls of 5 stamens) (Fig 2C, G) and in *C. heliotropiifolius* there are 20 stamens (4 whorls of 5 stamens) (Fig 2D, H). In the anthetic flower, the stamens are erect (Fig 2E-H) and the anthers are introrse, with longitudinal slit and pollen grains in monads (Fig 2E-L).

Figure 2. Morphology of *Croton* flowers. **A-D.** Floral formula and diagram of *C. lundianus*. **E-H.** Model of flower morphology highlighting the different number of stamens. **I-L.** *Croton* flowers of the studies species.

| *Croton lundianus* | *Croton fuscescens* | *Croton sphaerogynus* | *Croton heliotropiifolius* |

A

✲ K(5) C5 A5+5 G0
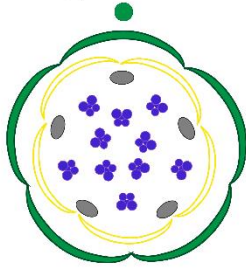
B

✲ K(5) C5 A5+5+1 G0
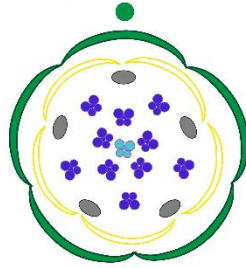
C

✲ K(5) C5 A5+5+5 G0

D
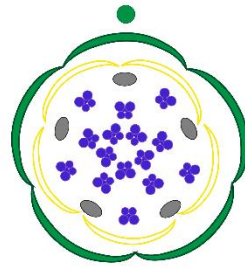
✲ K(5) C5 A5+5+5+5 G0
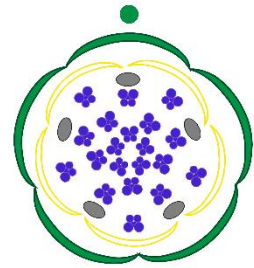
Androecium and nectaries development

The initiation of floral organs is similar in the four studied flowers and the sepals are the first primordia to arise in spiral. The sepals arise free and become fused early in the development (Fig 3A, B). Petals primordia arise free and at the same time, alternating with the sepals. There is a delay in petal's development when compared to sepals' (Figure 3C.D).

In the four species, early in the development, the first stamens primordia arise opposite to the petals and alternating with the sepals (Figure 3C-F). These stamens primordia compose the first and outer stamens whorl of the species. Inner to this whorl, we can observe the primordia of the second stamens whorl, which is oppose to the sepals and develop alternating with the petals and the first stamens whorl (Figure 3D-G).

In *C. fuscescens* one more stamen primordia arise in the centre of the receptacle and the stamens become slightly curved (Figure 3G-K). Adjacent to the outer stamen whorl, a nectary is observed opposite to the sepals and alternating with the petals and first stamen whorls of all the studied species (Figure 3K).

In *C. sphaerogynus* and in *C. heliotropiifolius* an extra third stamen whorl is formed (Figure 3L) and a fourth stamen whorl develops exclusively in *C. heliotropiifolius* (Figure 3O, P). In *C. sphaerogynus* the development of the third stamen whorls is irregular and some primordia elongate earlier than others (Figure 3M). In this stage, sepals and petals become larger and more expanded and stamens show young anthers in the beginning of thecae development, elongation and curvature of the filaments (Figure 3N-P).

Figure 3. Flower development: initiation and early elongation. **Fig 2A-K.** *C. fuscescens.* **A-B.** Development of sepals' primordia. **C.** Initiation of petals and sepals totally elongated (removed). **D.** Initiation of petals and first stamens' whorl antepetalous. **E-F.** Initiation of second stamens' whorl antesepalous. **G-H.** Stamens' primordia and initiation of a central stamen. Petals start elongation. **I-J.** Elongation of stamens, highlighting the central stamen. **K.** Stamens in elongation stage and nectary primordia in antesepalous position. **L-M.** *C. sphaerogynus.* **L.** Elongation of stamens with an available space in androecium area (*asterisk*). **M.** Late development of stamens primordia, highlighted in dark blue. **N-P.** *C heliotropiifolius*. Elongation of stamens and initial formation of thecae. (sepals = green; petals = yellow; stamens = blue; nectary = purple).
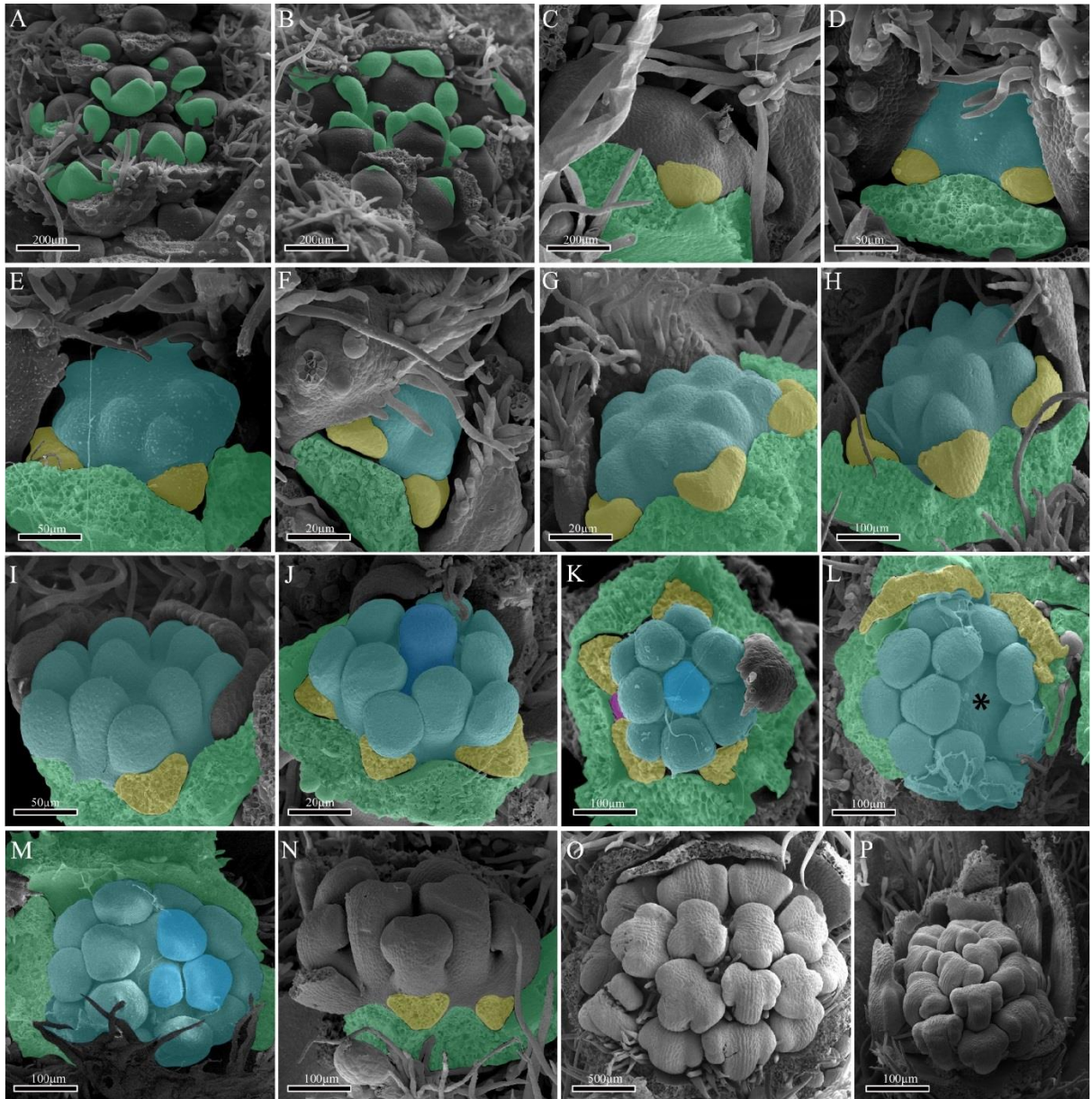
When anthetic, the stamens are elongated, with curved filaments and anthers completely developed with two thecae (Figure 4A-F) and four pollen sacs, two in each thecae (Figure 4E).

The developed and expanded nectaries maintain their initial position opposite to the sepals and closely associated with the androecium, thus adjacent to the outer stamen whorl (Figure 4D, G). In the transitory stage between anthetic and post-anthetic, the stamens become erect and the anthers show a flattened shape with longitudinal slit (Figure 4H).

Figure 4. Flower development: elongation and final shape. **A, F-G.** *C. heliotropiifolius*. Stamens elongated and curved with thecae completely developed. **B-C.** *C. lundianus*. Stamens completely elongated and nectary expanded in antesepalous position. **D, H.** C. *sphaerogynus.* **D.** Stamens elongated in the final shape of the flower. **E.** *C. fuscescens.* Flower completely developed with total elongation of the stamens. **F.** Stamens with a flattened aspect, almost in anthetic stage. **G.** Detail of the petals and nectaries. Petals are alternating with the sepals (removed) and nectaries are expanded and in antesepalous position. **H.** Detail of open anthers releasing pollen grains.
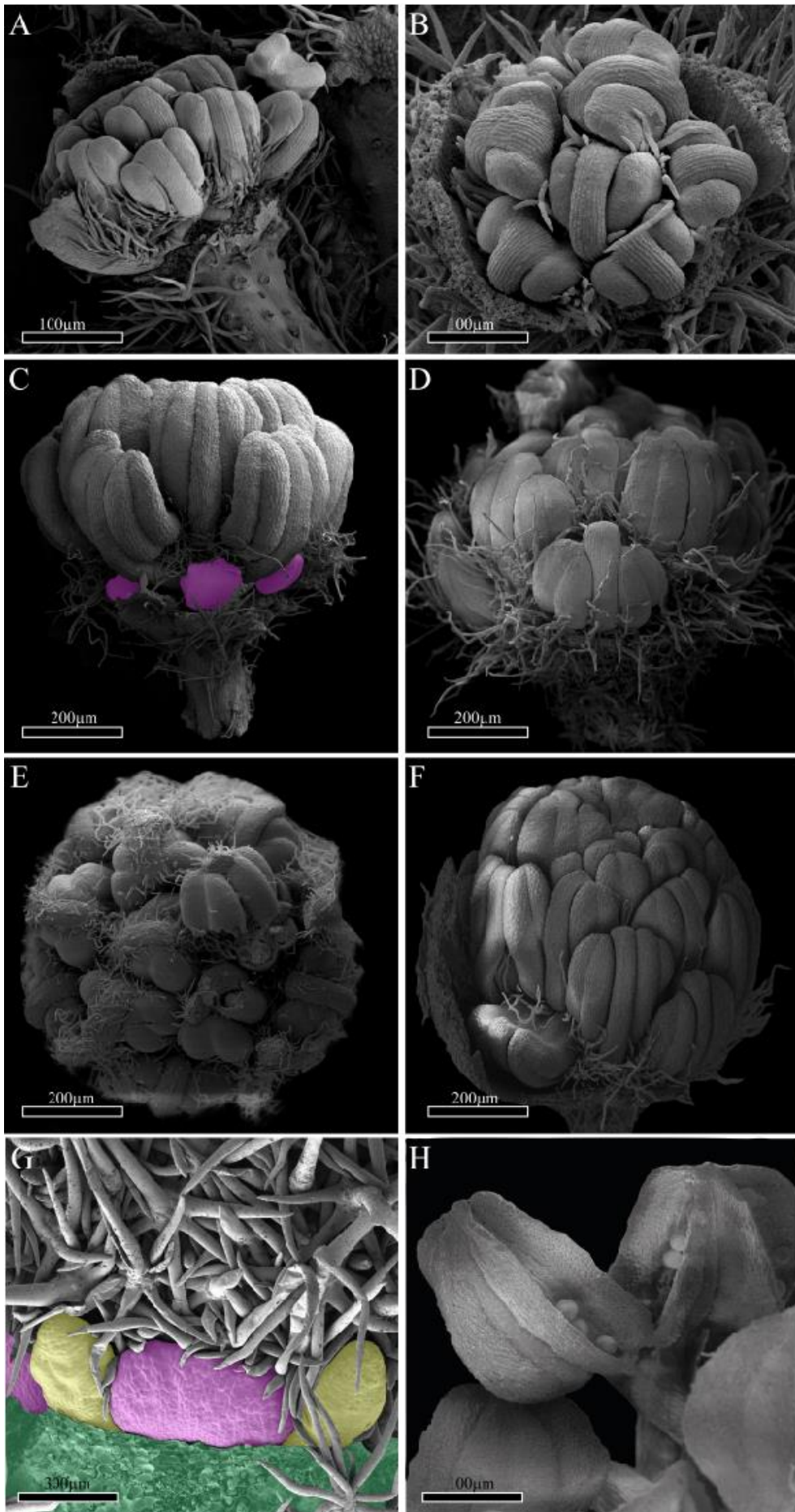
Vasculature of the staminate flowers

The floral steles of the studied flowers (Figure 5A-D) are composed of five vascular bundles in *C. lundianus* and *C. sphaerogynus* (Figure 5E, G) and ten vascular bundles in *C. fuscescens* and *C. heliotropiifolius* (Figure 5F, H).

In the receptacle, vascular traces diverge from the stele to vascularize the sepals. The number of traces is different among the species and there is one trace to each sepal in *C. fuscescens*, two in *C. lundianus* and *C. sphaerogynus* and five in *C. heliotropiifolius* (Figure 5I-L). The traces leave gaps in the floral stele, which are replaced by other vascular bundles (Figure 5I-L).

In the corolla level, vascular traces diverge to reach the petals and each one receives one vascular trace (Figure 5M-Q). Subsequently, the nectaries of *C. lundianus*, *C. fuscescens* and *C. heliotropiifolius* receive vascular traces which diverge from the stele and reach the glands (Figure 5M, N, P). On the other hand, the nectaries of *C. sphaerogynus* receive vascular bundles from the sepals (Figure 5O).

In the stamens' level, there are ten fundamental vascular bundles which will vascularize the androecium (Figure 5R-U). At this same level, the nectaries are expanded and compose a free whorl of single units alternating with the petals (Figure 5U, V, X). In *C. sphaerogynus* the nectaries become fused with the base of the filaments, though keep sharing vasculature with the sepals (Figure 5W). From the ten remaining central vascular bundles, the ones opposite to the petals diverge to vascularize the first stamen whorl (Figure 5W, 6A-D). Each stamen receives one vascular trace (Figure 6A-E). In the centre of *C. fuscescens* stele we can observe three vascular bundles (Figure 6B).

The five remaining vascular bundles which are opposite to the sepals diverge to vascularize the second stamens whorls and reach them without leaving spaces in the

Figure 5. Flower vasculature: calyx, corolla and nectaries. **A-D.** Longitudinal view of the flowers and delimitation of the stele region. **E-H.** Stele with fundamental vascular bundles. **I-L.** Divergence of sepals' traces. **M-P.** Divergence of petals' and nectaries' traces. Note the ramification of the sepals' trace in *C. sphaerogynus* to vascularize the nectary. **Q-T.** Sepals', petals' and nectaries' traces reach the structures and sepals' bundles are divided in *C. fuscescens*. **U-X.** Complete vasculature of the perianth and nectaries and initial divergence of stamens traces, especially in *C. sphaerogynus*.

A *Croton lundianus* B *Croton fuscescens* C *Croton sphaerogynus* D *Croton heliotropiifolius*
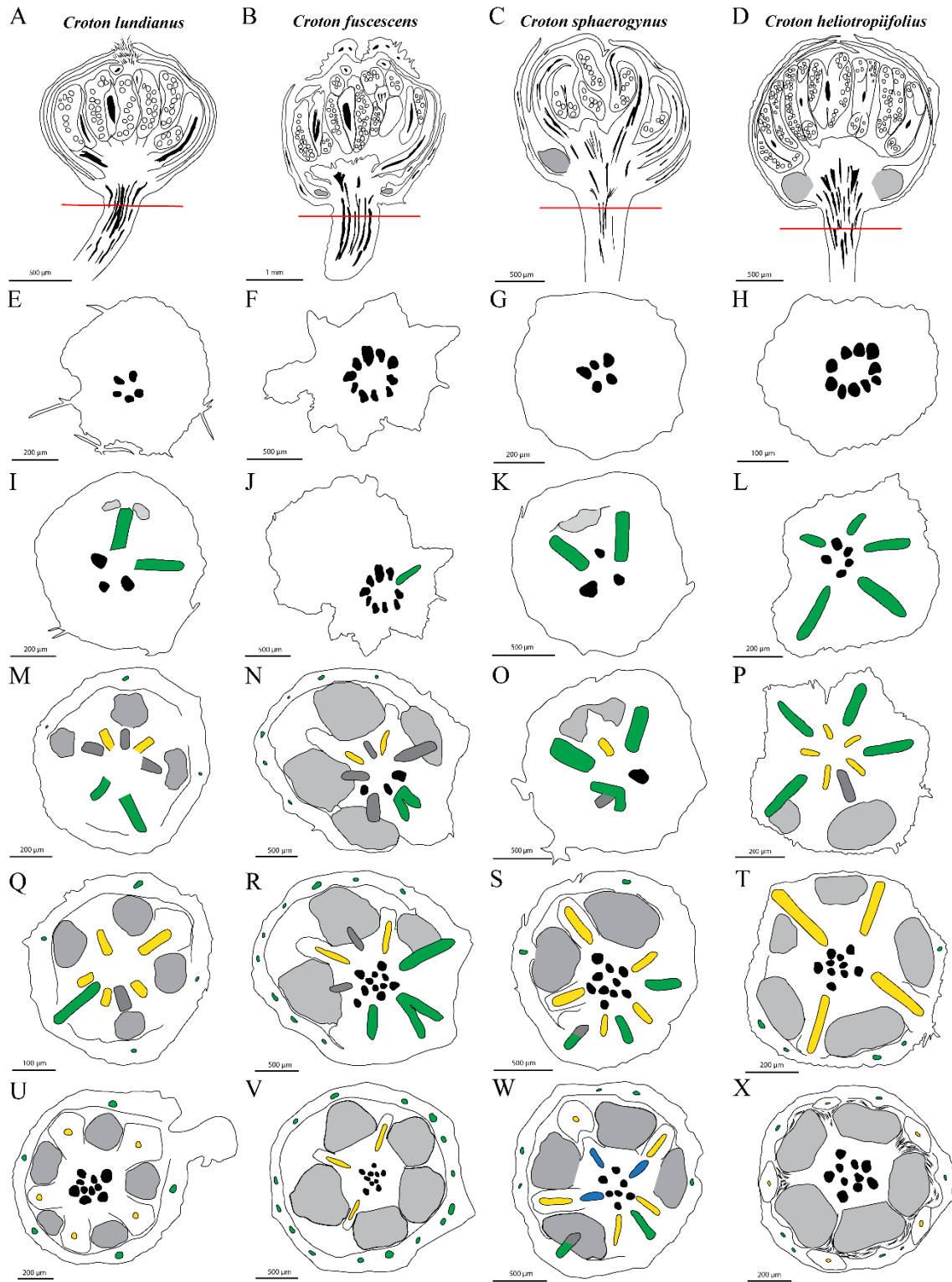
stele (6D, 6F, G). With the expansion of the stamens (Figure 6D-L), five vascular bundles opposite to the petals and first stamen whorl can be observed in the centre of *C. heliotropiifolius* and *C. sphaerogynus* (Figure 6D, G). These later bundles diverge to vascularize the third stamens whorl in these two species and reach the stamens base without leaving space in the stele and without ramification (Figure 6H, K, M-T).

The five remaining vascular bundles in the centre of *C. heliotropiifolius* stele diverge to vascularize the fourth stamen whorl (Figure 6P) and the three central vascular bundles present in *C. fuscescens* become fused to vascularize a central stamen (Figure 6R).

Figure 6. Flower vasculature: nectaries and androecium. **A-D**. Divergence of first stamens' whorl vascular traces in antepetalous direction. Note that in *C. heliotropiifolius*, the second stamens' whorl traces are diverging in antesepalous direction. The nectaries become fused with the stamens base and the sepals' vascular bundles are divided, except in *C. lundianus*. **E-H**. Nectaries expansion and divergence of second stamens' whorl in antesepalous direction. In *C. heliotropiifolius* the third stamens' whorl is diverging in antepetalous direction. **I-L.** Divergence of second and third stamens' whorl. Note three central vascular bundles in *C. fuscescens*. **M-P.** Stamens are totally vascularized, except for *C. heliotropiifolius* in which the fourth stamens' whorl traces are diverging in antesepalous direction. **Q-T.** Stamens vascularized by one single collateral vascular bundle each. Note that the three central vascular bundle in *C. fuscescens* become fused.

A **Croton lundianus** B **Croton fuscescens** C **Croton sphaerogynus** D **Croton heliotropiifolius**
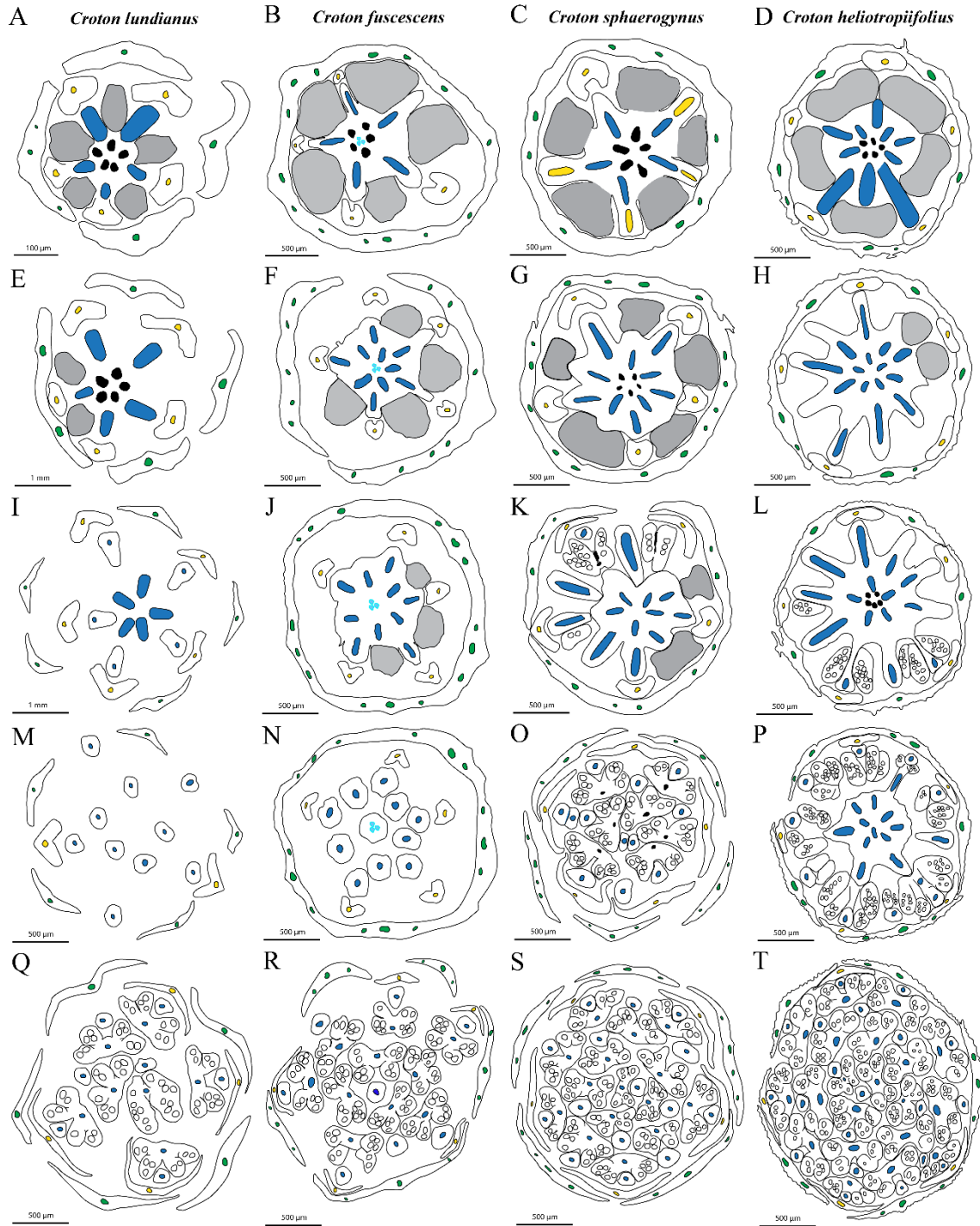
Auxin regulation in the staminate flowers development

The development of the studied staminate flowers is guided by the auxin flow and its different concentration and accumulation in the floral parts.

In floral meristem, we can observe a pool of auxin and the equal concentration of free auxin in the whole tissue (Figure 7A, B). Unidirectional flow of the hormone to the base and lateral region of the meristem results in an expansion of the floral meristem and in the development of sepals primordia (Figure 7C, D).

With the development of the calyx primordia, the free auxin flow becomes bidirectional and the hormone concentration gets higher in the central of the meristem, in the base and in the lateral sepals (Figure 7E). This concentration results in the development of petals and stamens primordia and induces the expansion of the perianth vascular tissue (Figure 7E-H). The stamens primordia show higher auxin concentration than the other floral parts and the development of the anthers can be observed (Figure 7F-H).

The free auxin flow changes with the development of the young anthers and it gets unidirectional and highly concentrated in the androecium region with a lower concentration in the vascular tissues (Figure 7I). The high accumulation of free auxin in the androecium region induces the development and elongation of the stamen filaments (Figure 7J-L) and also the development of nectaries primordia adjacent to the androecium (Figure 7I, J).

With the development of the stamens, the concentration of auxin keeps higher in the anthers than in the filaments and nectaries (Figure 7L-N). The accumulation of free auxin in the anthers induces the total development of these structures, since the formation of the thecae (Figure 7N, O) until the elongation and maturation of the anthers with the production of pollen grains (Figure 7P).

Figure 7. Auxin regulation in flower development. **A.** Model of floral meristem with high free IAA concentration. **B, D, H, K, L.** *C. sphaerogynus*. **B.** Floral meristem with high concentration of free IAA. **C, F, G, J, O, P.** *C. fuscescens*. **C-D.** Floral meristem with unidirectional IAA flow and sepals' primordia (*asterisk*). **E.** Model of flower bud with stamens primordia and different pools of IAA. **F.** Flower bud with stamens primordia and bidirectional IAA flow. **G-H.** High concentration of free IAA in the stamens' primordia and low concentration in petals and sepals. **I.** Model of pre-anthetic flower with high IAA concentration in the stamens and nectaries. **J-L.** Pre-anthetic flower with high IAA concentration in the stamens, especially thecae region and nectaries (*asterisk*). **M.** Model of anthetic flower with IAA pool in the anthers. **N.** *C. lundianus*. **N-O.** Anthetic flowers with IAA pool in the anthers. **P.** Detail of the anther with high IAA concentration.
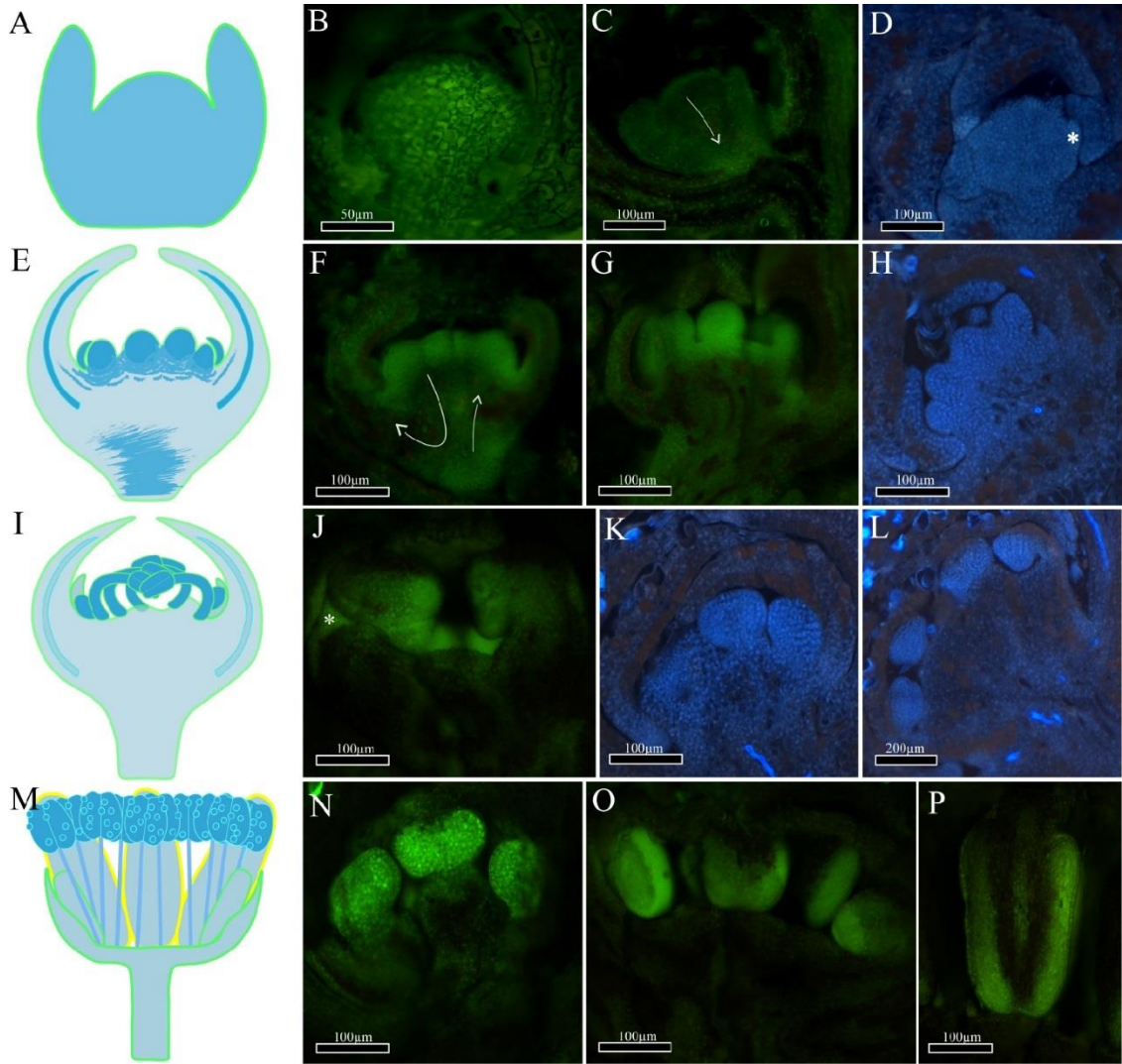
DISCUSSION

Merosity diversity in Croton L. androecium

The studied species showed polystemonous androecium with different number of stamens, ten in *C. lundianus* (*C.* sect. *Geiseleria*), eleven in *C. fuscescens* (*C.* sect. *Julocroton*), fifteen in *C. sphaerogynus* (*C.* sect. *Cleodora*) and twenty in *C. heliotropiifolius* (*C.* sect. *Adenophylli*). The diversity in the androecium merism seems to be a strong morphological character in *Croton* and different number of stamens has been observed for several species in different sections (Webster 1993, 1994).

According to our results the occurrence of 10-15 stamens seems to be an apomorphic character, such as in *C. lundianus* (*C.* sect. *Geiseleria*) and *C. fuscescens* (*C.* sect. *Julocroton*) and higher numbers >15 could be associated with a more pleisiomorphic state, such as observed in *C. sphaerogynus* (*C.* sect. *Cleodora*) and *C. heliotropiifolius* (*C.* sect. *Adenophylli*). The most frequent number of stamens in the genus was registered to be between 10 – 15, such as in the phylogenetically most recent sections *C.* sect. *Luntia, Lamprocroton, Luetzelburgiorum* and in the less recent *C.* sect. *Cleodora, Eluteria, Eutropia, Barhamia, Cuneati, Sampatik, Prisci*, *Nubigeni*, *Quadrilobi*, *Pachypodi*, *Alabamensis*, *Corinthii* and *Corylocroton.* Higher number of stamens such as 11 – 350 has been reported for *Croton* sect. *Cyclostigma*, also a phylogenetically less recent section (Webster 1993, Van Ee et al., 2011, Van Ee et al., 2015).

The evolutionary significance of the androecium merism diversity and the organization of the stamens in whorls have been questioned by different authors in studies with Euphorbiaceae (Mueller 1866; Venkato Rao & Ramalakshmi 1968; Webster 1993, 1994). The studied species showed stamens usually organized in two whorls of five stamens. *Croton fuscescens* showed a variation of this pattern with the

occurrence of a central stamen. *Croton sphaerogynus* and *C. heliotropiifolius* showed three and four whorls of five stamens, respectively. According to Michaelis (1924) a usual polistemone androecium with an indefinite number of stamens organized in many whorls could represent a primitive condition in Euphorbiaceae. On the other hand, Venkato Rao & Ramalakshmi (1968) and Webster (1994) suggested that and androecium with less than 10 stamens, organized in one or two whorls, could represent the primitive condition of Euphorbiaceae, thereby leaving an opened question about the evolutionary significance of the variety in *Croton* androecium merism.

The central stamen observed in *C. fuscescens* has been reported as a strong morphological character for *Croton* sect. *Julocroton*, as most of the species in the section present it in the center of other ten stamens (Webster 1993). The central stamen is not frequent in *Croton* and besides *C.* sect. *Julocroton* it was only registered in some species of *C.* sect. *Quadrilobi* (Webster 1993, Van Ee et al., 2011).

Flower development: obdiplostemony as a pattern

The development of the studied species showed similar initiation with sepals' primordia in spiral sequence. According to Rudall (2007) sepals' primordia usually arise in a spiral and simultaneously, what was observed for some other Euphorbiaceae, such as in species of *Jatropha* (Liu et al., 2008, Alam 2011) and *Croton* (De Paula., et al 2011, Gagliardi et al., 2017). However, sepal initiation in the form of a ring meristem surrounding the dome-shaped floral apex was also described for *Ricinus* (Prenner et al., 2008).

According to Kirchoff (2003) the position of the sepal will affect the flower orientation in the inflorescence and the different initiation patterns could be explained by the Hofmeister's rule described by Hofmeister's (1868). This model suggests that the

first sepal will appear in the first available space on the apex, as far as possible from the position of the subtending bract.

In the studied species the petals' initiation can also be explained by the mathematical model Hofmeister's rule (Hofmeister's 1868). The petals' primordia initiate simultaneously and with a relatively longer plastochron when compared to the sepals' and the primordia are radially displaced away from the center as they expand. The slower expansion rhythm of the petals is not stablished as a pattern and a faster development of petals and a slower expansion of sepals have also been reported for angiosperms (Reid 2005).

In the four species the first stamens primordia arise opposite to the petals and alternating with the sepals and the second stamen whorl arise opposite to the sepals and alternating with the petals. In *C. sphaerogynus* and *C. heliotropiifolius* the third and fourth stamens whorls follow the same orientation as the two first whorls. The development of the first stamen whorl opposite to the petals is uncommon and characterizes a state of obdiplostemony instead of the diplostemony usual pattern (Chatin 1855, Endress 1996, Ronse Decraene et al., 2014, Endress 2010, De Craene and Bull-Hereñu 2016) and here we corroborate our first hypothesis of this stud and considering the functional stamens, the obdiplostemony is true for the four studied species.

Obdiplostemony is described as an androecial configuration with two stamen whorls, with the outer whorl antepetalous and alternating with the sepals, and the inner whorl antesepalous (Ronse Decraene and Bull-Hereñu 2016). Obdiplostemony was early interpreted as an 'anomalous state' (Ronse Decraene & Smets 1987) and has been widely studied for different groups to understand the morphological and evolutionary implications of it, such as in Chatin 1855, Eckert 1966, Ronse Decraene 1989, Ronse

Decraene & Smets 1987, 1991, 1992, 1993, 1995, Endress 2010, Ronse Decraene et al., 2014, Ronse Decraene and Bull-Hereñu 2016 and Ronse Decraene 2018.

The studied species show the initiation of the outer stamens whorl as antepetalous and the development indicates that there is no shift in position of stamens, so that the outer stamens initiate as antepetalous and maintain this position during the development. The development of stamens in the studied species of *Croton* may be associated with the development of antesepalous nectaries and the petals' long plastochron. The development of nectaries opposite to sepals occupies the usual stamen space and the petal's delay fits an available space for stamens development.

The correlation between petals and stamens development observed in the studied species is corroborated by Endress (2010) in a study with *Geranium* and by Ronse Decraene and Bull-Hereñu (2016), where they state that obdiplostemony results from a reduction of the attachment area with a maximum space occupation in petals' area.

Ronse Decraene and Smets (1998) suggested that obdiplostemony is not only a developmental modification but most specially plays a key role in floral evolution, so that it could represent a change in progress and a transitional state of diplostemony, haplostemony or obhaplostemony.

Our study is the first record of obdiplostemony in *Croton* and based on flowers with different number of stamens and different sections we infer that obdiplostemony may be an ontogenetic pattern for the genus, though further studies with more species from different sections and different number of stamens would be necessary to understand the androecium development of the genus in an evolutionary context.

Vasculature of the staminate flowers

The vasculature of the studied flowers indicates a pattern in the number of vascular traits and bundles with five or ten vascular bundles in the stele, one to three vascular bundles in the sepals, one vascular bundle in the petals and one in each stamen filament, except for the central stamen of *C. fuscescens* which shows three vascular bundles in the early vasculature and later become fused in a single vascular bundle.

In a study with *Croton* and *Astraea* species, De Paula et al., (2011) verified a similar number of vascular bundles for the flower parts, except for not registering the three vascular bundles of *C. fuscescens* central stamen. According to Puri (1951) and Pandey and Chadha (1997) sepals are usually provided with three vascular traits, which may come from one or two sources, petals and stamens usually are provided with one vascular trait and carpels with three.

The occurrence of a central stamen with three vascular bundles in *C. fuscescens* can be interpreted as a homeosis case as the carpel features persisted in the stamen, what indicates an identity change of the structure. By definition, homeosis is the assumption by a structure of an organism of likeness to another structure, what in flower ontogeny means that at the site of one structure, another structure or its features are expressed (Sattler 1988). According to Baum and Donoghue's (2002) and Rudall and Bateman (2002), homeosis is a state of heterotopy, which comprises evolutionary changes in the position and expression of specific features. These authors define homeosis as a dynamic evolutionary transition in floral morphology where genetic identity is shared, such as exemplified in the studied species.

The vasculature of the nectaries occurred in different ways in the studied species. In *C. lundianus*, *C. fuscescens* and *C. heliotropiifolius* the species received vascular traces from the receptacle and in *C. sphaerogynus* the nectaries share

vasculature with the sepals. In the first case, the nectaries are adjacent to the androecium (stamens filament) during the whole development and we interpreted them as staminodes and in the second case we assumed the nectaries as calycinal.

The vasculature corroborates the obdiplostemony pattern observed in the development of *Croton* species, though the species show two different types of obdiplostemony. In the first case the nectaries are staminodes and part of the androecium, what turns the obdiplostemony true when we consider only the functional stamens, as the staminodal nectaries are antesepalous. In the second case, the nectaries are calycinal and we have a true obdiplostemony, in which the first stamen whorl is antesepalous.

The obdiplostemony pattern observed in *Croton* species was described as secondary (Ronse Decraene and Smets 1995). A secondary obdiplostemony was once reported for Euphorbiaceae by Venkata Rao Ramalakshmi (1968), who explained the occurrence of obdiplostemony by spatial shifts caused by the presence of large antesepalous nectaries. De Paula et al., (2011) have analyzed *Croton* flowers and stated that nectaries share vasculature with the sepals, though obdiplostemony had not been reported for the group yet.

Nectaries as part of androecium are interpreted here as a case of heterotopy, which by definition is an evolutionary change in the position of a structure (Baum & Donoghue 2002). Heterotopy is usually observed when there is morphic translocation (Leavitt 1905), transference of function (Corner 1958) or a spatial transference of a feature (Rudall and Bateman 2002). In the studied species, the staminodes assumed the role of nectaries and based on this we conclude that this heterotopy case is present in three of the four studied sections of *Croton*, with some more recent, such as *Julocroton*

and *Geiseleria* and in the less recent one, *Adenophylli* (Figure 8). Calycinal nectaries are

observed in the intermediate section *Cleodora* (Figure 8).

Figure 8. Simplified reconstruction of ancestral character state (nectaries and staminodes) in *Croton* phylogeny (modified from Van Ee et al. 2011).
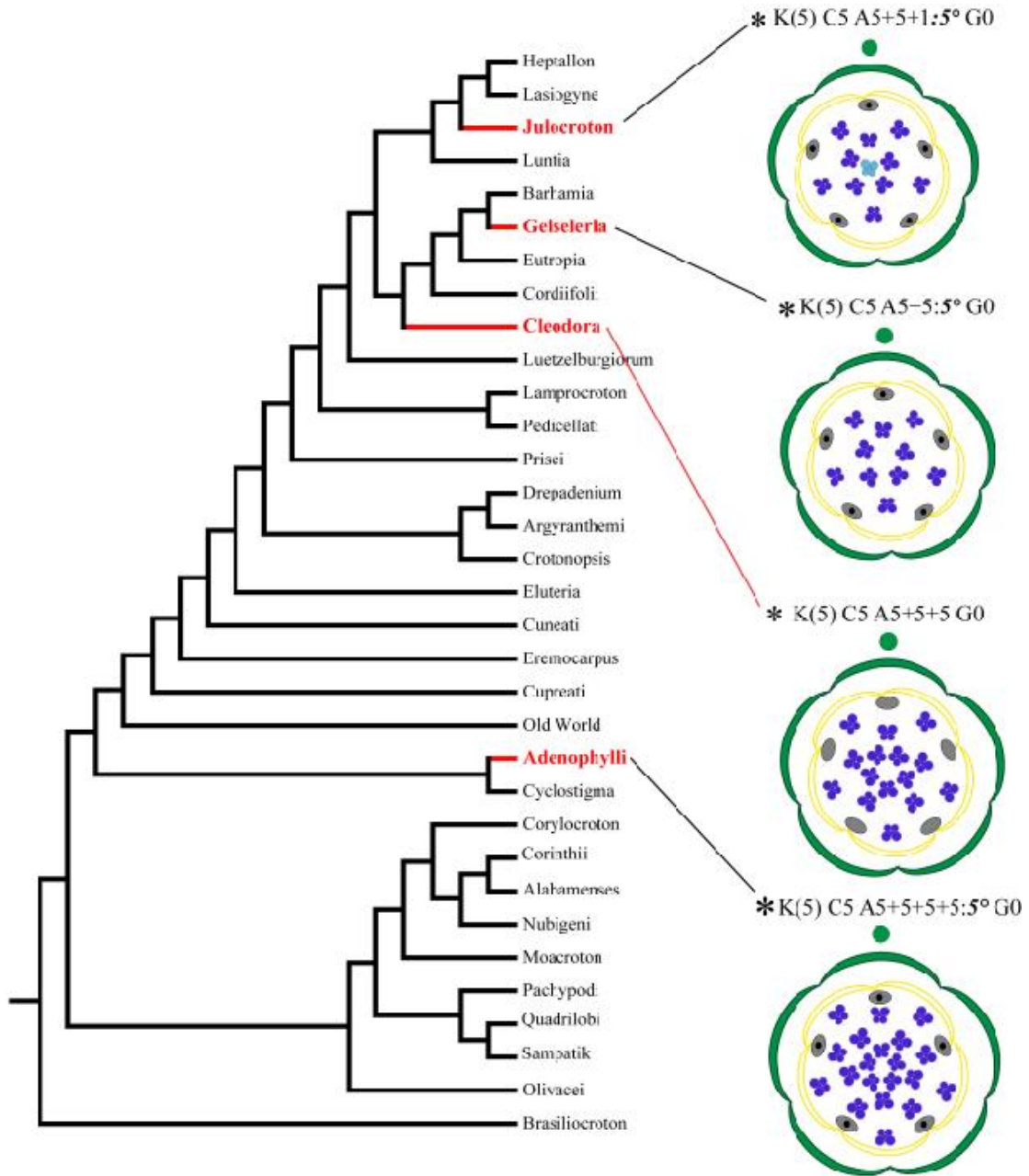
Although our study brings these novel information about a heterotopy case in *Croton*, further developmental and vasculature studies including species from different sections are important to conclude the role and origin of nectaries in the evolutionary history of *Croton*.

The vasculature of the androecium corroborates the obdiplostemony pattern observed in the flowers development. The outer vascular traces of the first stamens whorl diverge in an antepetalous direction, while the vascular traces of the second stamen whorl diverge into antesepalous way.

When totally expanded, stamens' filaments have one collateral vascular bundle each and according to Puri (1951) each flower whorl receives quite distinct vascular bundles from the stele. The widely discussed coevolution of petals and stamens (Ronse Decraene and Smets 1994, Ronse Decraene 2007, Ronse Decraene 2014, Gagliardi et al., 2017, Ronse Decraene 2018) could be an explanation for the presence of collateral vascular bundles in the stamens' filaments, instead of concentric vascular bundles, which are described as common for stamens (Puri 1951).

Auxin guides flower development in Croton

The flower development of the studied species showed different auxin concentration in different developmental steps.

The initial meristematic stage of *Croton* species showed a pool of auxin. According to Okada et al., (1991) and Aloni et al., (2006), high concentration of free IAA was also observed in *Arabidopsis* and the production of the hormone during floral-bud development suggests that high concentrations of free IAA could inhibit or retard organ-primordium initiation, what could be an explanation for the long plastochron of

petal's development observed in the studied species, thereby corroborating the hypothesis that auxin flow influences on the obdiplostemonal pattern of development.

The auxin basipetal IAA flow induces the development of stamens in *Croton*. In *Arabidopsis*, the major sites of high auxin concentration in developing floral buds are the young stamens, besides the apex of sepals and petals (Bowman et al., 1989, Aloni et al., 2006). The basipetal auxin flow is explained by Estelle and Klee (1994) and Nemhauser et al., (1998), who state that IAA fastly diffuses into cells, but it is specially exported at the base of cells, thereby polarizing them.

With the production of young stamens and the basipetal flow of IAA, high concentrations of the hormone are observed in the whole androecium region and in nectaries. The same concentration of auxin in stamens and nectaries can explain the fusion of these structures which was observed here in the flowers vasculature study. Opposite to the studied species, Oka et al., (1999) and Aloni et al., (2006) registered a basipetal IAA flow from the anthers to the nectaries, thereby reducing the hormone concentration in the anthers.

In *Croton* species the free IAA flow changes with the development of the young anthers and it gets unidirectional and highly concentrated in the androecium region. This high concentration induces the elongation of the stamen filaments and the total development of these structures with mature anthers. The same developmental steps were observed in *Arabidopsis* (Oka et al., 1999, Aloni 2004, Aloni et al., 2006).

The continuous IAA flow observed in the studied species seems to be fundamental in flower development and according to Cheng and Zhao (2007) any disruption of auxin pathways can profoundly affects flower the complete development of flower structures.

This is the first record of the auxin role in the development of *Croton* flowers. Considering that the staminate flowers of the genus show a wide range in the number of stamens and differences regarding the origin of nectaries, further investigations with IAA free flow are important to elucidate the morphological developmental differences in the genus.

CONCLUSIONS

Croton flowers have a great diversity regarding the androecium and this merism variety is a strong morphological character in the genus.

The development of the studied staminate flowers showed similar sepal initiation. Petals' development presented a longer plastochron when compared to sepals' and nectaries developed in antesepalous direction, both developmental steps which influenced in the androecium configuration as obdiplostemony.

The vasculature study of the flowers corroborates the obdiplostemony developmental pattern and clarifies two types of obdiplostemony for *Croton* species. The interpretation of nectaries as staminodes in three of the studied species was assumed here to be a heterotopy case and a homeosis case was evidenced in the central stamen of *C. fuscescens* with carpel features. The flower development of *Croton* species is guided by different auxin concentration in different developmental steps.

This is the first record of obdiplostemony and the role of auxin in *Croton* flower development. Further studies including species from different sections and with different merism are important to elucidate and the developmental patterns and the floral diversity in the evolutionary history of the genus.

ACKNOWLEDGMENTS

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Chapter 3 - Morphology and evolution of inflorescences in the large genus Croton L. (Euphorbiaceae)

Abstract

Background and Aims: Inflorescence morphology is a key trait in the systematics of angiosperms and directly influences their reproductive success by affecting flower display and pollination. The megadiverse genus *Croton* (Euphorbiaceae) shows a wide range of different inflorescence patterns, leading to contradictory systematic interpretations or interesting questions about reproductive biology. Morphological studies under a phylogenetic framework are key to the understanding of trait evolution, highlighting homoplastic features and diversification patterns. We aim to describe the predominant inflorescence patterns in *Croton* and associate these to systematic and evolutionary trends in the genus.

Methods: We studied morphological characters of the inflorescence in 213 species representing all major clades or sections of *Croton*. Inflorescence patterns were described, and traits were plotted on the most recent phylogeny of the genus for evolutionary and systematic discussion.

Key results: *Croton* inflorescences are variable especially regarding organization and distribution of pistillate and staminate flowers along the main axis and cymose subunits. Pistillate flowers vary more in their position and distribution along the inflorescence. They are usually clustered in the proximal region, a display associated to a temporal and spatial gap between sexual functions. Unisexual inflorescences are rarely described for *Croton*, and are associated to habitat and biogeographical history. The total number of flowers per inflorescence significantly increases in mesic environments as well as the proportion of pistillate flowers in xeric environments. These variations may be associated to changes in the dynamics of wind pollination between forests and open biomes.

Conclusions: Our results show that *Croton* has 17 inflorescence patterns that share similarities especially regarding the pistillate flowers. The inflorescence traits are very homoplastic, probably determined by convergent evolution in distantly related lineages distributed in similar habitats. Owning to their homoplasious nature, inflorescence traits can be used in systematics to diagnose sections in combination with other characters.

Keywords: habitat, homoplasy, pistillate flowers, selective pressure, sexual strategy, staminate flowers.

Introduction

Inflorescences, or the organization of multiple flowers on a plant axis during the reproductive phase (Troll and Weber 1953, Troll 1964, Weberling 1989, Prenner 2013), have long been of interest for botanists and evolutionary biologists (Kusnetzova 1988, Prenner et al. 2009, Endress 2010, Claßen-Bockhoff and Bull-Heren 2013, Harder and Prusinkiewicz 2013, Kirchoff and Claßen-Bockhoff 2013, Owens et al. 2016). They directly influence the reproductive success of a plant by affecting flower presentation in space and time (Kirchoff and Claßen-Bockhoff 2013) and a diversity of shapes and architectures characterize distinct angiosperm groups (Endress 2010).

The diversity of flowers and inflorescences is an important feature of Euphorbiaceae, which is composed of about 6300 species (Wurdach and Farfan-Rios 2017). This family is especially known for the highly reduced and condensed inflorescences, the pseudanthium, found in *Euphorbia* L. and *Dalechampia* L. (Webster 1994a). Nevertheless, basically cymose inflorescences with flowers either solitary or arranged in cymules or glomerules, often grouped into spiciform or condensed thyrses, are also well represented in the tribe Hippomaneae and genera *Tragia* L. and *Croton* L. (Webster 1994b).

Croton is a giant, monophyletic genus (Berry et al. 2005; Van Ee et al. 2011, Arevalo et al. 2017) and the second largest in the family between 1200 to 1300 species. It presents a cosmopolite distribution (Govaerts et al. 2000, Berry et al. 2005, Cordeiro et al. 2015), and is mostly found in tropical regions worldwide, especially in Brazil, the West Indies, and Mexico (Burger & Huft 1995), with some species distributed in subtropical and northern temperate areas. *Croton* shows great morphological diversity, with plant habit ranging from herbs to trees and a wide range of inflorescence architectures (Caruzo and Cordeiro 2013).

The organization of the small, unisexual flowers along the inflorescence axis has a historical systematic relevance in *Croton*. Flowers are often arranged in thyrses usually composed of solitary pistillate flowers proximally and cymules of staminate flowers distally (Webster 1967, Webster 1993), a pattern named as "agamoginaceous" by Radford (1986). Nevertheless, variations within this theme are frequently observed. Authors report, for instance, that instead of solitary pistillate flowers, there may also be pistillate flowers arranged in proximal cymules, as well as the occurrence of bisexual proximal cymules, with staminate and pistillate flowers composing the same cyme (Webster 1967, 1993, Cordeiro 1989, Caruzo 2005).

According to Webster (1993), there is an extensive variability in *Croton* inflorescences, which leads to a confusing interpretation in some groups (e.g. *Croton* sections *Cleodora* and *Cyclostigma* may have staminate only inflorescences described from staminate individuals, thereby suggesting dioecy). Caruzo (2005) organizes this variability and describes three different inflorescence patterns in Brazilian species of *Croton* based on the distribution of flowers: 1) flowers evenly distributed along the main axis of the thyrse, with proximal cymules bearing both pistillate and staminate flowers and distal cymules bearing exclusively staminate flowers; 2) flowers evenly distributed along the main axis of the thyrse, with proximal cymules bearing only pistillate flowers and distal cymules bearing only staminate flowers; 3) flowers unevenly distributed along the main axis of the thyrse, with an sterile area disconnecting the proximal pistillate cymules from the distal staminate ones. However, these patterns covered a reduced number of Brazilian *Croton* species, and have not yet been tested against recent phylogenetic frameworks in the genus (van Ee et al. 2011, Arevalo et al. 2017).

Studies of inflorescence diversity within smaller or larger clades show the diversity of forms and reveal which inflorescence forms are predominant in a group (Endress 2010). Associated with the morphological studies, the reconstruction of ancestral character states is a common approach used to understand the origins of key traits and their morphological evolution in different groups (Gruenstaeudl, 2016).

Studies about the evolution of morphological traits have been focused on a number of Euphorbiaceae taxa and characters, such as ovules and seeds in Euphorbioideae and Acalyphoideae (Tokuoka and Tobe 2002, 2003, Tokuoka 2007); cyathium (Park and Backlund 2002, Prenner and Rudall 2007, Prenner et al. 2008, 2011), growth form (Frajman and Schönswetter 2011, Horn et al. 2012), and photosynthetic pathway (Horn et al. 2014) in Euphorbieae; secretory structures and trichomes (Vitarelli et al. 2015) and wood anatomy (Wiedenhoeft et al. 2009, Arevalo et al. 2017) in *Croton*. The value of *Croton's* variable inflorescence patterns in discussing systematic and evolutionary trends in the genus, however, is yet to be unlocked.

The morphological study of this giant genus is essential to understanding the evolution of inflorescences, thereby highlighting their homoplastic and conserved features and the morphological diversification of these complex structures. Considering *Croton's* great variability in inflorescence architectures and the distribution and presence of unisexual (pistillate and staminate) flowers, the general aims of this study are: (1) to verify and describe in detail the predominant inflorescence patterns present in the genus and define the most common inflorescence arrangement for each *Croton* section; (2) to investigate the evolutionary patterns of individual inflorescence traits across *Croton's* evolutionary history; (3) to discuss selective pressures that may be important in the evolution of *Croton's* inflorescences diversity.

Material and Methods

We studied the morphology of *Croton* inflorescences by analyzing 220 species representing a thorough sample of the genus' phylogenetic, morphological and geographical diversity. Part of this sample (133 species) has been phylogenetically placed by recent studies and is highlighted in the original phylogenetic tree (Arevalo et al. 2017) in Fig. 1 and 2. Varieties and subspecies were not discriminated in our survey.

Herbarium analysis

We studied inflorescences directly from herbarium specimens with a Leica stereomicroscope. Some specimens were rehydrated for further investigation. In this case, samples were placed in boiling water for 10 min and kept in water overnight. After the hydration process, we performed dissections to clarify ramification patterns. We studied herbarium collections from K, BM, MA and SP (acronyms according to Thiers 2018). A table of the studied species, including taxonomic authorities, phylogenetic group (clade, section, genus), geographic distribution, species richness per group, and character state coding (habit, habitat, inflorescence traits) is provided in Tables 1, 2 and 3 (appendix of the thesis).

Reconstruction of characters

We plotted the morphological characters of the inflorescence on top of the most recent phylogeny of the genus (Arevalo et al. 2017) for ancestral state reconstruction (Fig. 1). This phylogenetic hypothesis is originally a dated tree of 312 tips. This tree was pruned to match the 133 species data information collected in our inflorescence survey using the function *drop.tip* from package *ape* in R (Paradis et al. 2004; R Core Team 2018).

Figure 1. Dated *Croton* phylogeny (Arevalo et al. 2017) with the studied species highlighted in red.
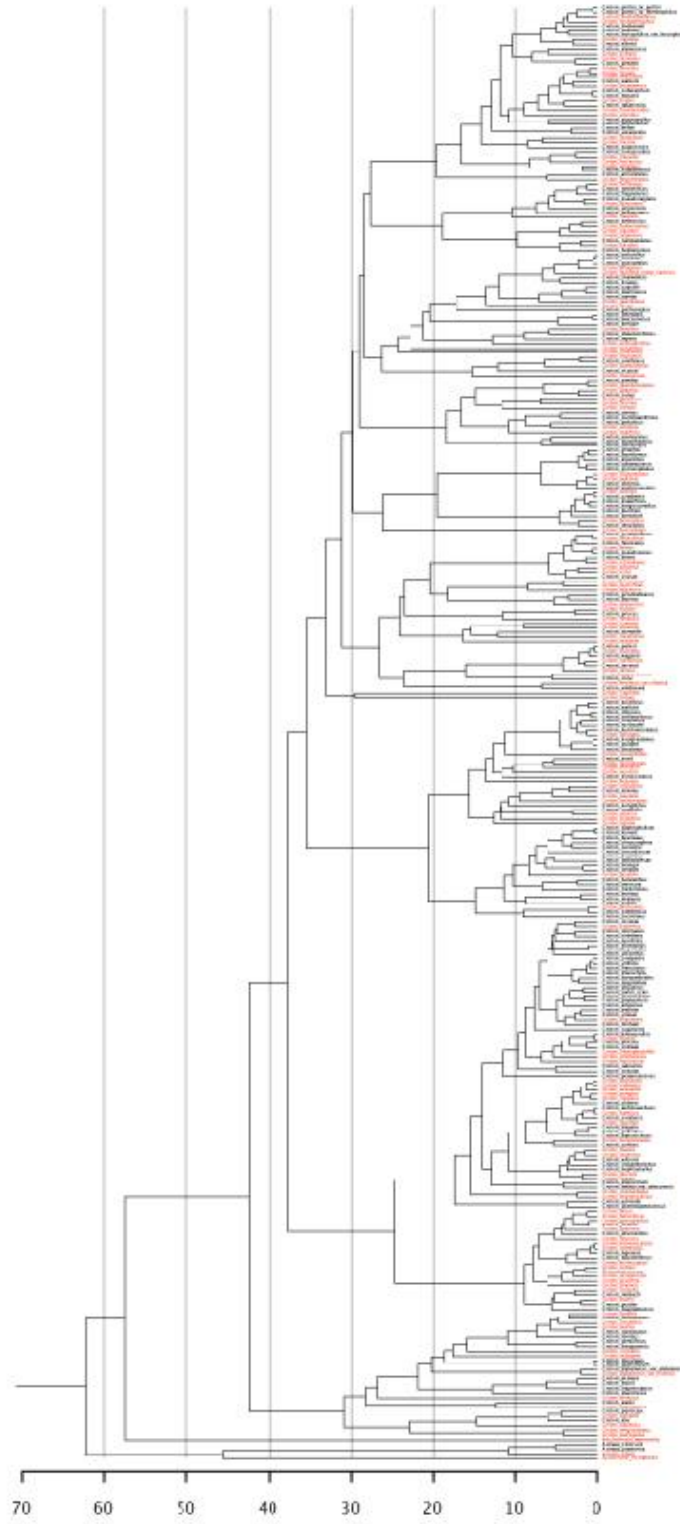
We used the results from our morphological survey to describe inflorescences in all 31 New World sections of *Croton* and the Old World clade or subgenus *Croton* sensu Van Ee et. al. (2011). We use the notation OW for the Old World clade (Table 1 and 2, appendix of the thesis). The sectional classification of the Old World species has not been clarified yet and the systematic knowledge of this clade is uneven in comparison to that of the New World species.

Characters used include (Table 1 and 2 - appendix of the thesis): total number of flowers per inflorescence, number of pistillate flowers per inflorescence, staminate flowers per inflorescence and proportion of staminate and pistillate flowers per inflorescence (number of pistillate flowers [fewer] divided per number of staminate [more numerous]). These were treated as continuous variables whilst the following ones were discrete (Table 2 appendix of the thesis): habitat and sexual mechanism. Habitat was scored according to herbarium labels, literature records, such as floras and taxonomic treatments, and personal field observations. To be consistent with a recent study on *Croton* contrasting wood anatomical characters with habitat and habit in a phylogenetic context, we follow the approach of Arevalo et al. (2017) using a simplified habitat characterization which reflects the predominant environmental preferences of the species: "xeric" (mostly open vegetation) and "mesic" (mainly closed vegetation types). Lastly, pistillate flowers clustered, staminate cymules, pistillate cymules and bisexual cymules were treated as binary variables.

Character reconstruction of discrete and binary traits was performed in a Bayesian inference approach using 1000 simulations using functions *make.simmap* and *describe.simmap* in package *phytools* (Revell, 2012). Continuous characters were reconstructed under a Brownian Motion model using function *contMap*, also in package *phytools*. These reconstructions were used to estimate the number of state changes,

Figure 2. *Croton* phylogeny containing only the studied species.
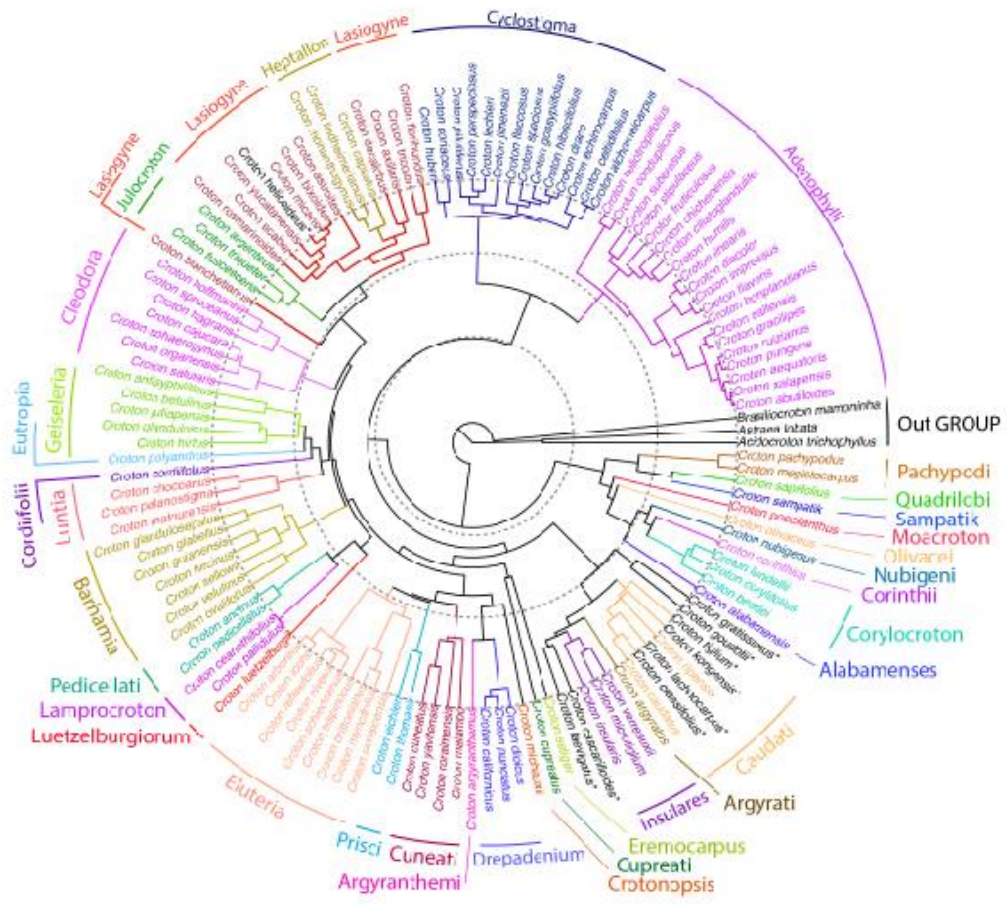
pleisiomorphic state, reversions and morphological trends for each section. All packages are available in the R platform (R Core Team, 2018).

Trait correlations

Trait correlations were used to infer environmental pressures that can act on inflorescence traits across evolutionary time. The gynoecium is fairly stable in *Croton* flowers (Webster 1975, 1993), whereas stamen number is quite variable. We therefore annotated stamen number and used it to estimate a proxy of investment in male function (number of staminate flowers multiplied by number of stamens). The average number of stamens per species was estimated by the arithmetic mean between the maximum and minimum number counted in the flowers.

To identify different sexual strategies, we discriminated the number of pistillate flowers, number of staminate flowers, pistillate/staminate proportion and the number of stamens in the male flowers. These traits were revised for phylogeny independence using function *pic* in package *ape* (Paradis et al., 2004), following the premise that phylogenetic related taxa tend to have more similar traits and data points cannot be treated as independent variables (Felsenstein, 1985). Investment in female and male functions was then tested for correlation against habitat variables (xeric and mesic) using a Kruskal-Wallis ANOVA rank sum test. A correlation matrix between continuous characters was also produced using function *corrplot* in package *corrplot* (Wei & Simko, 2016) to highlight the correlation between these traits (i.e. to test if changes in one trait affect other trait). Again, all analyses were performed in the R platform (R Core Team, 2018).

Results

General morphology of *Croton* inflorescences

The studied *Croton* species present flowers arranged in open thyrses, i.e. racemose inflorescences, with a racemose main axis and cymose branching and subunits (Fig. 3A). The inflorescences can be either terminal or axillar (Fig. 3B, C) and usually have a long first order main axis (Fig. 4A-C). The second order branch can be either long or short (Fig. 4A-C, 4F-H). In the first case, the inflorescences show a ramified aspect, such as observed in nine sections (Table 2 - appendix and Fig. 4D); when the second order branch is short, the inflorescences show a non-ramified, spike-like shape (Table 2 – appendix and Fig. 4E), such as observed in all the studied sections, except the ones mentioned above (Table 2 - appendix).

The shortening of the second order branch may lead to a very condensed shape of the inflorescences, such as observed in eight sections and in the outer group, *Acidocroton* (Table 2 - appendix, Fig. 4F-H). The condensed aspect can also be observed in *C. amentiformis* (*Croton* sect. *Cyclostigma*), which shows an exclusive spike shape with almost sessile flowers (Fig. 4I).

There are different leafy structures within the inflorescence. Prophylls are present along the main axis and precede lateral, second order ramifications. Prophylls also develop in the second order branches and precede the lateral cymules (Fig. 4J). The prophylls may be either reduced or rarely conspicuous (Fig. 4J), such as observed in *C. glaziovii* (*Croton* sect. *Cyclostigma*).

Figure 3. General morphology of *Croton* inflorescences. **A**. Thyrse. **B**. Terminal branches. **C**. Axillary branches. **D**. Long main axis (note the circle showing continuous growth). **E.** Long second order branches. **F**. Short second order branches. **G**. Small leafy structures. **H**. Conspicuous leafy structures. **I**. Bisexual inflorescence with pistillate flowers clustered. **J.** Unisexual inflorescence.

Figure 3. General morphology of *Croton* inflorescences. **A**. Thyrse. **B**. Axillary branches. **C**. Terminal branches.

A
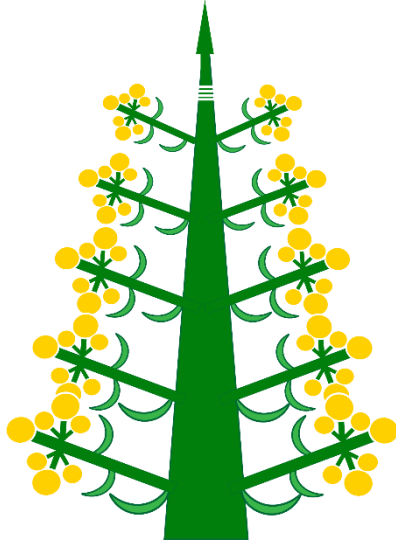
B

C

Croton inflorescences are bisexual and rarely unisexual (Table 2 - appendix, Fig. 4K-N). When bisexual, staminate and pistillate flowers are usually separated along the inflorescences, thus with a distinct staminate portion and a pistillate portion (clustered pistillate flowers). This d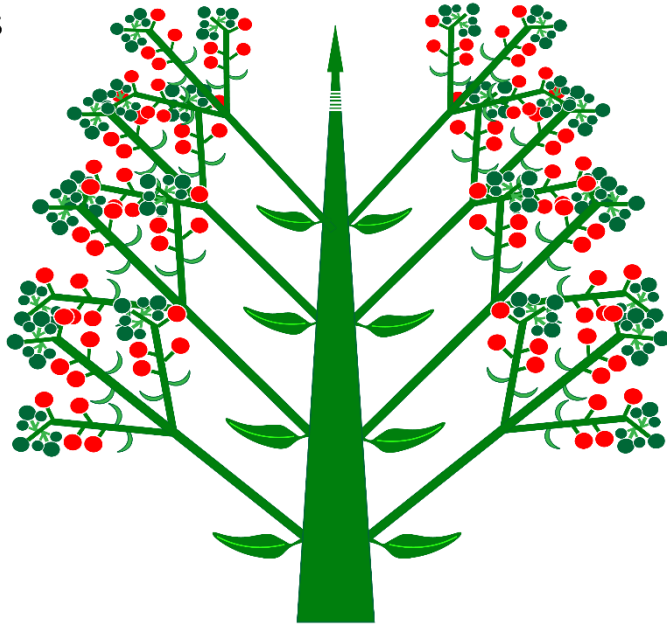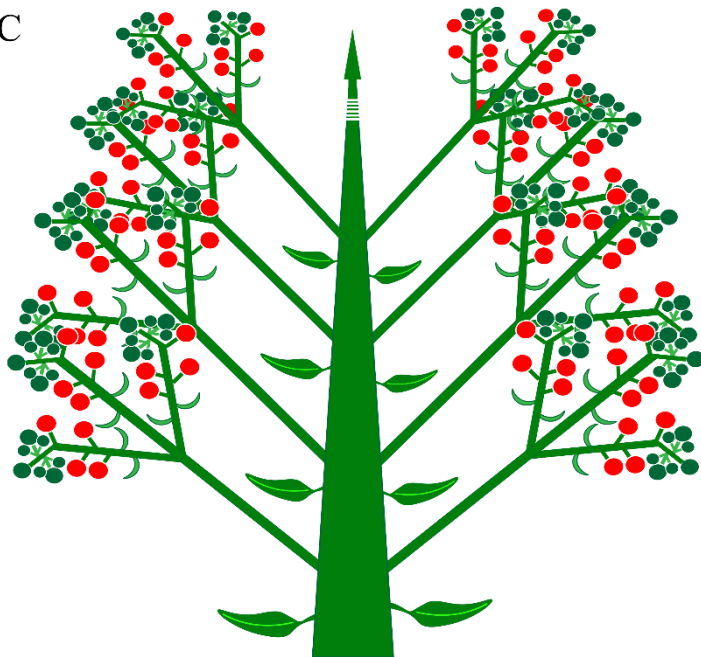istribution was observed in four Old World species and in New World species of 22 sections. *Brasiliocroton*, the sister genus of *Croton*, also showed concentrated pistillate flowers (Table 2 - appendix, Fig. 4K, L).

Staminate and pistillate flowers may be together composing the same cyme or arising from the same node, such as in six Old World species, 14 New World sections and in the other Crotonoid genera *Astraea* and *Acidocroton* (Table 2 - appendix, Fig. 4M, N).

Unisexual inflorescences were observed in the minority of the surveyed species, such as in *Croton* sect. *Drepadenium*, *C.* sect. *Adenophylli* (exclusively in *C. discolor* and *C. linearis*), *C.* sect. *Cordiifolii* and *C.* sect. *Quadrilobi* (Table 2 - appendix, Fig. 4O).

Croton **inflorescence patterns**

Croton inflorescences show different inflorescence patterns and the morphological variations are especially related to the distribution of flowers along the main axis, elongation or shortening of branches and the organization of staminate and pistillate flowers. The description of each observed pattern is presented below in three categories: elongated inflorescences, condensed inflorescences, and special inflorescences.

Elongated inflorescences

Pattern 1: This is the most predominant inflorescence pattern in *Croton.* The proximal region of the main axis is composed of single, pediceled, pistillate flowers and the distal

Figure 4. General morphology of *Croton* inflorescences. **A-C.** Elongated inflorescence (*Croton lechleri*). **B**. *Croton blanchetianus*. **C.** *Croton urucurana*. **D.** Inflorescence with ramified aspect (*Croton piptocalyx*). **E.** Inflorescence with non-ramified aspect (*Croton pavonis*). **F-G.** Condensed inflorescence in *Croton myrsinites*. **G.** *Croton setigerus*. **H.** *Croton humilis*. **I.** Spike in *Croton amentiformis*. **J.** Conspicuous bracts in *Croton glaziovii*. Note the bracts (arrowhead). **K, L.** Inflorescence with pistillate flowers clustered in proximal region in *Croton heliotropiifolius*. **L.** *Croton sellowii*. **M, N**. Inflorescence with bisexual cymules in *Croton celtildifolius*. **N.** *Croton echinocarpus*. **O.** Unisexual inflorescences with exclusive pistillate flowers (*Croton discolor*).

region has cymules with staminate flowers. The 2nd and 3rd order branches are elongated. This pattern was observed in some Old World species and in New World species of 14 sections (Table 2 – appendix, Fig. 5A).

Pattern 2: This pattern is a variation of pattern 1. The inflorescence's main axis is composed of mostly single, pediceled, pistillate flowers. At the apex of the main axis there is a ramification with a pistillate flower and cymules with staminate flowers. This pattern was observed in species of two New World sections (Table 2 – appendix, Fig. 5B).

Pattern 3: This pattern is a variation of pattern 2. The proximal region of the main axis is composed of single, pediceled, pistillate flowers and the distal region has pistillate flowers and cymules with staminate flowers (similar to the apex ramification of pattern 2). The 2nd order branch (composed of cymules with staminate flowers) elongates from the same node of a single pistillate flower (Fig. 5C). This pattern was observed in the Old World *Croton laevigatus* and in the New World *Croton* sect. *Eluteria* (*C. schiedeanus*) (Table 2 – appendix,).

Pattern 4: This pattern is a variation of pattern 3. The whole inflorescence is composed of pistillate flowers and cymules with staminate flowers (similar to the distal region of pattern 3) (Fig. 5D). This pattern was observed exclusively in *Croton* sect. *Crotonopsis* (Table 2 – appendix).

Pattern 5: The second most predominant pattern has staminate and pistillate flowers organized in cymules. The main axis proximal region is composed of cymules with pistillate flowers and the distal region presents cymules with staminate flowers. The 2nd and 3rd order branches are quite elongated (Fig. 5E). We found this pattern in eight New World sections (Table 2 – appendix).

Figure 5. Architecture of long inflorescences: patterns 1-9. A. Pattern 1. The most common pattern in *Croton*. **B**. Pattern 2. Variation of pattern 1 with mostly pistillate flowers. **C.** Pattern 3. Variation of pattern 2. **D.** Pattern 4. Variation of pattern 3. **E.** Pattern 5. The second most common pattern in *Croton*. **F**. Pattern 6. Variation of pattern 5 with a sterile space between pistillate and staminate region. **G.** Pattern 7. Third most common pattern in *Croton*. **H.** Pattern 8. Variation of pattern 7 with sterile spaces between staminate cymules. **I**. Pattern 9. Variation of pattern 8.
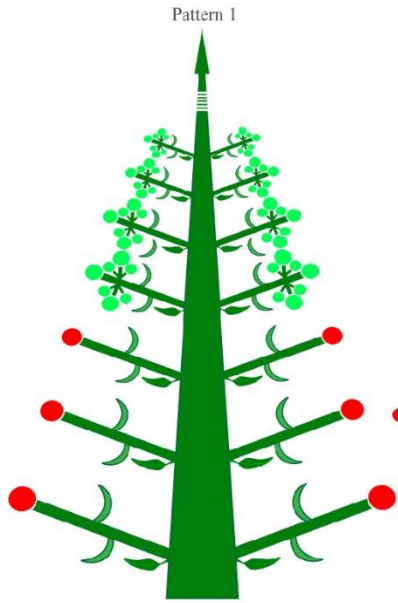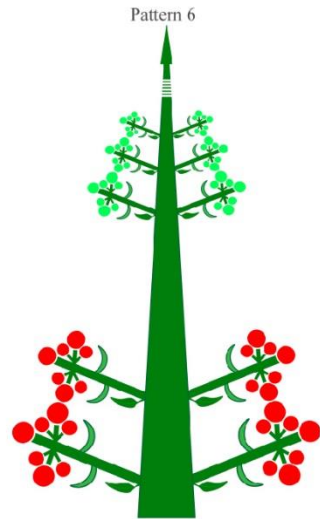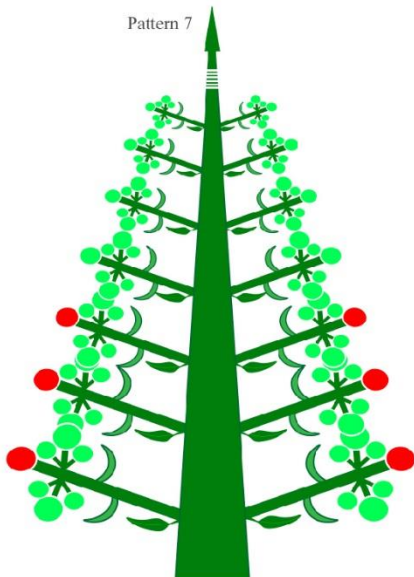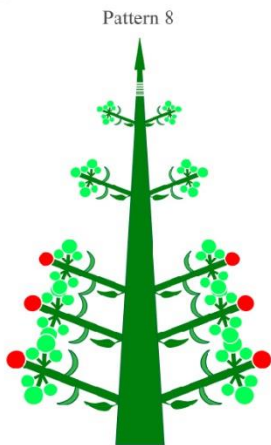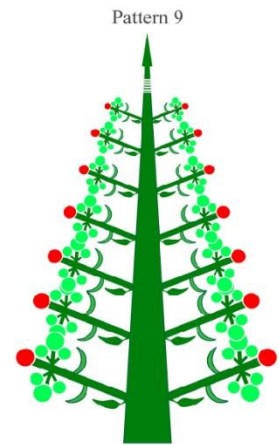
Pattern 6: This pattern is similar to pattern 5, except for the presence of a sterile space between the proximal pistillate region and the distal staminate one (Fig. 5F). This was observed in two New World sections (Table 2 – appendix).
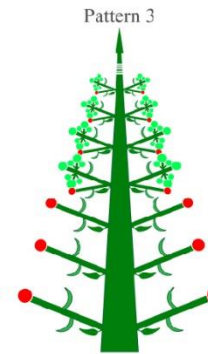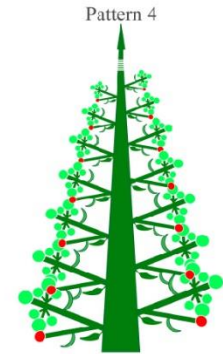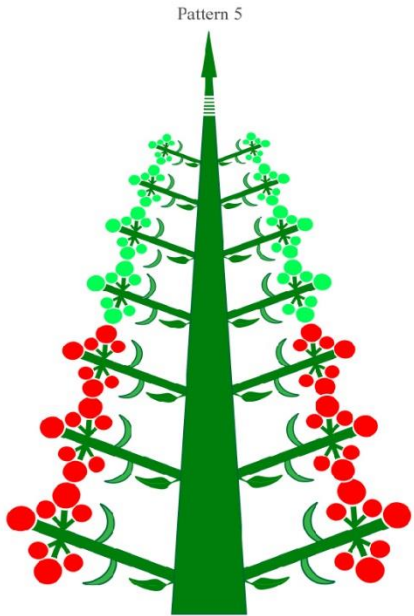
Pattern 7: This is the third most common pattern found in *Croton* inflorescences. The proximal region of the main axis is composed of bisexual cymules (with pistillate and staminate flowers in the same cymule) and the distal region is exclusively composed of cymules with staminate flowers. The 2nd and 3rd order branches are elongated (Fig. 5G). This pattern was found in some Old World species, in 11 New World sections and in the genus *Astraea* (Table 2 – appendix).

Pattern 8: This pattern is a variation of pattern 7. The proximal region of the main axis is composed of bisexual cymules, such as in pattern 7. The distal region is exclusively composed of cymules with staminate flowers. These cymules are clustered and there are sterile spaces separating them. The 2nd and 3rd order branches are elongated (Fig. 5H). This pattern was found in some Old World species and in three New World sections (Table 2 – appendix).

Pattern 9: This pattern is a variation of pattern 7. The whole inflorescence is composed of bisexual cymules (similar to the proximal region of pattern 7). The 2nd and 3rd order branches are quite elongated (Fig. 5I). This pattern was observed in *Croton* sect. *Cyclostigma* (*C. rusbyi*) and in the genus *Brasiliocroton* (Table 2 – appendix).

Condensed inflorescences

Pattern 10: This pattern has the same organization of pistillate and staminate cymules as pattern 5, though the whole inflorescence is very condensed. The 2nd and 3rd order branches are short, as well as the space between the nodes (Fig. 6A). This pattern was found in four New World sections (Table 2 – appendix).

Figure 6. Architecture of condensed (patterns 10-14) and unusual inflorescences (15-17). **A**. Pattern 10. A condensed version of pattern 5. **B.** Pattern 11. A condensed version of pattern 1. **C.** Pattern 12. A condensed version of pattern 4. **D**. Pattern 13. A condensed version of pattern 7. **E.** Pattern 14. Staminate and pistillate cymules randomly positioned in the inflorescence axis. **F**. Pattern 15. Bisexual inflorescence with a terminal pistillate flower. **G.** Patter 16. Unisexual pistillate inflorescence with flowers organized in a single cyme. **H.** Pattern 16. Unisexual staminate inflorescence with single staminate flowers, not organized in cymules. **I.** Unisexual pistillate inflorescence with a terminal flower. **J.** Unisexual staminate inflorescence with flowers organized in cymules.

A Pattern 10
B Pattern 11
C Pattern 12
D Pattern 13
E Pattern 14
F Pattern 15
G Pattern 16 ♀
H Pattern 16 ♂
I Pattern 17 ♀
J Pattern 17 ♂
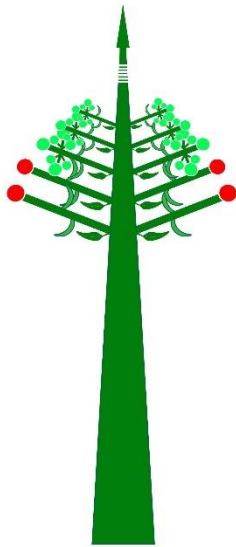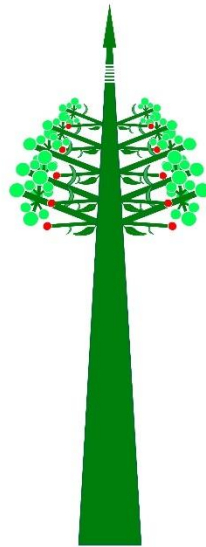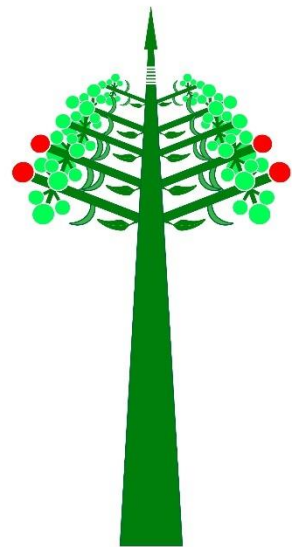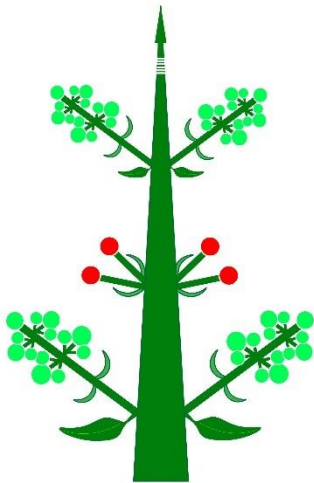
Pattern 11: This pattern is similar to pattern 1, though the whole inflorescence is very condensed and 2nd, 3rd order branches are short, as well as the space between the nodes (Fig. 6B). This pattern was observed in five New World sections (Table 2 – appendix).

Pattern 12: similar to pattern 4, though the whole inflorescence is very condensed and 2nd, 3rd order branches are short, as well as the space between the nodes (Fig. 6C). This pattern was found in two New World sections and in *Acidocroton* (Table 2 – appendix).

Pattern 13: similar to pattern 7, though the inflorescence is very condensed. The 2nd and 3rd order branches are short, as well as the space between the nodes (Fig. 6D). This pattern was observed exclusively in *Croton* sect. *Heptallon* (Table 2 – appendix).

Special inflorescence patterns

The patterns 14–17 were not included in any of the two main categories above, because they are very different from the other patterns.

Pattern 14: This pattern shows pistillate and staminate flowers organized in cymules which are randomly distributed along the inflorescence main axis. The cymules with staminate flowers are predominant and present in the proximal and distal region. Cymules with pistillate flowers are less frequent in the inflorescence and are distributed in the middle of the axis, between the staminate ones (Fig. 6E). This pattern was observed in two New World sections (Table 2 – appendix).

Pattern 15: This pattern has pistillate and staminate flowers differently organized along the different branches. The first order main axis is composed of single-pediceled pistillate flowers and there is a terminal pistillate flower. The 2nd order branches are elongated and composed of single pistillate flowers in the proximal region and cymules

with staminate flowers are in distal position (Fig. 6F). This pattern was exclusively observed in *Croton* sect. *Crotonopsis*, in which we also observed pattern 4 (Table 2 – appendix).

Pattern 16: This pattern shows unisexual inflorescences. The pistillate inflorescence has a long first order main axis and the flowers are arranged in single group of cymules (Fig. 6G). The staminate inflorescence has single pediceled flowers along the whole main axis (Fig. 6H). This pattern was exclusively observed in *Croton* sect. *Quadrilobi* (Table 2 – appendix).

Pattern 17: This pattern shows unisexual inflorescences. The pistillate inflorescence has a long first order main axis composed of single pediceled flowers and a terminal flower (Fig. 6I). The staminate inflorescence also has a terminal flower and the flowers are organized in cymules (Fig. 6J). This pattern was observed in three New World sections (Table 2 – appendix).

Trait reconstructions and correlations

Flower features and inflorescences sexual mechanism

Pistillate flowers show variation in their arrangement, i.e. single or in cymules, and in the distribution along the axis of *Croton* inflorescences.

Pistillate flowers predominantly occur as single flowers along the inflorescence axis (Fig. 7A-B), such as observed in patterns 1–4, 11–12, 15 and 17. They may also be organized in cymules of exclusively pistillate flowers (Fig. 7A, C), such as observed in patterns 5–6, 10, 14 and 16. Our analysis suggests that single flowers predominate as a pleisiomorphic state with sixteen independent evolution events of flowers organized in cymules in the genus *Brasiliocroton* and in *Croton* sections *Pachypodi*, *Quadrilobi*, *Nubigeni*, *Corinthii*, *Caudati*, *Crotonopsis*, *Drepadenium*, *Eluteria*, *Lamprocroton*,

Figure 7. Inflorescence traits in *Croton*: organization of flowers. **A.** Distribution of solitary pistillate flowers or pistillate flowers in cymules. **B.** Solitary pistillate flowers (diagram and photo). **C.** Pistillate flowers organized in cymules (diagram and photo). **D**. Distribution of bisexual cymules. **E**. Bisexual cymules (diagram and photo).
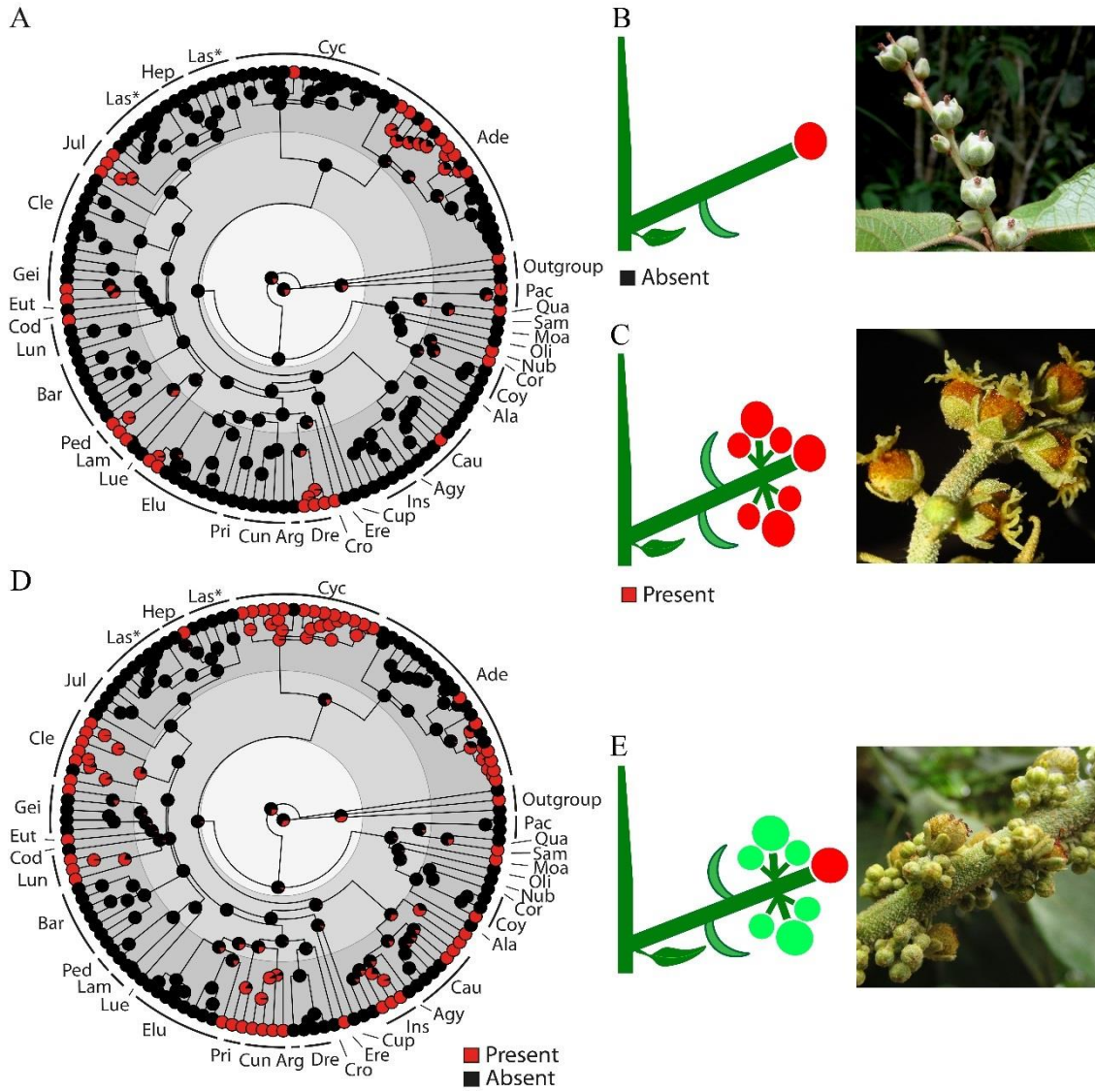
Pedicellati, *Cordiifolii*, *Geiseletia*, *Julocroton*, *Cyclostigma* and *Adenophylli* with at least three reversal events in section *Adenophylli*.

Together with staminate flowers, pistillate flowers may also compose bisexual cymules (Fig. 7D-E), such as described for patterns 7–9 and 13. The pleisiomorphic state is unisexual cymules with twenty-one independent evolution events of bisexual cymules in the genus *Astraea* and in *Croton* sections *Sampatik*, *Moacroton*, *Corylocroton*, *Caudati*, *Insulares*, *Eremocarpus*, *Cuneati*, *Prisci*, *Eluteria*, *Luntia*, *Eutropia*, *Geiseleria*, *Cleodora*, *Heptallon*, *Cyclostigma* and *Adenophylli*. There is evidence of at least one reversal event in sections *Caudati*, *Geiseleria*, *Cleodora*, *Heptallon*, *Cyclostigma* and at least three reversal events in *Adenophylli*.

Pistillate flowers may be clustered in one region (proximal or distal) of the inflorescence, or they may be found along the whole inflorescence (Fig. 8A-C). Our analysis suggests that clustered pistillate flowers is the pleisiomorphic state with evidence of nine independent events of evolution towards absence of this pattern in the outgroup genera *Brasiliocroton* and *Acidocroton* and in the *Croton* sections *Insulares*, *Crotonopsis*, *Eluteria*, *Luetzelburgiorum*, *Pedicellati* and *Heptallon* with two evidences of reversal in *Eluteria* and one in *Heptallon* (Fig. 8A).

Staminate flowers represent a stable character in *Croton* inflorescences, because they are in most cases arranged in cymules (Fig. 8A-B), such as observed in all the patterns described. They are only rarely solitary (Fig. 6H), exclusively found in pattern 16. Staminate flowers occur mostly in the distal region of the inflorescences (Fig. 8B), such as observed in patterns 1–3, 5–6, 10–11, though we also found them along the whole inflorescence (Fig. 8C), such as in patterns 4, 7–9, 12–14, 16 and 17.

Figure 8. Inflorescence traits: distribution of pistillate flowers. **A.** Distribution of clustered pistillate flowers. **B.** Pistillate flowers concentrated (diagram and photo). **C.** Pistillate flowers along the whole inflorescence axis (diagram and photo).
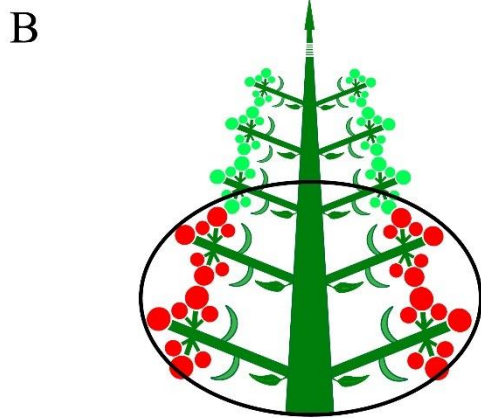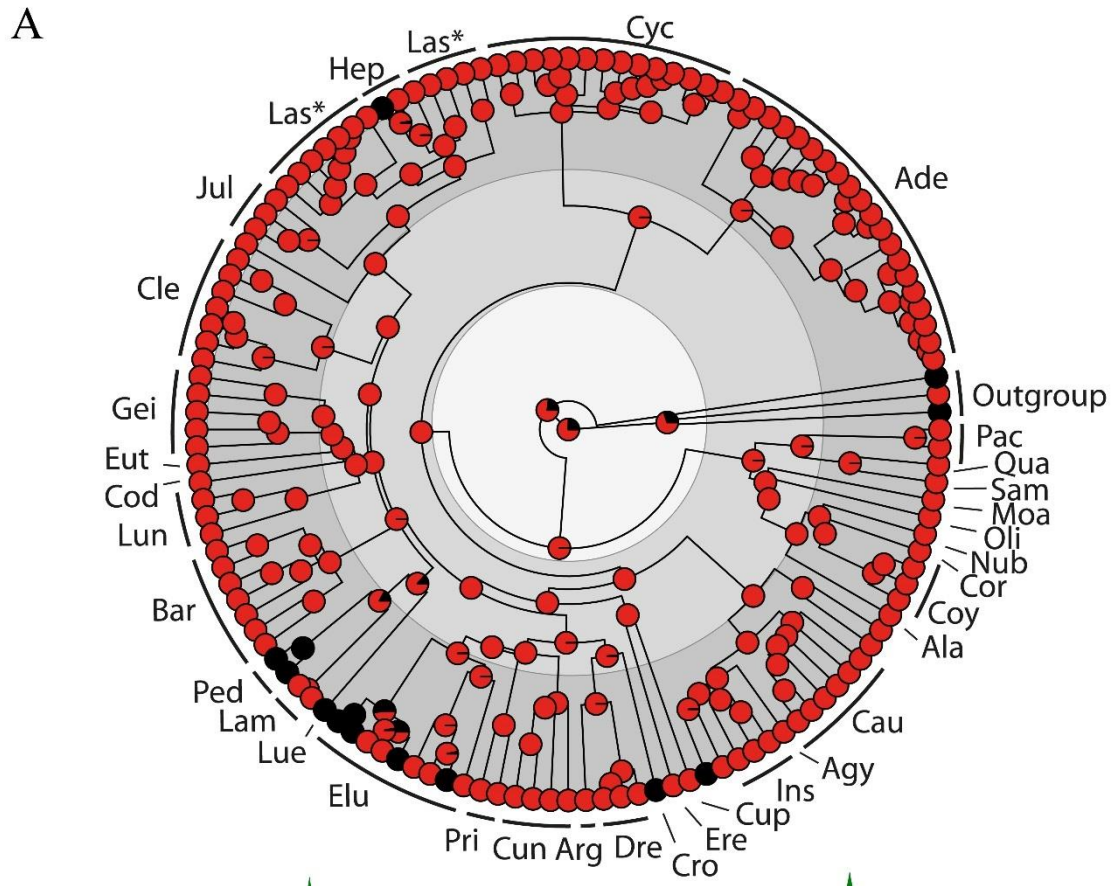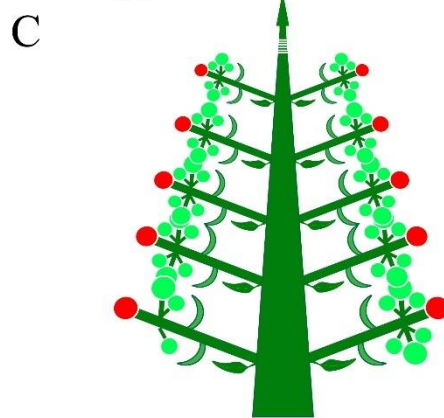
Bisexual inflorescences are predominant in *Croton* (Fig. 9A-C), and this is the pleisiomorphic state with four independent events of unisexual inflorescence evolution in sections *Adenophylli*, *Quadrilobi*, *Drepadenium* and *Cordiifolii*. There is no evidence for reversal for this state (Fig. 9A). From the four events of unisexual inflorescences, one of them is described here as pattern 16 (Fig. 9C) and the other three, as pattern 17 (Fig. 9B).

Figure 9. Inflorescence traits: sexual mechanism. **A.** Distribution of bisexual and unisexual inflorescences. **B.** Unisexual inflorescence schemes. Note the pistillate solitary flowers and staminate flowers in cymules. **C**. Unisexual inflorescence schemes. Note the pistillate single cymules and the staminate solitary flowers.
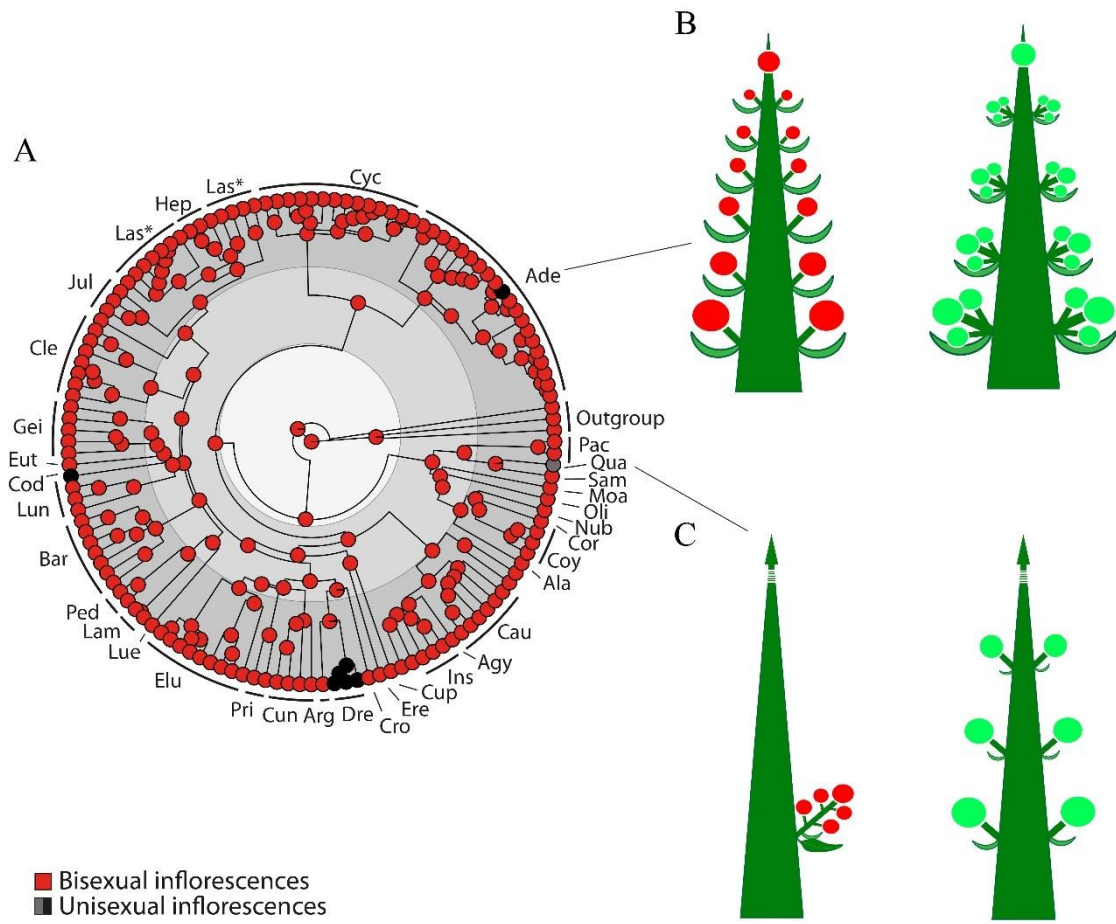
Reconstructions show that the ancestral inflorescence in *Croton* was likely to be a few flowered (Fig. 10A), with a total number of flowers around 25. The number of pistillate and staminate flowers shows a wide range among the sections. Pistillate flowers are present in low number and this seems to be the pleisiomorphic state (Fig. 10B), which is around five flowers, observed in *Croton* sect. *Eluteria*. Two hundred pistillate flowers was the highest number, observed in *C.* sect. *Cyclostigma*.

Staminate flowers are present in bigger numbers than the pistillate flowers, though the pleisiomorphic state is a low number of staminate flowers per inflorescence (Fig. 10C), which is around 15 flowers, observed in *Croton* sect. *Pedicellati*. The highest number registered was 1200 staminate flowers per inflorescence, observed in *C.* sect. *Cyclostigma*.

Our analysis suggests a low proportion of pistillate flowers against staminate flowers, which is the pleisiomorphic state for *Croton* inflorescences, with exception of the outgroup genus *Brasiliocroton*, which shows a high proportion of pistillate against staminate flowers (Fig. 10D).

Figure 10. Inflorescence traits: number of flowers and correlations. **A**. Total number of flowers per inflorescence. **B.** Number of pistillate flowers per inflorescence. **C.** Number of staminate flowers per inflorescence. **D.** Proportion of pistillate flowers vs. staminate flowers.
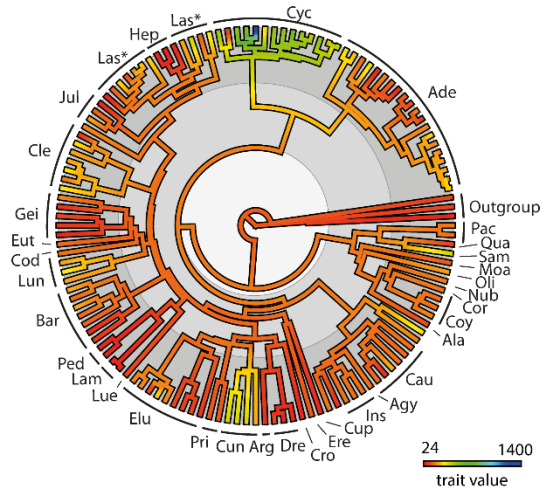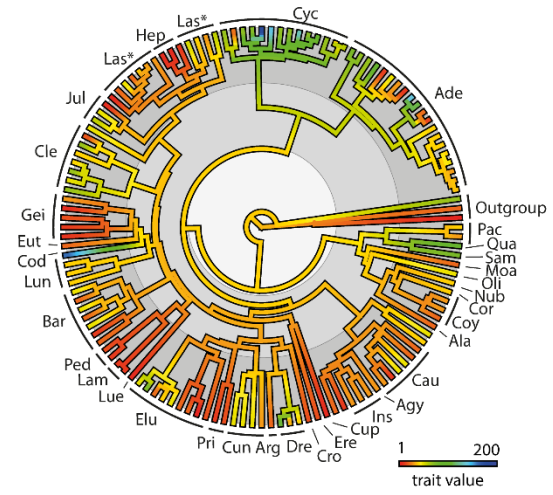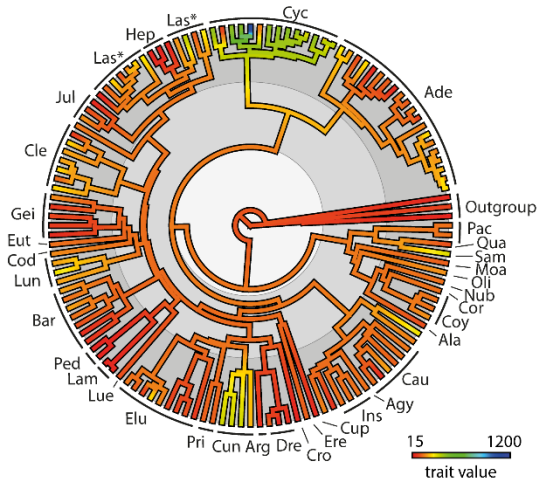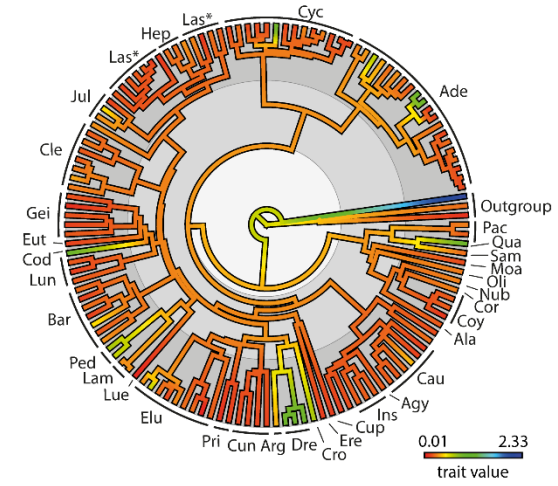
Number of flowers and stamens versus habitat

The number of flowers and stamens change according to the habitat they are found, which may go from xeric (open vegetation) to mesic (closed vegetation) habitats.

The total number of flowers per inflorescence increases significantly in mesic environment (Fig. 11A). The number of pistillate flowers is not significantly affected by the habitat and the average of flowers is similar in all types of environment, except for the xeric places, on which the average of flowers is lower than in the other habitats (Fig. 11B).

Similar to the pistillate flowers, the staminate flowers show a significant increase in mesic places and a drop in xeric environments (Fig. 11C). The proportion of pistillate to staminate flowers (number of pistillate flowers divided by number of staminate flowers) increases significantly in xeric environments (Fig. 11D).

The number stamens present a wide range among the sections, thereby varying from 6, such as in *Croton* sect. *Heptallon* (*C. monanthogynus*), *C.* sect. *Eluteria* (*C. schiedeanus, C. jamaicensis, C. myricifolius* and *C. brittonianus*), *C.* sect. *Eremocarpus* and *C.* sect. *Crotonopsis* up to 150 stamens, such as in *C.* sect. *Cyclostigma* (*C. speciosus*) (Table 3 – appendix). This is a variable character and our analysis suggests that both the number of stamens and the investment in male function (number of stamen in relation to the number of staminate flowers) are significantly affected by the environment. These last character increases in mesic environment, though the average is quite similar to the other habitats (Fig. 11E-F).

Figure 11. Number of flowers and stamens vs. habitat. **A.** Total number of flowers. Note high significance (P = 0.002). **B.** Total number of pistillate flowers with low significance (P = 0.14). **C.** Number of staminate flowers with high significance (P = 0.03). **D.** Proportion of pistillate vs. staminate flowers with significance (P = 0.0002). **E.** Number of stamens with low significance (P = 0.05). **F.** Investment in male function with low significance (P = 0.17). (D = dry; DW = dry to wet; W = wet).
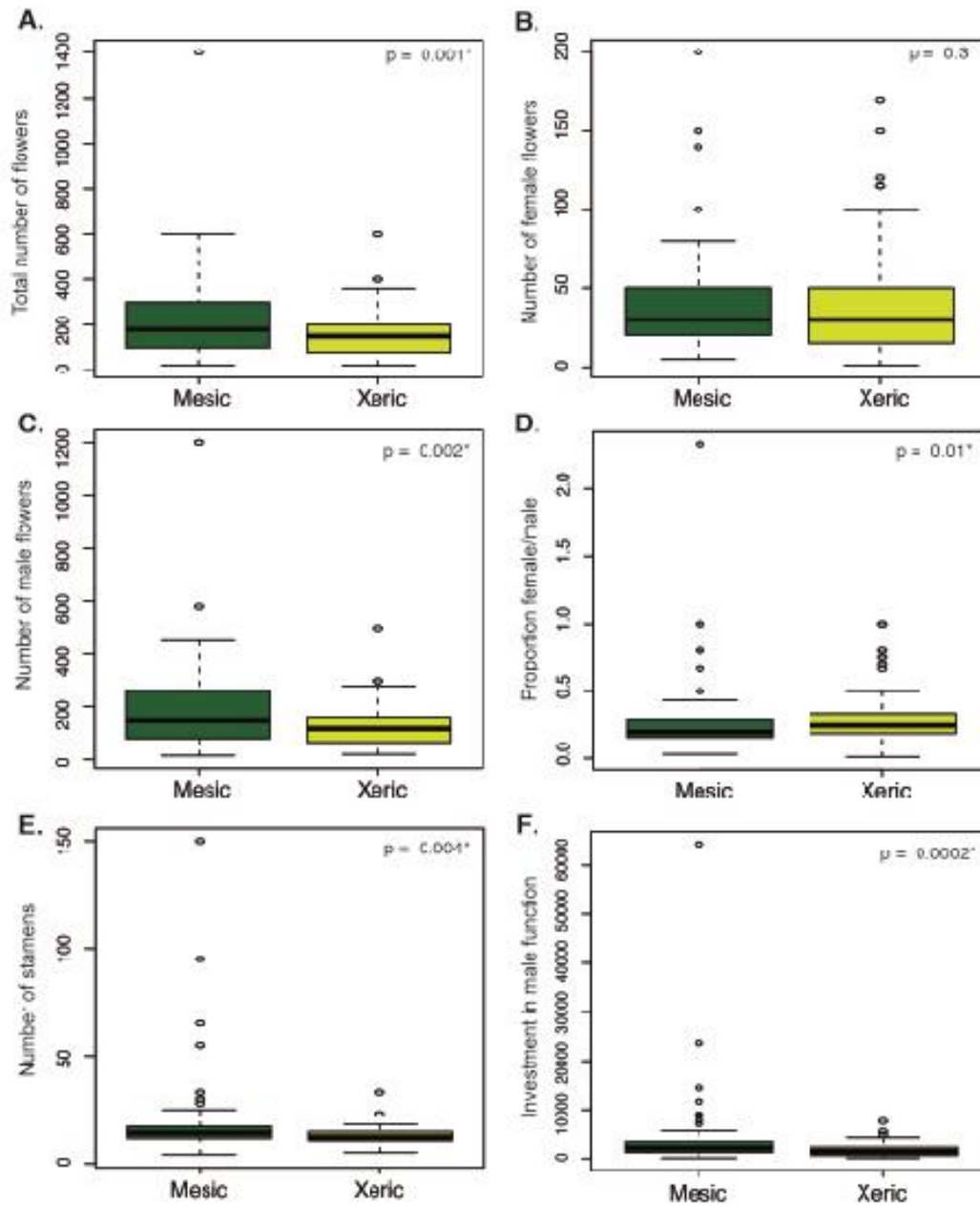
In contrast to the correlation of stamen number and environment, the number of stamens increases with the number of flowers and number of male flowers per inflorescence. Also a positive correlation is observed between the investment in male function and the number of stamens, which are directly proportional (Fig. 12).

Figure 12. Correlations and traits crossing.
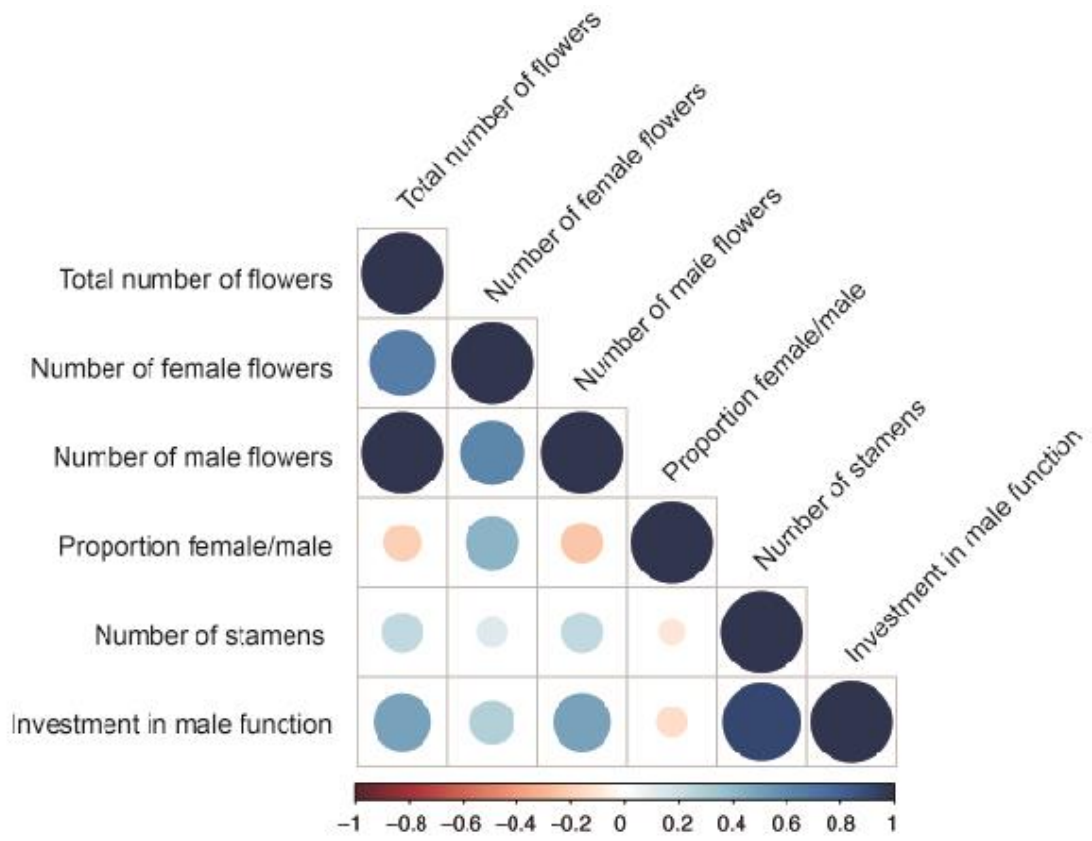
Discussion

General inflorescence morphology in *Croton* – growth and branching pattern

Even though inflorescence terminology and types are a debatable issue in the literature, with different interpretations given by different authors (Parkin 1913, Croizat 1943, Kusnetzova 1988, Weberling 1989, Prenner et al. 2009, Endress 2010, Kirchoff and Claßen-Bockhoff 2013, Harder and Prusinkiewicz 2013), *Croton* inflorescences are considered to be thyrses, due to the racemose and indeterminate main axis with cymose subunits (Caruzo and Cordeiro 2006). This corroborates Webster (1993), who also states that the thyrse is a common inflorescence type in the whole genus.

Inflorescence terminology by different authors partially supports this argument. Based on the ontogeny concept, Claßen-Bockhoff and Bull-Hereñu (2013) described thyrses as any type of inflorescence with cymules and highlighted that the term should be restricted to the inflorescence level and not be mixed with the branching of flowering shoot systems or cymose floral units, such as the *Euphorbia* cyathium (Prenner and Rudall 2007, Prenner et al. 2011). Following the developmental concept, Harder and Prusinkiewicz (2013) stated that thyrses arise by a switch from racemose initiation along the main axis to cymose initiation on higher order axes.

Based on the systematics concept, Troll (1964) and Weberling (1989) described a thyrse as an inflorescence with cymose partial inflorescences and cymose branching, defined as the branching from the axils of the prophylls. Prenner et al. (2009) have a similar definition for a thyrse, which is a compound inflorescence with lateral cymose inflorescences developed on a primary axis. Endress (2010) explores the definition of thyrse and argues that this inflorescence type is a combination of two contrasting branching forms, racemose and cymose.

Croton inflorescences are all composed of a first order main axis, usually indeterminate and rarely determinate, and several cymose second order branches. Rarely third order branches are present, what could be interpreted as a combination of racemose with cymose branching. According to Endress (2010), racemose branching has a maximum number of two branches, while the number of second order branches is variable. On the other hand, the cymose branching is characterized by a fixed number of lateral branches of each axis (usually two) and a variable number of branching orders. Most *Croton* inflorescences have an indeterminate main axis, except for patterns 15 and 17.

Thyrses may be either determinate or indeterminate, being considered as a closed thyrse or thyrsoid (terminal flower in the main axis) or open thyrse (no terminal flower in the main axis), respectively (Weberling 1898, Prenner et al. 2009, Endress 2010). The open thyrse has been commonly observed for *Croton* inflorescences (Webster 1993, Caruzo 2005, Caruzo and Cordeiro 2007, Carneiro-Torres et al 2011, Secco et al. 2012, Caruzo and Cordeiro 2013, Lujan et al. 2015, Kainulainen et al. 2017) and seems to be the most frequent pattern for the genus.

Thyrsoid inflorescences were observed here in a few *Croton* species, such as in the species of *Croton* sect. *Drepadenium*, *C. discolor, C. linearis, C. scouleri* and *C. rivinifolius* (*Croton* sect. *Adenophylli*), *Croton* sect. *Cordiifolii*, *Croton* sect. *Crotonopsis*. Caruzo et al. (2011), Caruzo and Santos (2015), Feio et al. (2016), Webster (1967); Berry et al. (2005) and Pereira et al. (2017) used the term thyrsoid to designate a thyrse-like inflorescence and not necessarily a closed thyrse. This type of thyrse with a terminal flower in the main axis is rarely present in *Croton* and as well as the species registered in this study, it was registered by Riina et al. (2010) for the

inflorescences with pistillate flowers of *C. sapiifolius Croton* sect. *Quadrilobi,* considered by us as an open thyrse.

The different interpretations about *C. sapiifolius* inflorescence suggest that more herbarium and field observations are necessary as well as developmental studies to conclude whether the thyrse is open or closed and also the possible interference of the environmental conditions in the inflorescence architecture. In our study we described this species in pattern 16, corroborating Riina et al.'s (2010) definition for *C. sapiifolius* as a rare inflorescence within *Croton,* with a single unisexual thyrse in an unusual long axis.

Similar to pattern 16 discussed above, patterns 14 and 15 are special cases which were never reported before for the genus. These patterns represent a very distinct architecture with a combination of pistillate and staminate flowers along the main axis. The occurrence of these special patterns shows the importance and necessity of more extensive study with *Croton* inflorescences with a systematic and evolutionary approach.

General inflorescence morphology in *Croton* – sexual strategies

Most of the studied species showed bisexual inflorescences. This is the common pattern described for the genus (Webster 1975, 1993) and it was confirmed in different studies in the group (Berry et al. 2005, Caruzo and Cordeiro 2007, van Ee and Berry 2009, Riina et al. 2010b, van Ee et al. 2011, van Ee and Berry 2011, van Ee et al. 2015, Kainulainen et al. 2016).

We found unisexual inflorescences in *Croton* sect. *Drepadenium*, *Croton* sect. *Adenophylli* (*C. discolor, C. linearis, C. scouleri, C. rivinifolius*), *Croton* sect. *Cordiifolii* and in *Croton* sect. *Quadrilobi.* This type of inflorescence is rare in the

genus and had been previously registered only for *Croton* sect. *Adenophylli* (*C. scouleri*, *C. alnifolius, C. pavonis* and *C. rivinifolius*) (Rumeu et al. 2016) and *Croton* sect. *Quadrilobi* (*C. sapiifolius*) (Riina et al. 2010). In the latter, the authors considered the species to be dioecious based on the absence of bisexual inflorescences and differential growth form of pistillate (determinate) and staminate inflorescences (indeterminate).

Another explanation for the unisexual inflorescences could be associated with the habitat where the species are found and its geographical history. Rumeu et al. (2016) links the morphological uncommon shape of *C. scouleri* to the radiation of this species in the Galápagos islands. The author also states that *C. scouleri* may share a biogeographical history with the three continental species (*C. alnifolius, C. pavonis* and *C. rivinifolius*) and that the unisexual inflorescences could be the result of colonization events in the archipelago.

About the island effect that can influence the production of unisexual inflorescences and dioecy, Baker and Cox (1984) state that the increased proportion of dioecious species in islands may be because these can reproduce in the absence of specialized pollinators. The more specialized reproduction becomes, the less likely it is possible to observe long distance dispersal events (Pannell, 2015).

Evolution and systematics of inflorescence patterns in *Croton*

Bisexual inflorescence is the pleisiomorphic state with four independent events of unisexual evolution and no evidence for reversal. This leads to the hypothesis that unisexual inflorescences are an evolutionary dead-end in *Croton*.

According to Barrett (2013), traits that evolve frequently but do not revert to the pleisiomorphic state are considered dead-ends, as well as traits that provide adaptive

advantages in a "short period of time" but increase sensitiveness to extinction in the long term, such as the Galápagos species, *C. scouleri* (Rumeu et al. 2016).

Clustered pistillate flowers predominate in the genus and represent a pleisiomorphic state while pistillate flowers along the whole inflorescence was found only in a few species and also in the sister groups *Brasiliocroton* and *Acidocroton*, thus potentially representing the ancestral state.

The hypothesis above could be corroborated by the presence of segmented nectaries in the staminate flowers (Freitas et al. 2001, De Paula et al. 2010, Gagliardi et al. 2017) usually confused with petals, thereby being very attractive to floral visitors. Although the pollination system of the large genus *Croton* is poorly known, it is expected to be diverse, including anemophily, entomophily and ambophily (Narbona and Dirzo 2010).

According to Webster (1993), the proximal distribution of pistillate flowers is a common habit of *Croton* species, as well as the mostly solitary pistillate flowers instead of cymules. According to our analysis solitary pistillate flowers are the ancestral state and pistillate flowers organized in cymules have evolved several times in the genus, thus being also a frequent state for the genus. The presence of single pistillate flowers or pistillate flowers organized in cymules could indicate the high or low investment in seed production for dispersal and species propagation (Freitas et al. 2001).

Bisexual cymules are commonly present in *Croton* inflorescences and represent an apomorphic state, which is characteristic of some sections, such as *Cyclostigma, Sampatik, Moacroton, Insulares, Eremocarpus, Cuneati, Prisci, Luntia, Eutropia* and *Cleodora*. The presence of pistillate and staminate flowers in the same cymules may be interpreted as a strategy for both pollination and seed dispersal (Freitas et al. 2001, Biswas et al. 2012)

We identified the following morphological traits: clustered pistillate flower, pistillate flowers in cymules, solitary pistillate flowers, bisexual cymules and sexual mechanism. All of these traits appear to be very homoplastic and the inflorescence diversity in *Croton* could be interpreted as a product of convergent evolution. Therefore, inflorescence characters tend to lose their systematic relevance in species rich sections, unless they are combined with environmental character, such as habitat and habit. There are no exclusive inflorescence patterns for individual sections though we found in some sections only a single pattern, such as in sections *Olivacei* (pattern 1), *Sampatik* (pattern 4), *Cordiifolii* (pattern 17), *Eremocarpus* (pattern 11), *Crotonopsis* (pattern 15), *Alabamenses* (pattern 1), *Nubigeni* (pattern 5), *Corinthii* (pattern 5), *Cupreati* (pattern 7), *Luetzelburgiorum* (pattern 2), *Eutropia* (pattern 8), *Quadrilobi* (pattern 16) and *Caudati* (pattern 1). Besides, two different patterns (pattern 4 and 15) were observed in the same monospecific section *Crotonopsis*, what could indicate the possible influence of the environment on the inflorescence architecture of the species.

In some sections we found dominant patterns which are nevertheless neither exclusive nor unique to those sections: *Croton* sections *Argyrati* (pattern 1), *Argyranthemi* (pattern 1), *Drepadenium* (pattern 17), *Lasiogyne* (pattern 1), *Cleodora* (pattern 7), *Cyclostigma* (pattern 7), *Pedicellati* (pattern 2), and *Cuneati* (pattern 7).

Besides the organization and distribution of flowers, the inflorescences shape is also affected by the presence of leafy structures, usually very reduced and in some cases very conspicuous such as in *Croton amentiformis* (*C.* sect. *Cyclostigma*). These conspicuous bracts were also observed by Riina et al. (2015) who described them as bracts with incurved apex. Kainulainen et al. (2017) and Berry and Kainulainen (2017) have also registered conspicuous bracts in Madagascar *Croton* as well as a variety of bract types, including ovate, triangular, lanceolate and awn-shaped bracts.

Together with the presence of bracts, the elongation and shortening of branches were also observed as variable features in *Croton* inflorescences. Most of the inflorescences studied here show a long main axis, what seems to be a pattern for the genus (Berry et al. 2005, Caruzo and Cordeiro 2007, van Ee and Berry 2009, Riina et al. 2009, 2010b, van Ee et al. 2011, van Ee and Berry 2011, van Ee et al. 2015, Kainulainen et al. 2016).

We also found condensed inflorescences in a few clades, such as in *Croton* sections *Geiseleria* (*C. guildinguii*) *Heptallon, Lasiogyne* (*C. rosmarinoides*), *Julocroton* (*C. argenteus*), *Eremocarpus*, *Lamprocroton* (*C. pallidulus*) and in the genus *Acidocroton.* Condensed inflorescences were also observed by Kainulainen et al. (2016) in the Madagascar species and it seems to be a common morphological character in Malagasy *Croton*. A phylogenetic study including a broad representation of all Old World *Croton* lineages would be extremely important to answer this question.

Systematic and developmental studies show that other genera in Euphorbiaceae have condensed inflorescences due to the shortening of the branches, such as *Euphorbia*, *Dalechampia* and *Joannesia* (Webster and Webster 1972, Armbruster 1984, Souza et al. 2010, Prenner and Rudall 2007, Gagliardi et al. 2016). Endress (2010) applied the term coenosome to condensed branching that appears as compact body. Coenosomes are associated with cymose branching and are the result of a rapid branching without axis elongation, commonly found in Moraceae and Urticaceae.

Selective pressures in the evolution of *Croton* inflorescences

Croton inflorescences show a multitude of distinct floral spatial arrangements and sexual strategies. Changes in inflorescence architecture and floral display influence how pollinators perceive flowers and, consequently, affect overall reproductive success of a

plant (Wyatt, 1982). Shifts in investment strategies and correlation between these and the environment can provide hints about external pressures and energetic trade-offs that have shaped these structures during evolutionary time (Garland 2014).

Staminate flowers are almost always more numerous than pistillate flowers, so it is no surprise that the increase in total number of flowers per inflorescence is more strongly correlated to staminate flowers than to pistillate flowers (Fig. 12). The unexpected result, however, is that the number of staminate flowers is also positively correlated to the number of stamens in each staminate flower; and the total number of flowers in an inflorescence is negatively correlated to the proportion of pistillate vs. staminate flowers.

Due to energetic trade-off rules (Garland 2014), it was expected that with an increased proportion of staminate flowers, these would present less stamens, keeping investment in male function neutral. Furthermore, it was also presumed that the proportion of pistillate vs. staminate flowers would be neutral to changes in flower number in an inflorescence. Nevertheless, our result shows that the larger the inflorescence the higher the investment in male reproductive functions, both in terms of stamen number per flower and proportion of staminate flowers in the inflorescence.

In the case of *Croton*, the environment seems to be a selective force in the evolution of such inflorescence strategies. Our results show that male investment in wet environments tends to be higher than in drier environments. Even though the total investment in male function is not correlated with habitat (Fig. 11F), the number of staminate flowers and the total number of flowers per inflorescence are significantly correlated to habitat, and they increase sharply in wet environments (Fig. 11A, C). Additionally, the proportion of female flowers is also significantly higher in dry environments (Fig. 11D).

This correlation may be associated with increasing pollination efficiency in different environment. Authors reported evidence for wind pollination (i.e. anemophily) in several *Croton* species (Reddi and Reddi 1984; Bullock 1994; Dominguez et al. 1989), and state that nectar production would act more often as a reward to plant defenders than to pollinators (Dominguez et al. 1989; but see also Narbona and Dirzo, 2010). Besides empirical evidence, *Croton*'s inflorescences and flowers show many characters commonly associated with wind pollinated species: the flowers are small, numerous and unisexual, with a spatial or temporal separation of pistillate and staminate flowers (i.e. "male" and "female" phase); perianth parts are reduced and investment in male function is increased (Ackerman, 2000).

Anemophilous species have to facilitate the aerodynamics of wind pollination (Niklas, 1985). If this is the case in *Croton*, it is intuitive to associate an increase in the proportion of staminate and total number of flowers in calm and less windy environments, such as in rainforests (Abrahamczyk 2011). Webster (1993) also suggests that a reduction in female flowers, both in size and number, is associated with shifts to wind pollination in Crotonoideae lineages. This could explain the decrease of pistillate flowers when compared to the staminate ones.

Conclusions

Croton inflorescences show distinct structural patterns and most of the differences are found in the distribution and organization of pistillate flowers. The morphological traits studied here (clustered pistillate flower, pistillate flowers in cymules, solitary pistillate flowers, bisexual cymules and sexual mechanism) are homoplastic and inflorescence diversity appears to be the product of convergent evolution, which would be expected in a species-rich and biologically diverse group such as *Croton*.

The inflorescence characters do not show systematic relevance in species-rich sections because there is no exclusive pattern for any individual section. However, in some sections only a single pattern occurs and other sections have predominant patterns. The organization and distribution of flowers in *Croton* inflorescences can be associated with the predominantly wind pollination mode and investment in seed production for dispersal and species propagation.

In wet environments the inflorescences show an increase in number of staminate flowers per inflorescence, whereas in dry environments the proportion of pistillate/staminate flowers increases. These are assumed here to change the inflorescences architecture, thereby influencing on how pollinators perceive a flower and, consequently, affect overall the reproductive success, besides the larger the inflorescence the more it invests in male reproductive functions.

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Chapter 4 - Inflorescence transcriptome of Croton fuscescens Spreng (Euphorbiaceae): an overview and first insights

Abstract

Background: *Croton* L. (Euphorbiaceae) is a large genus with about 1300 species. The genus is notable for the different types of inflorescence and especial arrangement of pistillate and staminate flowers distribution. Based on this, the aim of the present study is to through comparative transcriptome analysis between pistillate and staminate region of *Croton fuscescens* Spreng identify the genetic atlas of the inflorescence and its expression profile during floral development.

Results: Here we report the transcriptome sequence of *C. fuscescens* inflorescence. A total of 277.212 transcripts were assembled of which 104.804 as good Transcripts and 172.408 as bad Transcripts. *Flex* experimental drawing showed a better clustering of the samples and a differential expression level was observed in the four inflorescence zones.

Conclusions: The transcriptome of *C. fuscescens* inflorescence showed that the different zones have their development guided through the same transcripts set, though each zone has different expression levels.

Keywords: flower development, inflorescence, pistillate flowers, RNAseq, staminate flowers.

Introduction

The diversity of inflorescence architecture within angiosperms illustrates the extreme evolutionary plasticity of reproductive structures (Harder and Prusinkiewicz 2013). Increased knowledge of phylogeny, sexual reproduction and especially developmental genetics has raised interest in the evolutionary and functional significance of inflorescences (Harder et al., 2004; Prenner et al., 2009; Endress, 2010; Feng et al., 2011).

Among angiosperms, Euphorbiaceae is a wide diverse family regarding inflorescences architecture and is characterized by flowers in cyme or racemose inflorescences, which are sometimes reduced in a structure similar to a single flower, named pseudanthia. Non-reduced inflorescences are also present in the family and *Croton* L. is notable for the diversity of different types of ramification and distribution of flowers along the inflorescence axis (Webster, 1993).

As the second largest genera of Euphorbiaceae, *Croton* is composed of about 1.300 species with pantropical distribution and about 350 species located in Brazil (Govaerts et al. 2000; Berry et al. 2005). Most of *Croton* flowers are distributed in thyrse inflorescences, in which the basal pistillate flowers may be solitary, in cymules or arranged in bisexual cymes, nevertheless also existing staminate cymes distally located (Webster, 1993, Berry et al. 2005, Caruzo and Cordeiro (2005, 2007, 2013).

Studies about inflorescence architecture, ontogeny and gene expression are important to elucidate the great reproductive structures diversity observed in angiosperms. The evolutionary developmental area, evo-devo, have already revealed relevant information for the comprehension of several groups, such as *Arabidopsis thaliana* and *Antirrhinum majus* (Coen and Meyerowitz 1991), *Petunia* (Colombo et al.

1995), *Euphorbia* (Prenner et al. 2011), *Passiflora* spp. (Cutri et al. 2013, Rosa et al. 2013, Rosa et al. 2014, Scorza, LCT. & Dornelas, MC 2014).

Associated with evo-devo, sequencing of mRNA using next-generation sequencing (NGS) technologies (RNA-seq) has the potential to reveal unprecedented complexity of the transcriptomes, which provides insights into the gene space, opportunity to isolate genes of interest, development of functional markers, quantitation of gene expression, and comparative genomic studies (Garg and Jain 2013).

Transcriptome studies have been performed for different groups and different organs, such as in Brassicaceae, *Arabidopsis* (Buchanan-Wollaston et al. 2005), Cucurbitaceae (Guo et al. 2010), Asteraceae (Lulin et al. 2012), Rosaceae (Chen et al. 2014, Li et al. 2017, Sánchez-Sevilla et al. 2017) and Gesneriaceae (Roberts and Roalson 2017). In Euphorbiaceae, there are transcriptome studies with seeds of *Sapium* (Divi et al. 2015) seedlings of *Hevea* (Deng et al. 2018), roots, steam, leaf and latex of *Euphorbia* (Barrero et al. 2011, Qiao et al. 2018), flower bud of *Sapium* (Yang et al. 2015) and inflorescence meristem of *Jatropha* (Pan et al. 2014).

Considering the different distribution of pistillate and staminate flowers in *Croton* inflorescences, a transcriptome analysis of different inflorescence regions would reveal the molecular architecture and genetic factors which determine the space boundary between the flowers of different sexes and developmental stages.

Based on this motivation, the aim of this study is to perform a comparative transcriptome study between pistillate and staminate region of *Croton fuscescens* Spreng in order to identify the genetic atlas of the inflorescence and its expression profile along the same inflorescence axis.

Material and methods

Plant Material and RNA Extraction

To obtain a comprehensive picture of *C. fuscescens* inflorescence transcriptome we selected four representative stages of development to perform the expression analyses by RNA-seq (Fig. 1). The studied zones were: pistillate adult flowers (FA), pistillate meristematic region (FM), staminate adult flowers (MA) and staminate meristematic region (MM) (Fig. 1). We selected 3 samples (different organisms) of each zone and the material was collected in the Botanical Garden of São Paulo.

After collection of racemes and separation of flowers from each zone, flowers were sprayed with liquid nitrogen and macerated. Total RNA was isolated from each of the 4 previously determined zones using plant RNA extraction kit Rneasy_Qiagen, which was complemented with 100 µl of PEG 4000 10% to RLT buffer.

The RNA was further digested with DNase (TURBO^{TM} Rigorous Treatment) to remove genomic DNA contamination and visualized by electrophoresis on 1% agarose gel. RNA integrity and quantity was determined by Nanodrop spectrophotometer (absorbance ratios at 260/280 nm and 260/230 nm) and Agilent 2100 Bioanalyzer (CEFAP – University of São Paulo) with RIN values above 6. The cDNA library was built using Illumina kit (cDNA- TruSeq Stranded mRNA Library Preparation Kit Set A).

Transcriptome Sequencing and de Novo Assembly

The sequencing was performed with Illumina NextSeq equipment (NextSeq® 500/550 High Output Kit v2 - 300 cycles with 75bp reading length).

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For the assembly, we chose the *de novo* strategy rather than the reference-based strategy (Rahman et al. 2013) and we hired the company TAU GC Bioinformática to perform this step.

Figure 1. Inflorescence of *Croton fuscescens* and its maturation gradient, indicating the four zones used in this study.
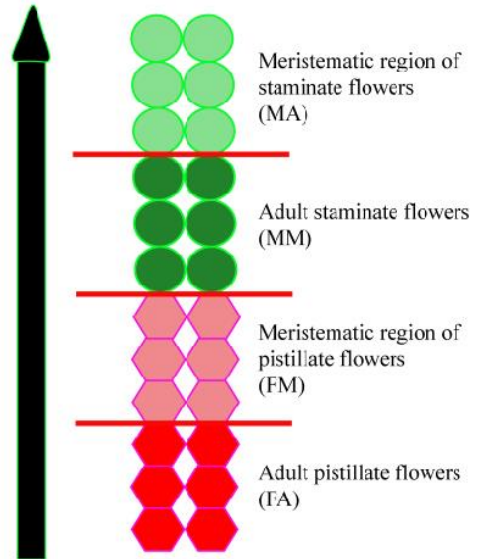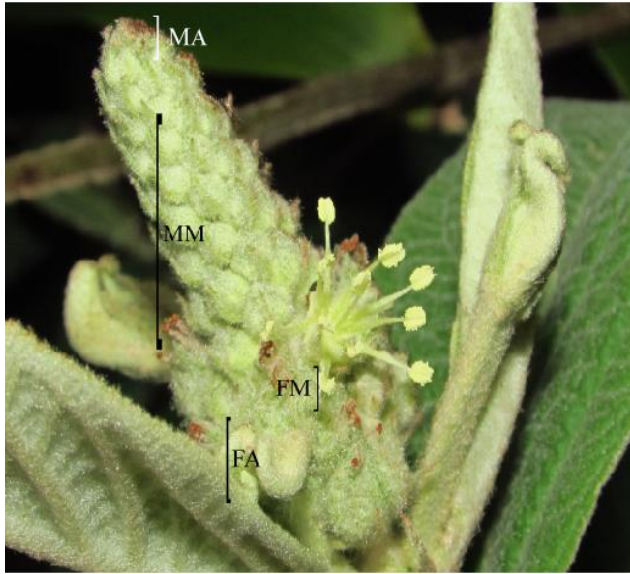
FastQC v0.11.7 was used for the quality control of the sequences, Pear v0.9.11 to evaluate the fragments size, FastQ_Screen v0.11.1 with Bowtie v2.3.4.1 for filtering the control sequences, and Illumina phiX and TrimGalore v0.4.4 with CutAdapt v1.13 to remove adaptors sequences and split for quality.

The resulting data from the 4 inflorescence zones of the 3 samples were compiled with Trinity, annotated with Trinotate v3.1.0 and analyzed for differential expression with RSEM (RNA-Seq by Expectation-Maximization) v1.3.0 and edgeR v3.16.5.

Results and Discussion

Transcripts annotation

It was possible to assemble 277.212 transcripts from the RNAseq which correspond to 114.727 transcripts with ORF and 156.476 without ORF. In the annotation process, 159.833 transcripts were annotated, from which 102.215 had predicted ORF whereas 51.618 had no predicted ORF (Table 1). Transdecoder considers an ORF if it is longer than 100 bases, if a candidate ORF in fully encapsulated by the coordinates and if it has a start and stop codon prediction.

Analysis of the assembly resulted in 104.804 transcripts that were considered of "*good quality*" (goodTranscripts) and 172.408 were annotated as "*bad quality*" (badTranscripts) (Table 1). Transrate goodTranscrip definition is based on 4 criteria as follows: both members of the pair are aligned, in the correct orientation, on the same contig and without overlapping either end of the contig. BadTranscripts are defined where any of the above criteria fail.

	TransRate Good	TransRate Bad	Trinity Total
Annotated with ORF	27.994	74.221	102.215
Annotated w/o ORF	17.603	40.015	57.618
Not annotated with ORF	5.240	7.281	12.521
Not annotated w/o ORF	53.967	50.891	104.858
Total	104.804	172.408	277.212

TABLE 1. Distribution of transcripts according to the annotation, ORF and quality assembling

Principal component analysis (PCA) of the different inflorescence regions

The PCA was performed in order to analyze the correlation among the transcripts of each sample and thus demonstrate the uniformity among the samples.

The first analysis compared the four inflorescences zones in PC1 = 42,73% and PC2 = 15,85% and we observe a non-uniform distribution of the transcripts (Fig. 2A).

The highest gene expression variation was observed in two samples of the staminate zones MA, MM, which also showed similar transcripts. One of the samples of each zone (MA and MM) is not clustered with the others and showed a different gene expression variation (Fig. 2A).

Samples of the pistillate zones showed a lower gene expression variation when compared to the staminate zones. Two samples of FA showed the lowest gene expression variation and quite similar transcripts. A third FA sample revealed a higher gene expression variety when compared to the other two. Zone FM presented high gene expression variation in all the samples from which two showed very similar expression, though one of them was fairly distant from the other two (Fig. 2A).

The second analysis compared the four inflorescences zones in PC2 = 15,85% and PC3 = 14,55% and we observe a non-uniform distribution of the transcripts (Fig. 2B).

The highest gene expression variation observed here was also in the staminate zone MA and the samples showed distinct transcripts. The staminate zone MM showed

lower gene expression variation when compared to MA and the samples showed different transcripts too (Fig. 2B).

The pistillate zone (FA and FF) presented smaller gene expression variation. FA zone revealed two samples with similar transcripts, and the same was observed for FM (Fig B).

Figure 2. Principal component analysis (PCA) demonstrating the correlation among the transcripts of the different inflorescence zones.

A
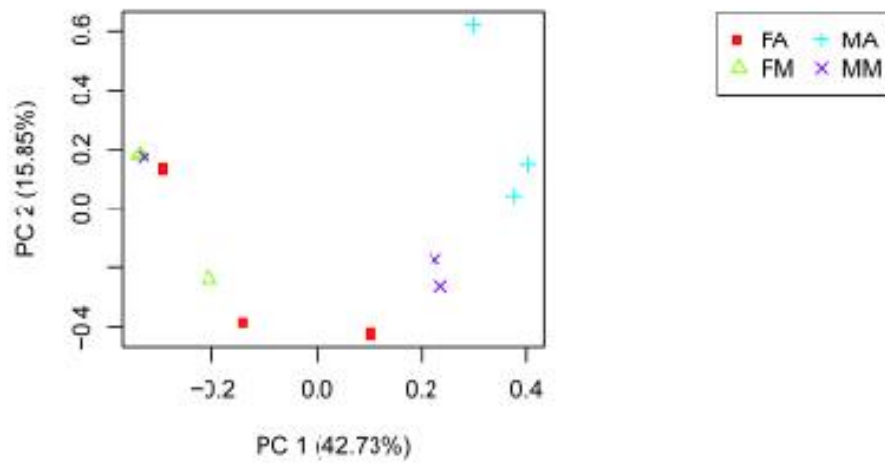
B
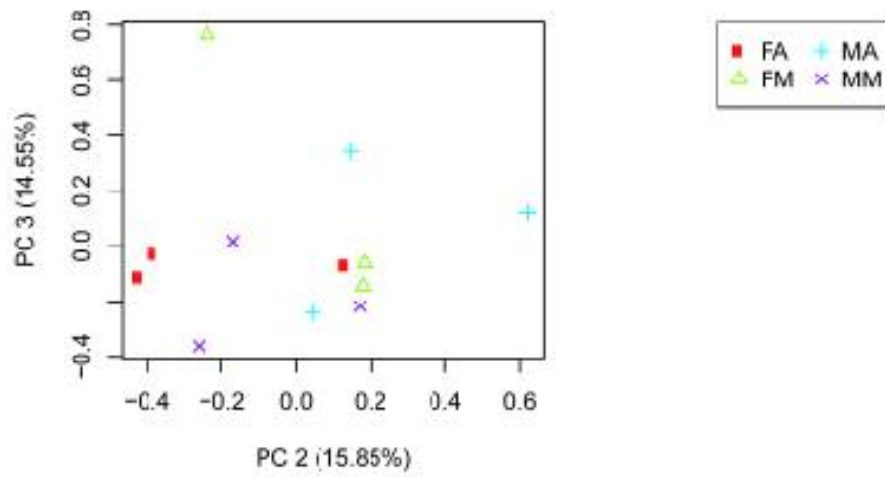
Differential gene expression analysis across different Inflorescence regions

Clustering analysis of the expression patterns of *C. fuscescens* inflorescence is shown in Fig. 3. The genes display higher expression levels at some regions of all the inflorescence zones. The inflorescence has a set of genes, though in each zone the expression levels of each transcript are different. In this first Heatmap we can observe that an unexpected result occurs between samples MM_rep1 and FM_rep1. Both samples diverge from the cluster of pistillate and staminate zones and their exclusion would turn the cluster fairer (Fig. 3).

We would associate these results with the persistence of female features in staminate flowers, what was explored in chapter 2 of this thesis. In this chapter we studied the development of *Croton* staminate flowers and observed the occurrence of a central stamen in *C. fuscescens* with three vascular bundles, what according to Puri et al. (1951) is a carpel feature and not a stamen, which usually shows one single vascular bundle. This development and vasculature data would explain the position of sample MM_rep1 in the pistillate zone. Though, further transcripts annotation and molecular studies would be necessary to check the truthful of this hypothesis.

Figure 3. Heatmap showing the gene expression pattern of different Inflorescence zones. Red boxes indicate the re-grouping of the experimental drawing flex. Coloring scale (value) according to the distance among samples.
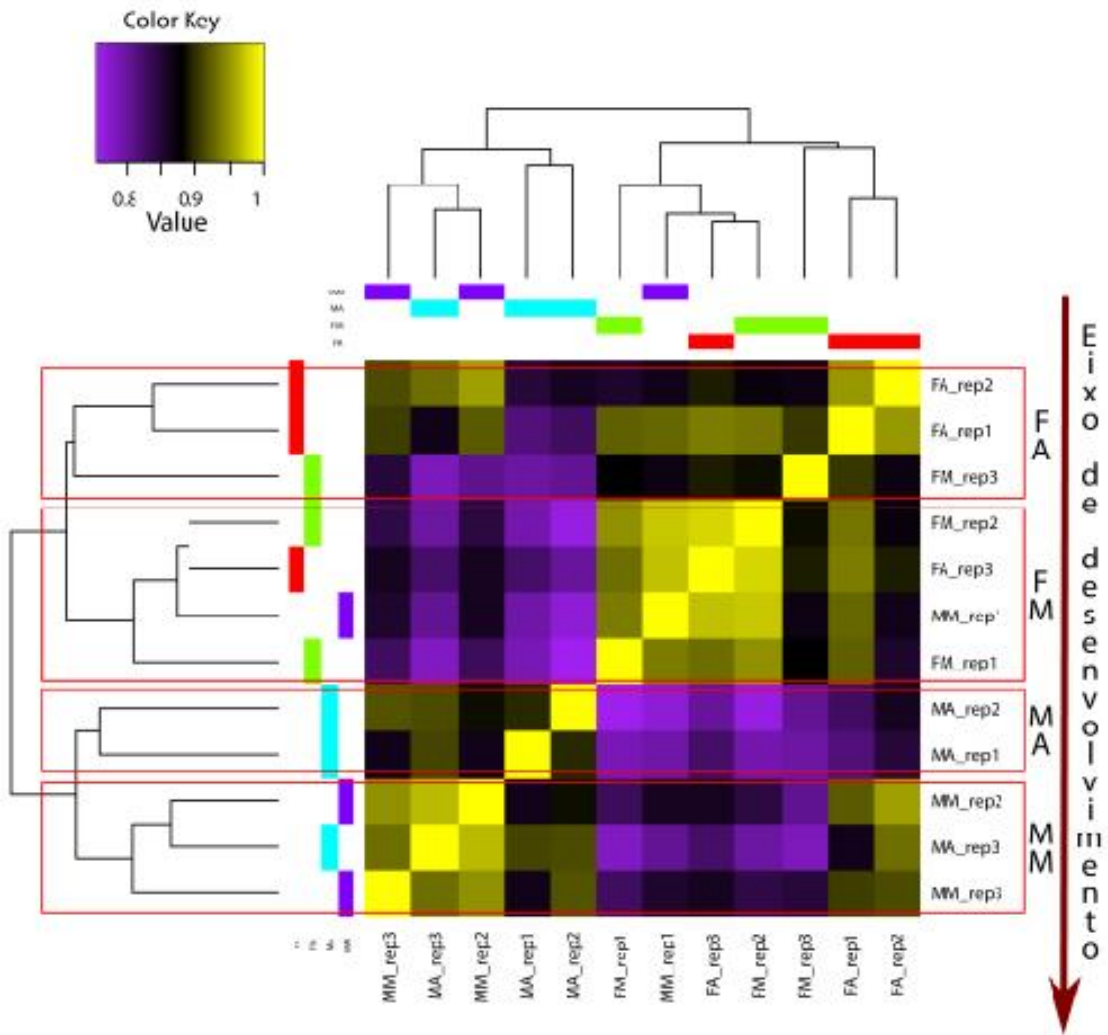
Due to the unexpected clustering of these samples, new analyses using alternative experimental designs were performed to better correspond the correlations among the zones, a conservative (*consv*) and a flexible (*flex*) analysis. The results were plotted in a new PCA and Heatmap (Figs 4-7).

In the conservative experiment the outlier samples (MM_rep1 and FM_rep1) were removed, thereby remaining two samples of each zone. The PCA (PC1 x PC2) showed a better clustering between the samples of each zone (Fig. 4A), which indicates similar transcripts between the samples. The zones with highest gene expression variation were FM and MA, whereas FA and MM showed lower gene expression variation (Fig. 4A).

Figure 4. Principal component analysis (*consv*) demonstrating the correlation among the transcripts of the different inflorescence zones.

A
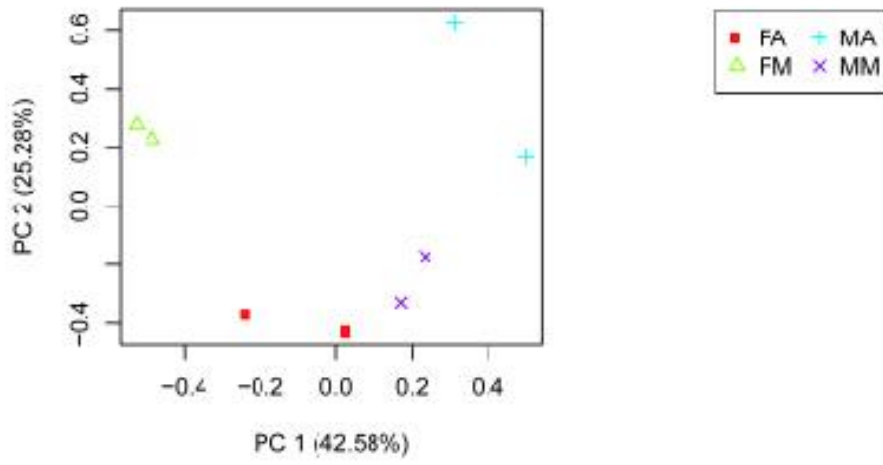
B
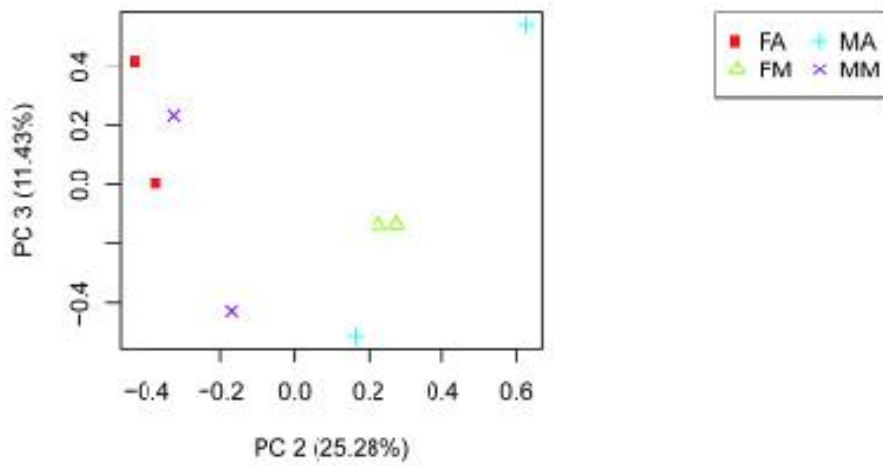
The PCA (PC2 x PC3) showed a lower clustering between the samples of the zones, except for the two samples of FM. Samples of FA were slightly clustered, though MM and MA showed very distinct transcripts between the species and high gene expression variation (Fig. 4B).

The conservative Heatmap corroborated PCA and shows a transcript set and different gene expressions in each zone. Besides, we can observe the closest position between zones MM and FA demonstrated in PCA (Fig. 5), which was an unexpected result considering that the inflorescence development is acropetal and goes from pistillate proximal zones to the staminate distal ones (Fig. 1).

The experimental design named flexible (*flex*) re-groups the samples (red boxes showed in Fig. 3) in order to consider the proximity of transcripts, although each zone comes to be composed of a different number of samples.

Figure 5. Heatmap (consv) showing the gene expression pattern of different inflorescence zones. Coloring scale (value) according to the distance among samples.
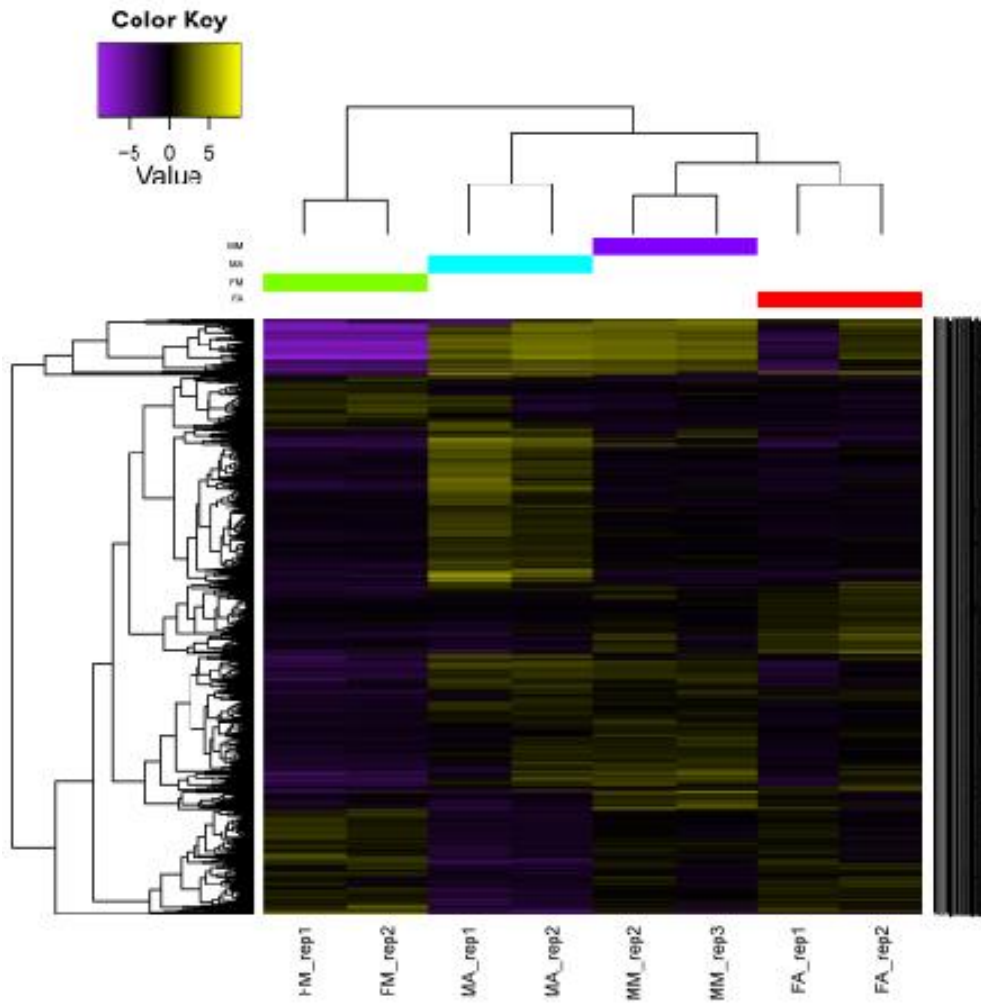
The PCA (PC1 x PC2) based on *flex* experiment shows a high level of clustering among the samples of each zone, which indicates similarities in the samples transcripts. The highest gene expression variation is observed in FA and FM, whereas MA and MM show lower gene expression variation when compared to the pistillate zone (Fig. 6A). The PC2 x PC3 correlation shows high level of clustering in the samples of the zones, except for FA, which shows the highest gene expression variation and thus, more different transcripts among each other. Zones FM, MA and MM present a low gene expression variation (Fig. 6B).

Figure 6. Principal component analysis (*flex*) demonstrating the correlation among the transcripts of the different inflorescence zones.

A
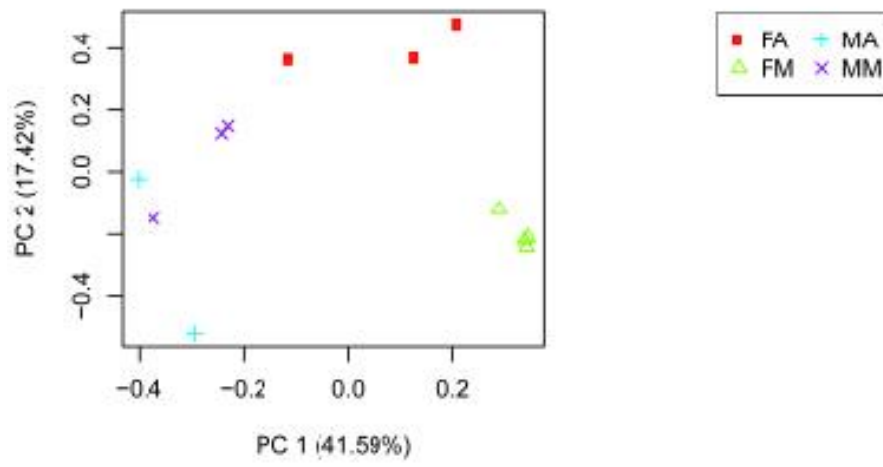
B
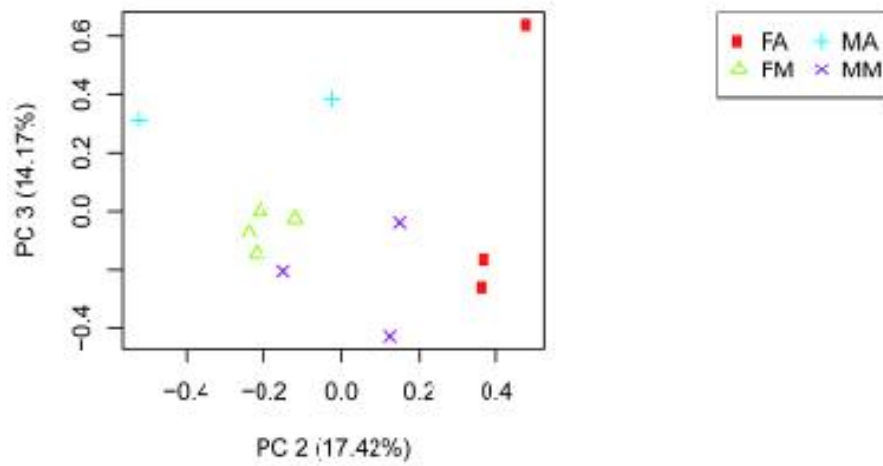
The flexible Heatmap corroborates the PAC analysis and shows a more constant transcripts' map with gradual gene expressions variations from zone to zone. The clustering is more consistent considering the acropetal inflorescence development. The two pistillate zones (FM and FA) demonstrated proximity, as well the staminate zones, with a minor divergence in the samples MA_rep2 and MM_rep3 (Fig. 7).

Figure 7. Heatmap (*flex*) showing the gene expression pattern of different inflorescence zones. Coloring scale (value) according to the distance among samples.
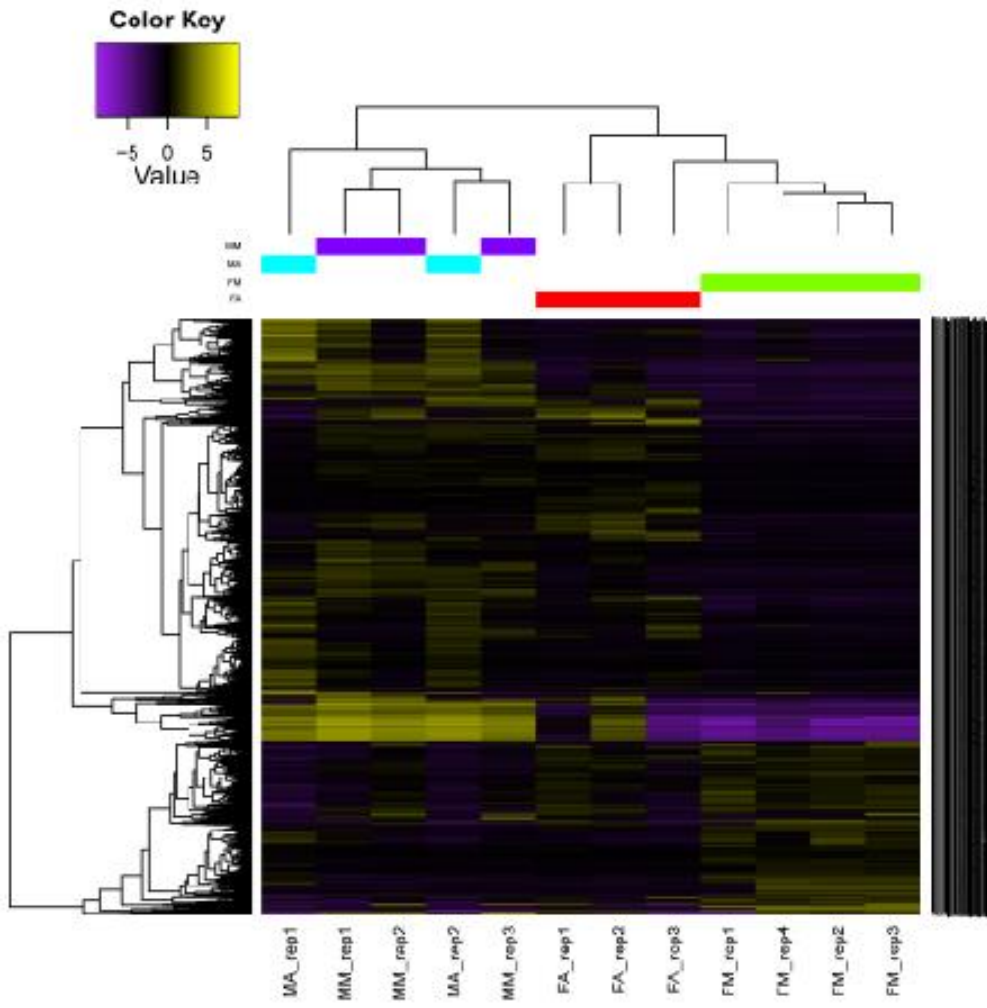
Transcripts annotation comparing the different experimental design

Considering the "*goodTranscripts*" were observed that 14.386 transcripts differently expressed in the *consv* experiment design, 15.379 in the *flex* and 14.386 were common between the two. From the common transcripts, 8.327 had predicted ORF against 6.059 without. The total of the transcripts obtained from each experiment is discriminated in Table 2.

	FA vs FM	FA vs MA	FA vs MM	FM vs MA	FM vs MM	MA vs MM	Common
Consv CDS	9.855	10.277	10.199	9.415	9.555	9.673	8.327
Flex CDS	10.720	10.740	10.860	10.041	10.321	9.969	8.805
Consv NC	8.539	8.930	8.938	7.477	7.857	7.915	6.059
Flex NC	9.808	9.738	10.032	8.294	8.962	8.433	6.574
Consv Total	18.394	19.207	19.137	16.892	17.412	17.588	14.386
Flex Total	20.528	20.478	20.892	18.335	19.283	18.402	15.379
Common Total	18.394	19.207	19.137	16.892	17.412	17.588	14.386

TABLE 2. Total of "*goodTranscripts*" differently expressed according to the experimental design, presence of ORF and comparison. *Common* regards to common transcripts between the categories or comparisons.

A very preliminary analysis reveals that Programmed Cell Death is probably involved in the transition of flower identity the inflorescence axis as both SAG39 (senescence cysteine protease) and CEP1 (KDEL-tailed cysteine endopeptidase) are differentially expressed being not expressed in Pistillate flowers (FA and FM) but expressed in Staminate flowers (MA and MM). Conversely, in the Staminate flowers increased expression of both the transcription factor bHLH75 and ARP1 (RNA-binding protein).

Conclusion

The transcriptome analysis of *C. fuscescens* inflorescence showed that the different zones have their development guided through the same transcripts set, though in each zone the expression level is different. Slight expression variations were observed in the transcriptome, what could be interpreted as the boundary between each inflorescence zone.

Acknowledgments

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General Conclusions

The flowers and inflorescences of *Croton* studied in this thesis revealed key answers to the diverse morphology of this giant genus, besides motivating us to keep learning and studying about this group.

Starting with the diversity in flower morphology, which we explored in the first chapter, the studied species represented the different morphological patterns found in the flowers perianth. We corroborated the two hypotheses which motivated this study; the first one was the confirmation that the morphologically different flowers result from different developmental steps, especially the first steps, which include sepals' initiation and elongation. The second hypothesis corroborated in this study was that the filaments of pistillate flowers, usually described as reduced petals, were interpreted here as staminodes, firstly described for *C. sphaerogynus.* These structures represent cases of transference of function and heterochrony and are considered a conserved characteristic.

Besides the diversity in the perianth, *Croton* flowers show notable differences regarding androecium merism, which seems to be a strong morphological character in the genus, a topic which was studied with detail in the second chapter of this thesis.

The development of the staminate flowers showed two key developmental steps which influenced in the androecium configuration. The first one was the petals' development, which presented a longer plastochron when compared to sepals', and the second one was the nectaries development in antesepalous direction. Both ontogenetic steps influenced in the androecium configuration turning it into obdiplostemony and thus we corroborated our first hypothesis.

The vasculature study of the staminate flowers corroborated the obdiplostemony developmental pattern and reveals two types of obdiplostemony for *Croton* species. The interpretation of nectaries as staminodes in three of the studied species was assumed here to be a heterotopy case and a homeosis case was evidenced in the central stamen of

C. fuscescens with carpel features. The flower development of *Croton* is guided by different auxin concentration in different developmental steps and this is the first record of obdiplostemony and the role of auxin in *Croton* flower development.

Besides flowers, *Croton* inflorescences also show a vast diversity, especially regarding flowers distribution and arrangement, what was widely studied in the third chapter. Most of the differences found in *Croton* inflorescences were concentrated on the distribution and organization of pistillate flowers and thus 17 inflorescence patterns were proposed. The morphological traits were all considered as homoplastic characters and *Croton* inflorescence diversity appears to be the product of convergent evolution, which would be expected in a species-rich and biologically diverse group such as *Croton*.

The inflorescence characters explored here did not show systematic relevance in species-rich sections because there was no exclusive pattern for any individual section. However, in some sections only a single pattern occurred and other sections had predominant patterns. The study crossing inflorescence features with habitat showed that there may be a change in inflorescences architecture depending on the habitat conditions.

The differences regarding flower distribution motivated us to develop the fourth chapter of this thesis, in which we studied the transcriptome of *C. fuscescens* inflorescence. The RNAseq analysis showed that the different zones have their development guided through the same transcripts set, though in each zone the expression level is different. Slight expression variations were observed in the transcriptome, what could be interpreted as the boundary between each inflorescence zone.

The evolutionary links identified here, such as the occurrence of staminodes in pistillate flowers, staminodal nectaries and obdiplostemony in staminate flowers, a diversity of inflorescence patterns and differences in gene expression within the inflorescence, raise the importance of future floral studies with the genus. *Croton* is one of the largest genus among angiosperms and studies involving all the areas mentioned above would bring a better understanding on how the reproductive structures evolved in the history of the group.

Appendix

Flower development in species of *Croton* (Euphorbiaceae) and its implications for floral morphological diversity in the genus

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Abstract.   The Euphorbiaceae are notable for floral diversity and evolutionary complexity. *Croton* is the second largest genus in the family and exhibits particular diversity in its flowers. The aim of this study was to investigate the floral ontogeny and structure of three *Croton* species with distinct morphologies, with a focus on testing the hypothesis that the filaments of female flowers, which have received different interpretations in the literature and are currently described as reduced petals, are staminodes and part of a vestigial androecium. With the ontogenetic study we can understand the origin of the organs and associate these with flower evolution in the genus. Flowers in several stages of development were analysed using light microscopy and scanning electron microscopy. In the early stage of development, the sepals are the first structures to be formed, although they do not continue to grow in female *Croton fuscescens* Spreng. flowers. Petals are absent in female flowers, with filamentous, petaloid structures, interpreted here as staminodes, alternating with the sepals in *Croton lundianus* (Didr.) Müll. Arg. In *Croton sphaerogynus* Baill., the staminodes are located between the nectary lobes. The stamens exhibit centripetal development in the flower bud stage, and the carpels are post-genitally connate, with differences in style branching. Besides the ontogenetic interpretation for the filamentous structures, the genus shows transitional structures that we consider evolutionary reductions. Our results can explain how developmental alterations have influenced the suppression and modification of floral organs in the genus.

Additional keywords: Crotonoideae, evolution, ontogeny, perianth, petals, staminodes.

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Introduction

Among the angiosperms, families such as the Euphorbiaceae are notable for their floral and inflorescence diversity. Many types of cymose inflorescences are found in this family, and some of them are unique and morphologically different from other groups, such as the pseudanthia of *Dalechampia* and *Euphorbia*.

Croton, which is the second largest genus of Euphorbiaceae, includes between 1200 and 1300 species of herbs, shrubs, trees, and lianas; it is pantropical, with ~300 species in Brazil (Berry *et al*. 2005; Cordeiro *et al*. 2016). Its inflorescences are terminal thyrses that have proximal cymules with either female or both female and male flowers, and distal cymules usually with only male flowers.

Croton has female and male flowers with particular differences, especially concerning the presence or absence of perianth whorls (Webster 1967). *Croton* female flowers have the combination of lobed, entire, pinatissected calyx, usually imbricate or valvate and petals are usually reduced or absent; filaments are usually common, though their interpretation is debatable, sometimes

described as petals or glands (De-Paula *et al*. 2011; Caruzo and Cordeiro 2013). The male flowers have valvate calyx, as many petals as there are sepals and stamens with no filaments (Webster 1967).

Caruzo *et al*. (2011) studied the evolution of characters and the phylogeny of *Croton* section *Cleodora* (Klotzsch) Baill., a group widely spread through America but which has not been revised recently. Caruzo *et al*. (2011) described the pistillate flowers of this group as generally apetalous, or with greatly reduced petals and rarely conspicuous petals. They argued that when filaments or glandular structures are present in the position of petals, these structures should be referred to as reduced petals. They also noted that the presence of filaments is shared by a majority of New World *Croton* species, where two-thirds of the species occur (Van Ee *et al*. 2011).

De-Paula *et al*. (2011) analysed the flowers of two genera of the tribe Crotoneae, *Croton* and *Astraea* and interpreted the male flowers as having five sepals and five petals and the female ones as having five sepals and five filamentous structures, which are unvascularised and interpreted as reduced petals. Nectaries

were interpreted as staminodial nectaries due to their external position in relation to the first staminal whorl.

The identification of petals in these flowers requires a deep study on the origin of the organs. In particular, the term 'petals' is imprecise and has been applied to a diverse range of showy, non-homologous structures in the second whorl of a perianth. Several degrees and different forms of perianth allied to the numerous cases of perianth organs loss and gain could result in the misinterpretation of perianth parts (Ronse De Craene and Brockington 2013). These represent cases where morphological structures derived from different whorls may develop different functions, a phenomenon generally called heterotopy or transference of function (Baum and Donoghue 2002).

Considering the floral variations described for *Croton*, especially with respect to sepals, petals and filaments, the study of flower development allied to evolutionary perspectives is important to understand heterotopy and transference of function. Ontogenetic studies also help understand differences in floral shape, particularly explaining the differences in primordia development and meristem activity (cell division, expansion and differentiation patterns).

The aim of this study is to test the hypothesis that the filaments of female flowers currently described as reduced petals are staminodes, and like the nectaries considered staminodes by De-Paula *et al.* (2011), these filaments are also part of a vestigial androecium. In order to test the hypothesis above we chose three species: *Croton sphaerogynus* (*Croton* sect. *Cleodora* (Klotzsch) Baill.), *Croton fuscescens* (*Croton* sect. *Julocroton* (Mart.) G.L. Webster) and *Croton lundianus* (*Croton* sect. *Geiseleria* (A. Gray) Baill.). These species were chosen because they show contrasting flower morphologies, especially concerning the number of whorls of the perianth, the shape of the sepals, petals and filaments, as well as the presence or absence of these structures. The chosen species represent much of the floral morphological variation found in *Croton*, they belong to different sections and were comparatively investigated in relation to the development and structure of the flowers, correlating our data with the available phylogeny in an evolutionary context.

Materials and methods

Inflorescences and flowers in several stages of development were collected in São Paulo at the Instituto de Botânica and in the city of Itanhaém. Voucher specimens were deposited in the Herbarium of the Universidade de São Paulo (SPF): *Croton fuscescens* Spreng. (*Gagliardi and Demarco 9* [SPF]), *Croton sphaerogynus* Baill. (*Gagliardi and Demarco 10* [SPF]), *Croton lundianus* (Didr.) Müll. Arg. (*Gagliardi and Demarco 11* [SPF]).

Flower meristems, flower buds, and pre-anthetic, anthetic, and post-anthetic flowers were isolated, fixed under vacuum in FAA (formalin, acetic acid, 50% ethyl alcohol) for 24 h (Johansen 1940) and in NBF (neutral buffered formalin) for 48 h (Lillie 1965), and then stored in 70% ethyl alcohol.

The material was dehydrated in a butyl series (Johansen 1940), embedded in Paraplast, and transversely and longitudinally sectioned every 10–12 μm in a rotary microtome (Microm HM340E). The sections were stained with astra blue and safranin (Gerlach 1984) and the blades mounted in synthetic resin. Photomicrographs were taken using a light microscope (Leica DMLB).

For the ontogenetic study, micromorphological analyses were performed using the material fixed in FAA. After isolation of the floral parts, the material was dehydrated in an ethanol series, critical point dried with CO_2 (Balzers CPD 030), mounted on aluminium stubs, and sputter coated with gold (Balzers SCD 050). Observations were then made and images taken using a scanning electron microscope (Zeiss DSM 940) with a digital camera attachment.

Results

Morphology of the inflorescences and flowers

The inflorescences of all three studied species are thyrses, with cymules of female and male flowers differently arranged (Fig. 1a–c). In *C. sphaerogynus*, the flowers are continuously distributed along the axis of the thyrse, and the proximal cymules have both female and male flowers and the distal ones have exclusively male flowers (Fig. 1a). In *C. fuscescens*, the flowers are also continuous, with proximal cymules containing only female flowers and distal ones male flowers (Fig. 1b). In *C. lundianus*, the cymules containing female flowers are separated from the ones containing male flowers through a sterile area on the main axis of the thyrse (Fig. 1c).

The female flowers are apparently monochlamydeous in the three species (Fig. 1e, f), but *C. lundianus* presents an extra whorl composed of petaloid, white, and slightly expanded filamentous structures alternating with the sepals (Fig. 1f). The female flowers are hexamerous in *C. lundianus* and pentamerous in *C. sphaerogynus* and *C. fuscescens.*

The male flowers (Fig. 1d) are all pentamerous, but the androecium varies in the number of stamens: eleven stamens in *C. fuscescens*, fifteen in *C. sphaerogynus*, and 10 in *C. lundianus*.

Development of the inflorescences and cymules

The cymules containing female flowers are the first to initiate development in the inflorescences of all three species, and the terminal flower is the first to initiate differentiation in each cymule. This characterises the development as basipetal for the cymule and acropetal for the main axis (Fig. 2a).

Development of female flowers

Calyx

The first whorl to start differentiation is the calyx (Fig. 2b, c), and the sepals develop in an anticlockwise direction (Fig. 2b). The species have sepals with similar size and shape, except for *C. fuscescens*, which has larger, anterior sepals first differentiated, followed by small sepals (Fig. 2c).

When the elongation is almost complete, it can be observed in *C. sphaerogynus* and *C. lundianus* that the sepals develop continuously and all at the same rate (Fig. 2d), different from *C. fuscescens* (Fig. 2e). During the development of the calyx in *C. fuscescens*, the different rate of sepal elongation is notable and there is a delay in the elongation of the two posterior sepals allied to a laciniation of the three anterior ones, resulting in a calyx
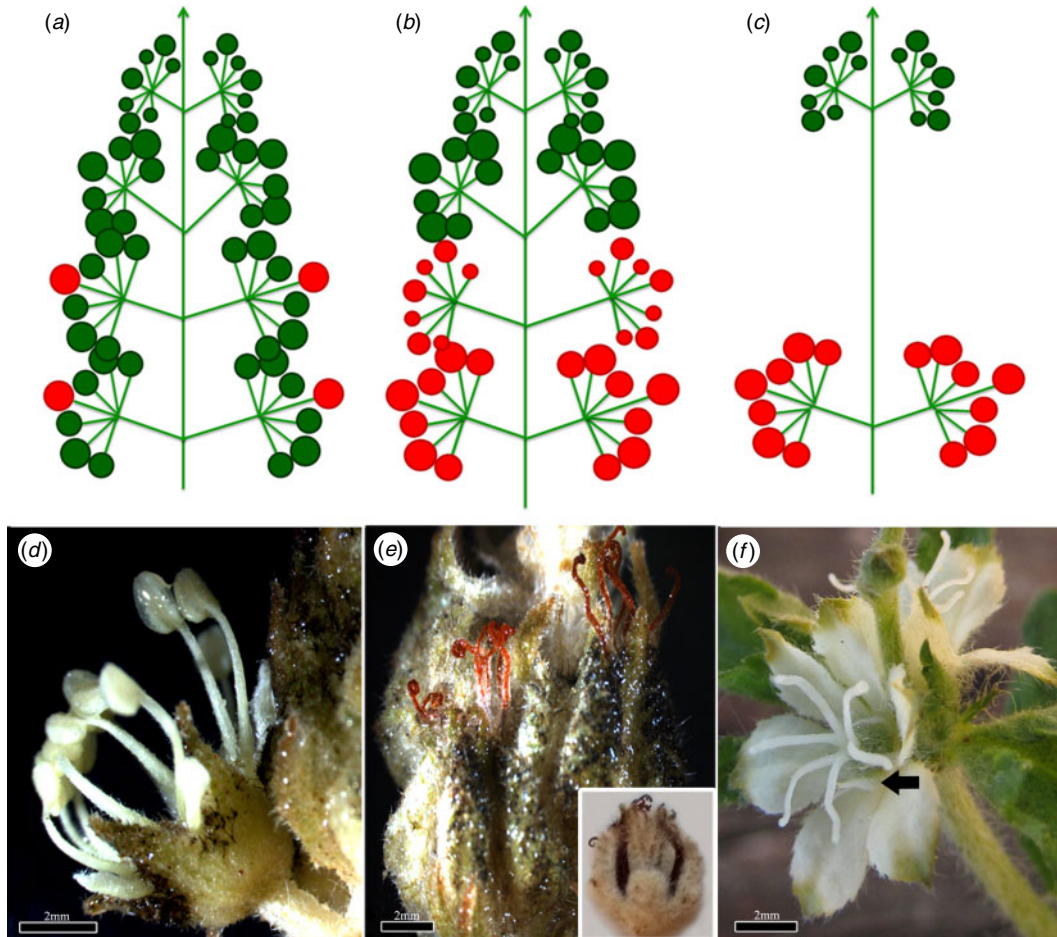
Fig. 1. Inflorescences and flowers of *Croton*. (*a*) Inflorescences of *Croton sphaerogynus*, (*b*) inflorescence of *Croton fuscescens*, (*c*) inflorescence of *Croton lundianus*, (*d*) staminate flower of *C. fuscescens*, (*e*) proximal region of the inflorescence of *C. fuscescens*, detail of the pistillate flower, and (*f*) pistillate flower of *C. lundianus*, note the petaloid structures (arrow).

with two reduced sepals and three that are longer and laciniate (Fig. 2*e, f*).

Filamentous-petaloid whorl

The development of filamentous, petaloid structures alternating with the sepals was observed in *C. sphaerogynus* and in *C. lundianus* (Fig. 2*g, k*). Following the elongation of the calyx, these filamentous are alternate with the sepals (Fig. 2*g*), and in *C. lundianus* the petaloid structures become more elongated and exhibit two distinct regions: a long basal portion, and a slightly flattened apical region (Fig. 2*h*). With the elongation of the sepals, the petaloid structures become spatulate, with a thin elongation at the apex in *C. lundianus* (Fig. 2*i*), but short and pointed in *C. sphaerogynus* (Fig. 2*k*).

Nectaries

During the elongation of the organs in the three species studied, the adaxial-basal meristematic cells adjacent to the sepals start differentiating into nectaries (Fig. 2*i–l*). The development of these glands around the ovary is followed by elongation. In *C. sphaerogynus*, the nectaries become lobed

and develop around the ovary with filamentous structures between the lobes (Fig. 2*k*); in *C. lundianus*, the nectaries are segmented into small, rounded structures, associated with the sepals (Fig. 2*i*). *C. fuscescens*, by contrast, exhibits deeply 3-lobed nectaries that develop only opposite and adjacent to the three large sepals, thereby surrounding part of the ovary (Fig. 2*j, l*). When the flowers are completely developed, the nectaries become larger and secretory (Fig. 2*i–l*).

Gynoecium

After the elongation of the calyx, the meristematic cells internal to the calyx start developing into the carpels. These are initially separate and then become fused during the course of development (post-genital fusion) in all three species (Fig. 2*m, n*), with evident ovule primordia inside the young ovaries (Fig. 2*n*). The elongation of the carpels is followed by the formation of the styles (Fig. 2*j, k, o, p*). A complete gynoecium is observed at this stage, with a hairy stigma in all the species, glandular and nonglandular trichomes (Fig. 2*o*); the styles are in division and are quite elongated in *C. fuscescens* and in *C. lundianus* (Fig. 2*o, p*), and also curved in the latter species

Fig. 2. Ontogeny of inflorescences and female flowers. (*a–c, e, f, j, l, m–o*) Young inflorescence of *Croton fuscescens*, (*b*) reproductive bud with sepals' primordia (*arrow*), (*c*) reproductive bud with sepals in development, long sepals and reduced sepals, (*d, k*) flower bud of *Croton sphaerogynus* with elongated sepals, (*e*) flower bud with sepals in different rhythm of elongation, (*f*) detail of the abaxial long sepals and reduced sepals (arrow), (*g–i, p*) flower bud of *Croton lundianus* with sepals removed (asterisk), note the petaloid structures (arrow), (*h*) anthetic flower with sepals removed (asterisk) and petaloid structures in elongation process (arrow), (*i*) mature flower with sepals removed (asterisk), elongated petaloid structures (arrow) and nectary (highlighted) associated with the removed sepal, (*j*) gynoecium with nectary (asterisk) in the basis of the ovary, (*k*) gynoecium with nectary (asterisk) in the basis of the ovary and filament structure (highlighted and in detail), (*l*) detail of the horse-shaped nectary around the ovary, which was removed (asterisk), (*m*) flower buds with carpels in early stage of development, (*n*) detail of the gynoecium with carpels almost fused (arrow) and ovule primordia (asterisk), (*o*) anthetic flower with elongation of styles (arrow) and ramification of stigmatic region, and (*p*) mature flower with elongated styles. Abbreviations: Ls, long sepal; Rs, reduced sepal.

(Fig. 2*p*), with a globose, hairy ovary. With elongation and complete development of the gynoecium, the styles become long and hairy, bifid in *C. lundianus* and *C. fuscescens* (Fig. 2*o*) and multifid in *C. sphaerogynus*. They have further curved branches in *C. fuscescens* and *C. sphaerogynus*, in contrast to *C. lundianus*, in which the stigmas become more elongate and less curved (Fig. 2*p*).

Anatomy of female flowers

Calyx

The sepals present a uniseriate epidermis composed of round-shaped cells in all the studied species (Fig. 3*a*) and slightly elongated cells in *C. sphaerogynus* and in the small sepals of *C. fuscescens* (Fig. 3*b*). In some regions of the abaxial epidermis

Fig. 3. Anatomy of pistillate flowers. (*a, e, g, h*) Transverse section of *Croton fuscescens* sepal with vascular traits (arrows), (*b, d, f, i*) transverse section of *Croton sphaerogynus* sepal with mucilage cells (asterisk), (*c, j, k*) longitudinal section of petaloid structure of *Croton lundianus* (asterisk), (*d*) longitudinal section of filament structure of *C. sphaerogynus* (asterisk), (*e*) longitudinal section of style and stigma, (*f*) longitudinal section of the curved and ramifies styles, (*g*) transverse section of style, note the stylar canal (asterisk) and vasculature of the ramified stylar branches (arrows), (*h*) division process (arrow) of the styles/stigmas, (*i*) transverse section of the ovary, (*j*) details of the ovary and ovule, note the ovary wall (bar) and vasculature (arrow), and (*k*) ovule with nucellar beak (arrow) and placentary obturator (asterisk). Abbreviation: Ov, ovule.

of *C. sphaerogynus* there are large and round crystal idioblasts and mucilage cells (Fig. 3*b*). The mesophyll is homogeneous, with chlorophyllous parenchyma, and the vasculature is formed by one to three collateral vascular bundles or even more, as in *C. fuscescens* (Fig. 3*a*).

Petaloid whorl and filamentous structures

The petaloid structures of *C. lundianus* and the filamentous structures of *C. sphaerogynus* are anatomically similar to the sepals, although the mesophyll is thinner, with fundamental parenchyma and no vasculature (Fig. 3*c, d*).

Gynoecium

The gynoecium has long stigmas with a uniseriate and papillose epidermis in all three species (Fig. 3*e*); stigmas are slightly elongated in *C. sphaerogynus* (Fig. 3*f*) and more pronounced in *C. fuscescens* and *C. lundianus* (Fig. 3*e*). The style branches are hollow in all three species and consist of a uniseriate epidermis of small, cubic cells on both faces, and a central region with a stylar canal composed of secretory palisade cells (Fig. 3*g, h*) that are present through the whole style branch, from the stigma to the ovary. In the apical region of the ovary, the styles are united in a short extension that divides into three in the larger distal portion (Fig. 3*h*); each one of these parts divides again into two stigmata, creating a total of six branches. In *C. sphaerogynus* the styles are more curved than those in the other species and crystal idioblasts and mucilage cells are widely present in the style cells (Fig. 3*g*).

The ovary of all three species is tricarpellate and trilocular, with one ovule in each locule (Fig. 3*i*). It presents a uniseriate outer epidermis composed of small, isodiametric cells in *C. fuscescens*, a biseriate epidermis with cubic cells in *C. lundianus* (Fig. 3*j*), and a uniseriate epidermis with slightly elongated cells in *C. sphaerogynus* (Fig. 3*i*). Nonglandular trichomes are found in all three species, and crystal idioblasts are present in *C. sphaerogynus* (Fig. 3*i*). The mesophyll is homogeneous, with dorsal and ventral collateral vascular bundles (Fig. 3*j*). The inner epidermis is composed of elongated cells, uniseriate in *C. sphaerogynus* and *C. lundianus*, and biseriate in *C. fuscescens* (Fig. 3*i, j*).

The ovules are bitegmic (Fig. 3*j*) and anatropous, with a thicker (3–5 cell layers) outer integument and a thinner (2–3) inner integument. The micropyle is composed of both integuments; there is also a placentary obturator and nucellar beak present in all three species (Fig. 3*k*).

Development of male flowers

The cymules containing male flowers show later development compared with the ones with female flowers (Figs 2*a*, 4*a*).

Calyx

The sepals are the first structures to be formed, with five protuberances in anticlockwise direction (Fig. 4*b*). After differentiation and the beginning of elongation, the sepals undergo post-genital fusion (Fig. 4*c*). The elongation of the sepals is followed by the formation of trichomes on their surface, covering the stamens (Fig. 4*c*).

Corolla

Following the development of the sepals and alternating with them, the free primordia of petals begin their differentiation in all three species (Fig. 4*d*). The petals exhibit slow and delayed elongation (Fig. 4*d, e*), and whereas the calyx is quite elongated, the corolla is still reduced. With the elongation of the perianth, the petals reach their final shape and size.

Androecium

Internal to the calyx and corolla primordia, a group of meristematic cells begins to differentiate and some stamen primordia may be observed in a centripetal formation in the marginal, outer region of the receptacle (Fig. 4*e, f*). Followed by cell divisions, in bud stage, the primordia of the stamens show elongation of the filaments and formation of the anthers, which are rounded before thecal differentiation. The anthers are quite immature with early differentiation of their walls (Fig. 4*f*). As they elongate, from bud stage to pre-anthesis, the filaments begin to curve and the anthers become completely formed, with pollen grains that are initiating their own development (Fig. 4*g*). With elongation, the stamens become more curved, with longer connectives that expand over the anthers (Fig. 4*h, i*). The anthers present only two layers of cells in their walls (epidermis and endothecium), with the secretory tapetum consumed for the production of the pollen grains, which are now completely formed (Fig. 4*l*). In the post-anthetic stage, the stamens show longer filaments, and the anthers are characterised by flattening of the thecae (Fig. 4*j, k*).

Nectaries

During the elongation of the perianth, the nectaries around the androecium differentiate (Fig. 4*g*). These glands go through the elongation process and develop as five small and cubic structures closely associated and almost adnate to the stamens (Fig. 4*g*). When the flowers are completely developed, the nectaries become fully differentiated and secretory (Fig. 4*k*).

Anatomy of male flowers
Calyx and corolla

The sepals and petals of the male flowers in all three species present a uniseriate epidermis, composed of round-shaped cells with abundant nonglandular trichomes only on the abaxial surface (Fig. 5*a*). The mesophyll is homogeneous with phenolic idioblasts and crystalliferous idioblasts containing druses. The vasculature is formed by collateral vascular bundles, with one medium vascular bundle and one or two lateral bundles in the sepals (Fig. 5*a*). The petals have a similar anatomical structure, with one collateral vascular bundle; the abaxial epidermis of *C. sphaerogynus* is exceptionally composed of large, rounded, crystal idioblasts and mucilage cells (Fig. 5*b*). On the apex of the sepals and petals of all three species, there are nonglandular trichomes (Fig. 5*c*) and in *C. fuscescens* there are long nonglandular trichomes at the base of the petals also (Fig. 5*f*). These trichomes intercalate to each other and keep the calyx and corolla closely attached and associated with each other.

Fig. 4. Ontogeny of staminate flowers. (*a–f, h, j*) Young inflorescence of *Croton fuscescens*, (*b*) flower buds with sepals (thick arrow) and petals formation (middle arrow), (*c*) flower bud with sepals' elongation and initial trichomes (arrow), (*d*) flower bud with sepals removed (asterisk) and petal in early stage of development (arrow), (*e*) flower bud with petals starting elongation (arrow) and stamens initiating development (asterisk), (*f*) longitudinal section of flower bud with stamen filaments in elongation process (arrow), (*g, k*) longitudinal section of *Croton sphaerogynus* flower with the stamens and anthers in differentiation, note the nectary (highlighted), (*h*) detail of the elongated and curved stamen, (*i*) detail of the long connective (asterisk) which involves the anthers, (*j*) anthetic-flower with long and developed whorls, (*k*) post-anthetic flower with the maximum elongation of the whorls and mature stamens, note the developed nectaries (highlighted), and (*l*) detail of a developed anther with pollen grains of *Croton lundianus*.

Fig. 5. Anatomy of staminate flowers. (*a*) Sepal of *Croton fuscescens*, (*b*, *d*, *e*) petal of *Croton sphaerogynus*, note the mucilage cells (asterisk), (*c*, *f*) anthetic-flower of *Croton lundianus* with developed stamens, though sepals and petals still united by trichomes (arrow), (*d*) developed stamen with long and curved filament, (*e*) detail of developed anther, note the connective with idioblast cells (asterisk), and (*f*) detail of the trichomes on the base of petals and stamens (arrow).

Androecium

The filaments exhibit a uniseriate epidermis and are composed of parenchyma cells (Fig. 5*d*). The connective is long and presents an apical region composed of cells with a secretory aspect (Fig. 5*e*, *e*). The anther is tetrasporangiate with longitudinal dehiscence of the pollen grains (Fig. 5*e*). The anther walls

present epidermal cells that are tangentially elongated, an endothecium with palisade cells and wall thickenings forming trabeculae; the secretory tapetum is totally consumed during the production of the pollen grains, which are organised in monads (Fig. 5*e, f*).

Discussion

Perianth, petaloid whorl, and transitional structures

The results obtained in this study for the development of *Croton* flowers corroborate our hypothesis that the filaments in the female flowers of *C. lundianus* are staminodes, thus part of a vestigial androecium, such as the nectaries studied by De-Paula *et al*. (2011).

Croton flowers have similarities in the initiation developmental step and differences in origin of the whorls and in elongation. In the initiation, the first structures to develop in all the species were sepals, followed by the petals and androecium in male flowers, staminodes and gynoecium in the female flowers. The differences are focussed on the elongation developmental step, which we assume to be the responsible for the high morphological floral diversity in *Croton*, especially the elongation of the sepals and filaments of the female flowers. In *C. sphaerogynus* and *C. lundianus* the five sepals elongate in the same way and rhythm, while in *C. fuscescens* the three exposed sepals become thicker faster and irregularly elongated, resulting in a deeply laciniate calyx, with large three sepals and two smallest ones. In the other species, the sepals are not as thick and large as in *C. fuscescens*, although they are present around the whole flower, thereby providing protection to the fertile whorls.

Croton male flowers have a whorl of petals that alternate with the sepals. The female flowers are all monochlamydeous but sometimes show an extra whorl of filaments or petaloid structures, which were initially, described as reduced petals (De-Paula *et al*. 2011). Although, the elongation of the petaloid structures of *C. lundianus* is different from a petal development and characterises a stamen with two different regions, one basal and more elongated, such as a stamen filament and an apical portion slightly flattened, such as an anther. The flattening in the apical portion characterises these structures as staminodes that did not complete anther development. In the mature stage, they exhibit an elongated and thin connective region and a more flattened antheroid.

Staminodes bearing antheroids are relatively uncommon, but widely distributed taxonomically (Walker-Larsen and Harder 2000). Rijpkema *et al*. (2006) studied the ontogeny of *Petunia* flowers and suggested that the modification of petals into staminodes could be associated with a mutation of BLIND-BL gene, as *bl* mutants displayed a homeotic conversion of the corolla into antheroid structures in the second whorl. The development of *Croton* staminodes observed here corroborates our hypothesis that petals in the core eudicots have a staminodial nature as previously suggested by Ronse De Craene (2007).

In taxonomical studies, *C. lundianus* has been described as a monochlamydeous flower with reduced petals (Lima and Pirani 2003; Caruzo and Cordeiro 2007; Silva *et al*. 2010), but according to our ontogeny results, *C. lundianus* should be interpreted as a monochlamydeous flower with a whorl of

staminodes. In *C. sphaerogynus* filamentous structures were also observed between the nectary lobes, and based on their position (opposite to the sepals) these structures are described and interpreted here for the first time as staminodes. The same interpretation was used for De-Paula *et al*. (2011), who have described nectaries as staminodes based on the position of these structures opposite to the sepals.

Besides the elongation developmental step which explains the high morphological diversity in *Croton*, the presence of staminodes in female flowers can be interpreted as transference of function and heterochrony cases. The staminodes of *C. lundianus* provide an example of transference of function, in which the staminodes assumed the role of petals. This is similar to the case of the coloured staminodes of *Jacquinia macrocarpa* (Theophrastaceae), which are morphologically very similar to the petals, but represent an aborted stamen whorl (Ronse De Craene 2003). The same author studied Papaveraceae flowers and concluded that the organ identity can switch at the boundary of petals and stamens, thereby culminating in the transition of petals into stamens and *vice versa*.

Heterochrony is observed in the development of *C. sphaerogynus* staminodes, a process defined by Baum and Donoghue (2002) as a temporal developmental change (a phenotypic modification of pre-existing structures). This phenotypic modification is the loss of function of filamentous structures, observed through the initial development of the stamens filament, which showed an early and premature maturation of the tissues, resulting in no further development of the stamens, thereby anthers and pollen grains were not developed.

Contrary to our conclusion, De-Paula *et al*. (2011) suggested that these filamentous/petaloid structures of *Croton* may be interpreted as reduced and transformed petals; further ontogenetic and vasculature analysis is necessary to draw firm conclusions on the origin of these structures.

Based on our observations and the available literature, Crotonoideae flowers exhibit sterile whorls and fertile whorls in the composition of their flowers, though with different patterns of development including transitional structures, such as the staminodes, that give rise to different floral morphologies. Staminodes are considered transitory structures, and according to Walker-Larsen and Harder (2000) and Ronse De Craene and Smets (2001), these structures point to an evolutionary change, either the loss or modification of a whole whorl of petals, such as in *C. lundianus* in which the petals were modified in staminodes, though keeping the attraction role. *C. sphaerogynus* presented the partial reduction of stamens within a whorl, which was also observed in other angiosperms, such as in Geraniaceae, Primulaceae, Myrtaceae, Scrophulariaceae and Verbenaceae (Ronse De Craene and Smets 2001).

Evolutionary interpretation of the floral developmental patterns

The species analysed belong to different sections of *Croton*. *Croton fuscescens* belongs to *Croton* sect. *Julocroton* (Mart.) G.L. Webster; *C. sphaerogynus* is included in *Croton* sect. *Cleodora* (Klotzsch) Baill.; and *C. lundianus* belongs to *Croton* sect. *Geiseleria* (A. Gray) Baill. (Van Ee *et al*. 2011). *Croton* sect.

Julocroton is the most recent section when compared with *Cleodora* and *Geiseleria*, and based on the results discussed above we could assume that the irregular sepal development in the female flowers of *C. fuscescens* explains the zygomorphic morphology of the flower, which results from the different sized, laciniate sepals. The zygomorphy and the laciniate calyx would be considered an apomorphic character.

Croton sphaerogynus belongs to *Croton* sect. *Cleodora* (Klotzsch) Baill, a phylogenetically oldest lineage section when compared with *Geiseleria* and *Julocroton* (Van Ee *et al.* 2011). *Croton* sect. *Geiseleria* (A. Gray) Baill. (Van Ee *et al.* 2011), in which *C. lundianus* is included, is an intermediate lineage section when compared with *Cleodora* and *Julocroton*. The petaloid structures, interpreted here as staminodes, and the filamentous structures in *C. sphaerogynus*, also interpreted as staminodes, are assumed to be transitional structures in the evolution of *Croton* flowers. These staminodes are absent in *C. fuscescens* (sect. *Julocroton*), which represents the most recent lineage species here, and thus the presence of staminodes could be expected to be a conserved characteristic in *C. lundianus* sect. *Geiseleria* and in *C. sphaerogynus* sect. *Cleodora*.

Other species of Euphorbiaceae show perianth initiation similar to what we observed in *C. sphaerogynus* and *C. lundianus*. Liu *et al.* (2008) analysed the ontogeny of *Jatropha* flowers, which also develop simultaneous and continuous sepals and petals. In other species of *Croton* and *Astraea*, such as *Croton glandulosus*, *Croton piptocalyx*, *Croton urucurana* and *Astraea lobata*, the pattern of sepal initiation is similar, although in *Croton triqueter* the sepals show unidirectional development (De-Paula *et al.* 2011), similar to the pattern we observed in *C. fuscescens* and both species belong to *Croton* sect. *Julocroton*.

In contrast to our observations, some Euphorbiaceae flowers exhibit vestigial or even no perianth formation (Prenner and Rudall 2007; Narbona *et al.* 2008; Cacho *et al.* 2010; Prenner *et al.* 2011). Gagliardi (2014) studied the pseudanthia of Peraceae and Euphorbiaceae (Acalyphoideae and Euphorbioideae), and the Acalyphoideae inflorescences showed female and male flowers with developed sepals, whereas the perianth is entirely lacking in Euphorbioideae.

The loss of petals in female flowers, as documented here in *C. fuscescens* and *C. sphaerogynus*, and the modification of staminodes into petaloid structures, as in *C. lundianus*, have occurred numerous times in flower evolution and may characterise large groups, such as the Ranunculales, Fagales and Cyperaceae (Endress 2011*a*). The developmental study of *Croton* flowers can help us to understand floral evolution in the genus and in Euphorbiaceae, since *Croton* is an intermediate flower morphotype in the whorl reduction issue in comparison to many other Euphorbiaceae. In addition to the filamentous structures (staminodes) assuming the role of petals, *Croton* flowers maintain all the whorls present during ontogeny, unlike what has been described for *Calycopeplus*, *Euphorbia* and *Dalechampia* (Prenner and Rudall 2007; Gagliardi 2014).

With respect to the fertile whorls, the studied species are similar in the connation of the carpels, which has also been reported in other Crotonoideae species (De-Paula *et al.* 2011; Gagliardi 2014), and which may be considered a common ontogenetic characteristic for these phylogenetically associated flowers. The gynoecia of the species studied exhibit long and secretory stigmata, transmitting tissues, and anatropous ovules with nucellar beaks and placentary obturators. The long stigma is important to attract pollinators and maximise pollen grain deposition. All the characteristics above, except for the long stigma, have been frequently reported in the Euphorbiaceae (Tokuoka and Tobe 1995, 1998, 2002, 2003, 2006; Tokuoka 2007; Souza *et al.* 2010; Gagliardi *et al.* 2012, 2014; Gagliardi 2014).

The development of the stamens in all the species studied here was found to be centripetal, and according to Rudall (2007), this may be considered a basal characteristic of the eudicot clade, one also described by Johri *et al.* (1992) when understanding the evolutionary history of angiosperms.

Anatomy of flowers

The sepals and the petals of the species studied show indumentum in the abaxial epidermis with a larger number of trichomes in the apex that closely associate the calyx and corolla together through their intertwining. According to Weberling (1989), this may be interpreted as a special type of gamosepaly, described as capillinection, in which a close intertwining of trichomes maintains the adhesion of the perianth. This mechanism keeps the fertile whorls protected.

Crystal druses and phenolic idioblasts occur in the epidermis and mesophyll of the sepals, petals and anthers, which is associated with a special mechanism of protection against herbivores, dehydration and calcium control (Fahn 1979, 2000).

The gynoecium is quite similar in all *Croton* flowers and its structure seems to be a pattern for other flowers in Euphorbiaceae (Haber 1925; Souza *et al.* 2010; De-Paula and Sajo 2011; Gagliardi 2014; Martins *et al.* 2016).

The ovule characteristics of *Croton* flowers, such as curvature (antitropous), number of integuments (bitegmic), the thickness of integuments (thicker outer integument and a thinner inner integument), and the presence of nucellus are common in apparently all Euphorbiaceae (Endress 2011*a*) and in some Malpighiaceae as well.

The androecium exhibits a tetrasporangiate anther with an epidermis and endothecium similar to those described for other *Croton* species (De-Paula and Sajo 2011). The curvature and elongation of the filaments and the position of the anther are also similar to other *Croton*. This is an important character to differentiate *Croton* from *Brasiliocroton*, which shows erect filaments when in flower bud (Berry *et al.* 2005).

The structural characteristics described here have not been previously reported for the studied species and may be able to play an important role in the classification of *Croton* species and in the clarification of the differences among them.

Conclusions

The flowers of *Croton* studied here represent the different morphological patterns found in the genus and we corroborated the two hypotheses which motivated this study. The morphologically different flowers of *Croton* result from different developmental steps, especially the first steps, which include sepals initiation and elongation, emphasised by the

laciniation process of *C. fuscescens*, considered an apomorphic character for the genus. Based on our ontogenetic analysis, the filaments of female flowers usually described as reduced petals are interpreted here as staminodes, first described for *C. sphaerogynus.* These structures represent cases of transference of function and heterochrony and are considered a conserved characteristic.

The flowers of *Croton* exhibit intermediate conditions with respect to whorl reduction when compared with many other Euphorbiaceae; this is due to the modifications in their floral developmental patterns. These evolutionary modifications gave rise to an extensive diversity of floral morphologies in the large genus *Croton*, though future floral development studies including different species are important to verify the application of the patterns described here or also report unknown developmental alterations. Considering that *Croton* is one of the largest genus among angiosperms, studies concerning floral vasculature and molecular analysis are also essential to understand the floral evolution of the genus.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Species	Vouchers
Croton cascarilloides Raeusch.	Beechey;
Croton crassifolius Geiseler.	Kurz, S. 2485
Croton kongensis Gagn.	Balansa, B. 4533
Croton lachnocarpus Benth.	Champion, J.G. 473
Croton laevigatus Vahl.	
Croton tiglium L.	Wallich 7722
Croton goudotii Baill.	R. CAPURON 27073
Croton gratissimus Burch.	Burchell, W.J. 2154
Croton confertus Bak.	Bent, J.T. 231
Croton curyphyllus L.	
Croton hainanensis Men.	
Croton laetifolius Baill.	
Croton scabiosus Bedd.	Beddome, R.H.
Croton megalobotrys Müll. Arg.	MRS. H. M. Richards 13369
Croton argyratus Blume	Schultz, F. 609
Croton schultzii Benth.	
Croton argyranthemus Michx.	Berlandier, J.L. 1554
Croton arnhemicus Müll. Arg.	Dallachy, J.
Croton insularis Baill.	Caldwell, E.
Croton microtiglium Burkill	Crosby, C.S. 150
Croton verreauxii Baill.	
Croton phebalioides F. Muell. ex Müll. Arg.	
Croton capitis-york Airy Shaw	
Croton acronychioides F. Muell.	Dallachy, J.
Croton ripensis Kaneh & Hatus	
Croton tomentellus F. Muell.	Mueller, F.J.H.von.
Croton choristadenius K. Schum.	
Croton dockrilli Airy Shaw	
Croton hirtus L` Hér.	Hinton, G.B. 4552
Croton glandulosus L.	Lindheimer, F. J. 691
Croton antisyphiliticus Mart.	Harley, R.M.; Giulietti, A.M.; Stannard, B.L.; Hind, D.J.N.; Kameyama, C.; Prado, J.; Rudall, P.J.; Simão-Bianchini, R.; Taylor, N.P.; Zappi, D.C. 24843
Croton guildinguii Griseb.	
Croton jutiapensis Croizat	P. C. Standley 74971
Croton betulinus Vahl	Ledru, A. P.
Croton lundianus (Didr.) Müll. Arg.	Elias Contreras 8680
Croton sincorensis Mart.	
Croton virgultosus Müll. Arg.	
Croton adamantinus Müll. Arg.	Sasaki, D.; Camargo, A.C.; Rosa, S.A.; Piva, J.H. 1196
Croton sclerocalyx (Didr.) Müll. Arg.	Riedel 1122
Croton goyazensis Müll. Arg.	Riedel
Croton tetradenius Baill.	
Croton triangularis Müll. Arg.	
Croton teucridium Baill.	

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Croton costaricensis Pax (= C. Ortholobus)	Kuntze, O. 2237
Croton punctatus Jacq.	Pringle, C.G. 6355
Croton dioicus Cav.	Hartweg 83
Croton californicus Müll. Arg.	Hinds
Croton lindheimerianus Scheele	Wright, C. 641
Croton capitatus Michx.	Peter, R.
Croton monanthogynus Michx.	Pringle, C.G. 11164
Croton humilis L.	
Croton fruticulosus Torr.	Pringle, C.G.
Croton stipulaceus Kunth	
Croton suberosus Kunth	Hinton, G.B. 10853
Croton heliotropiifolius Kunth	Gardner 1400
Croton conduplicatus Kunth	
Croton flavens L.	Macnab, G.
Croton gracilipes Baill.	M. Serrano; S. Churchill; J. Vilalobos; D. Villarroel
Croton bonplandianus Baill.	Omondi W.; Kiamba J & Obunyali C & Odunga S
Croton xalapensis Kunth	Percy H. Gentle 2218
Croton discolor Willd.	Pollard, B.J. 1272
Croton pungens Jacq.	Fendler, A. 1213
Croton ruizianus Müll. Arg.	Paul C. Hutchison & J. Kenneth Wright 7148
Croton abutiloides Kunth	Felix Woytkowski 7789
Croton linearis Jacq.	Hamilton, M.A. et al. 671
Croton aequatoris Croizat	Eggers, H.F.A. von 15489
Croton saltensis Griseb.	Lorentz, P.G.; Hieronymus, G. 231
Croton impressus Urb.	Urban, I. 3893
Croton ciliatoglandulifer Ortega	Pringle, C.G. 1914
Croton bixoides Vahl	
Croton chichenensis Lundell	
Croton scouleri Hook. f.	
Croton rivinifolius Kunth	
Croton fishlockii Britton	Fishlock, W.C. 311
Croton alloeophyllus Urb.	
Croton angustatus Urb.	
Croton azuensis Urb.	
Croton poitaei Urb.	
Croton polytomus Urb.	
Croton origanifolius Lam.	Wright, C. 564
Croton subferrugineus Müll. Arg.	Pohl 1622
Croton campestris A. St. - Hil.	Harley, R.M.; Bromley, G.L.; Carvalho, A.M.; Nunes, J.M.S.; Hage, J.L.; Santos, E.B. 22376
Croton betaceus Baill.	Gardner 1840?
Croton pycnadenius Müll. Arg.	Burchell 8988
Croton thurifer Kunth	
Croton frieseanus Müll. Arg.	
Croton lehmannii Pax	Lehmann, F.C. 4821
Croton adipatus Kunth	
Croton lucidus L.	Emery C. Leonard & Genevieve M. Leonard

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Croton ciliatoglandulosus Ortega	
Croton cortesianus Kunth	Berlandier 2244 = 824
Croton schiedeanus Schltdl.	Ibarra Manríquez, G.; Sinaca C., S. 1361
Croton jamaicensis B.W. van Ee & P.E. Berry	
Croton myricifolius Griseb.	E. L. Ekman.
Croton brittonianus Carabia	C. Wright
Croton bispinosus C. Wright	Wright, C.
Croton niveus Jacq.	A. C. Smith 10179
Croton icche Lundell	
Croton arboreus Millsp.	
Croton reflexifolius Kunth	Triana, J. 3644
Croton eluteria (L) W. Wright	Wright, C. 1971
Croton schiedeanus Schltdl.	Ibarra Manríquez, G.; Sinaca C., S. 1361
Croton "glabellus" L. [sic.]	
Croton decalobus Müll. Arg.	
Croton micans Sw.	Blanchet 3655
Croton floribundus Spreng.	Chagas e Silva, F. 1751
Croton tricolor Klotzsch ex Baill.	Sellow
Croton blanchetianus Baill.	Blanchet 3094
Croton rosmarinoides Millsp.	Wright, C. 1968
Croton astroites Aiton	Walsh, J.J.
Croton scaber Willd.	Fendler, A. 1234
Croton decalobus Müll. Arg.	
Croton axillaris Müll. Arg.	
Croton yucatanensis Lundell	Lundell, C.L.; Lundell, A.A. 7400
Croton compressus Lam.	
Croton sucrensis Steyerm.	
Croton hoffmannii Müll. Arg.	
Croton sphaerogynus Baill.	Riedel
Croton salutaris Casar.	
Croton spruceanus Benth.	Spruce, R. 2205
Croton cajucara Benth.	Spruce, R. 528
Croton organensis Baill.	
Croton fragrans Kunth	Purdie
Croton billbergianus Müll. Arg.	Purdie 118
Croton javarisensis Secco	
Croton draco Schltdl. & Cham.	Chavarria, M.M.; Solis, A. 882
Croton coriaceus Kunth	
Croton gossypiifolius Vahl.	Holton, I.F. 868
Croton celtidifolius Baill.	Gardner 618
Croton jimenezii Standl. & Valerio	
Croton hibiscifolius Kunth ex Spreng.	Timothy Plowman 1935
Croton huberi Steyerm.	Fendler, A. 1221
Croton perspeciosus Croizat	C. Vargas 8549
Croton pilulifer Rusby	O. W. Stutter 11
Croton lechleri Müll. Arg.	Wilson Quizhpe; V. Granda; D. Veintimilla; H. Salas & P. Wampash 1501

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Croton speciosus Müll. Arg.	Linden, I. 34
Croton floccosus B.A. Sm.	van der Werff, H.; Gray, B.; Fuentes, P. 13379
Croton alchorneicarpus Croizat	Kuhlmann, M.
Croton echinocarpus Baill.	non especified
Croton rusbyi Britton ex Rusby	Rusby, H.H. 1224
Croton amentiformis Riina	
Croton ichthygaster L.B. Sm. & Downs	Hatschbach, G. 14976
Croton caldensis Müll. Arg.	Regnell, A.F. III 1080
Croton vulnerarius Baill.	Glaziou, A.F.M. 4916
Croton urucurana Baill.	
Croton pedicellatus H.B.K. (Det. Croizat)	Gardner 2308
Croton andinus Müll. Arg.	
Croton ovalifolius Vahl in H. West.	
Croton sellowii Baill.	J. S. Blanchet 1803
Croton velutinus Baill.	Blanchet 3660
Croton hircinus Vent.	
Croton guianensis Aubl.	Aublet, J.B.C.F.
Croton glandulosepalus Millsp.	
Croton atrorufus Müll. Arg.	Pohl 1636
Croton timandroides (Didr.) Müll. Arg.	Martius 958
Croton decipiens Baill.	
Croton garckeanus Baill.	F. Sellow 2363
Croton chaetophorus Müll. Arg.	
Croton muscicapa Müll. Arg.	
Croton glutinosus Müll. Arg.	
Croton urticifolius Lam.	Burchell 705
Croton chaetocalyx Müll. Arg.	Burchell 6496
Croton palanostigma Klotzsch	Zappi, D.C.; Sasaki, D.; Milliken, W.; Biggs, N.; Silveira, E.A.; Philippsen, M.; Bessa, M.A.; Piva, J.H. 873
Croton matourensis Aubl.	Brito, J.M.; Ribeiro, J.E.L.S.; Pereira, E. da C. 16
Croton chocoanus Croizat	Killip, E. P. 35482
Croton costatus Kunth	
Croton cuneatus Klotzsch.	Schomburgk
Croton yavitensis Croizat	L. O. Williams 1942
Croton roraimensis Croizat	A. S. Pinkus 122
Croton malambo H. Karst.	Karsten,H.
Croton olivaceus Müll. Arg.	
Croton sampatik Müll. Arg.	
Croton cordiifolius Baill.	Blanchet 3719
Croton triqueter Lam.	
Croton argenteus L.	
Croton fuscescens Spreng.	
Croton setiger Hook.	
Croton michauxii G. L. Webster	
Croton poecilanthus Urb.	Urban, I. 1172
Croton alabamensis E.A. Sm. Ex Chapm.	Mohr, C.
Croton corylifolius Lam.	Wright, C. 566

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Species	Voucher
Croton lundellii Standl.	
Croton beetlei Croizat	Elbert L.; Little Jr. 6712
Croton caracasauus Pittier	
Croton corymbulosus Engelm	
Croton nubigenus G. Webster	A. Grijalva 313
Croton corinthius Poveda & J.Á. González	
Croton cupreatus Croizat	
Croton eichleri Müll. Arg.	
Croton thomasii Riina & P.E. Berry	
Croton luetzelburgii Pax & K. Hoffm.	
Croton polyandrus Spreng.	
Croton ceanothifolius Baill.	Riedel 391/407
Croton pallidulus Baill.	
Croton splendidus Mart. ex Colla	
Croton julopsidium Baill.	
Croton cinerellus Müll. Arg.	Riedel
Croton pachypodus G.L. Webster	Cid Ferreira, C.A. 1076
Croton megistocarpus J. A. González & Poveda	Morales, Juan Francisco 3915
Croton sapiifolius Müll. Arg.	
Croton caudatus Geiseler.	Harmand, F.J.
Brasiliocroton mamoninha P.E. Berry & Cordeiro	Souza, V. 266
Astraea lobata (L.) Klotzsch	Harley, R.M. 25311
Astraea comosa (Müll. Arg.) B. W. Van Ee	Glaziou, A.F.M. 15390
Astraea divaricata Klotzsch	Sellow
Acidocroton sp. Griseb.	Ekman, E.L. 16896

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Species	Clade	Phylo	Infloresc	Pattern	Ramif.	Elong.	F.flowers.concent	M.cymes	F.cymes	M/F. cymes
Astraea comosa (Müll. Arg.) B. W. Van Ee	Genus *Astraea* (14)	no	Bisexual	7	no	yes	no	yes	no	no
Astraea divaricata Klotzsch	Genus *Astraea*	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton acronychioides F. Muell.	OW (450)	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton adamantinus Müll. Arg.	Geiseleria (82)	no	Bisexual	10	No	yes	yes	yes	no	no
Croton adipatus Kunth	Adenophylli (223)	no	Bisexual	1	No	yes	yes	yes	yes	no
Croton alloeophyllus Urb.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton amentiformis Riina	Cyclostigma (46)	no	Bisexual	1	No	no	yes	yes	no	no
Croton angustatus Urb.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton arnhemicus Müll. Arg.	OW-Arnhemici (5)	no	Bisexual	7	No	yes	no	yes	no	no
Croton atrorufus Müll. Arg.	Barhamia	no	Bisexual	10	No	yes	yes	yes	yes	no
Croton azuensis Urb.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton betaceus Baill.	Adenophylli	no	Bisexual	7	No	yes	yes	yes	no	no
Croton billbergianus Müll. Arg.	Cleodora	no	Bisexual	7	No	yes	yes	yes	no	no
Croton caldensis Müll. Arg.	Cyclostigma	no	Bisexual	5	No	yes	yes	yes	yes	no
Croton campestris A. St. - Hil.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	yes	no
Croton capitis-york Airy Shaw	OW	no	Bisexual	7	No	yes	yes	yes	no	yes
Croton caracasanus Pittier	Corylocroton (11)	no	Bisexual	5	No	yes	no	yes	yes	no
Croton chaetocalyx Müll. Arg.	Barhamia (84)	no	Bisexual	7	No	yes	yes	yes	yes	no
Croton chaetophorus Müll. Arg.	Barhamia	no	Bisexual	6	No	yes	yes	yes	yes	no
Croton choristadenius K. Schum.	OW	no	Bisexual	7	No	yes	yes	yes	no	yes
Croton ciliatoglandulosus Steud (= C. ciliatoglandulifer)	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton cinerellus Müll. Arg.	Lamprocroton	no	Bisexual	11	No	no	yes	yes	no	yes
Croton compressus Lam.	Lasiogyne	no	Bisexual	1	No	yes	yes	yes	no	no
Croton confertus Bak.	OW	no	Bisexual	1	No	yes	yes	yes	yes	no
Croton cortesianus Kunth	Adenophylli	no	Bisexual	16	No	yes	yes	yes	no	no
Croton costaricensis Pax (= C.	Geiseleria	no	Bisexual	1	No	yes	yes	yes	no	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

ortholobus)										
Croton costatus Kunth	Luntia	no	Bisexual	3	no	yes	yes	yes	no	no
Croton curyphyllus L.	OW	no	Bisexual	7	no	yes	yes	yes	no	no
Croton decipiens Baill.	Barhamia	no	Bisexual	5	yes	yes	yes	yes	no	yes
Croton dockrilli Airy Shaw	OW-Dockrilliorum (4)	no	Bisexual	5	no	yes	yes	yes	no	yes
Croton eluteria (L) W. Wright	Eluteria	no	Bisexual	1	yes	yes	yes	yes	yes	no
Croton fishlockii Britton	Adenophylli	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton frieseanus Müll. Arg.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	yes	no
Croton garckeanus Baill.	Barhamia	no	Bisexual	1	yes	yes	no	yes	no	no
Croton glutinosus Müll. Arg.	Barhamia	no	Bisexual	3	no	yes	yes	yes	yes	no
Croton goyazensis Müll. Arg.	Geiseleria	no	Bisexual	7	no	yes	yes	yes	yes	no
Croton hainanensis Men.	OW	no	Bisexual	1	no	yes	yes	yes	no	yes
Croton ichthygaster L.B. Sm. & Downs	Adenophylli	no	Bisexual	5	no	yes	yes	yes	yes	no
Croton javarisensis Secco	Cleodora	no	Bisexual	7	no	yes	yes	yes	no	no
*Croton julopsidium*Baill.	Lamprocroton	no	Bisexual	10	yes	yes	yes	yes	no	no
Croton laetifolius Baill.	OW	no	Bisexual	5	no	yes	yes	yes	no	no
Croton laetifolius Baill.	OW	no	Bisexual	1	no	yes	yes	yes	yes	no
Croton lehmannii Pax	Adenophylli	no	Bisexual	5	no	yes	yes	yes	yes	no
Croton lucidus L.	Adenophylli	no	Bisexual	10	no	yes	yes	yes	no	yes
Croton lundianus (Didr.) Müll. Arg.	Geiseleria	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton megalobotrys Müll. Arg.	OW	no	Bisexual	7	no	yes	yes	yes	no	no
Croton muscicapa Müll. Arg.	Barhamia	no	Bisexual	1	no	yes	yes	yes	yes	no
Croton origanifolius Lam.	Adenophylli	no	Bisexual	5	no	yes	yes	yes	no	yes
Croton phebalioides F. Muell. ex Müll. Arg.	OW	no	Bisexual	7	no	yes	yes	yes	no	no
Croton poitaei Urb.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	no	no
Croton polytomus Urb.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	no	yes

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Species	Section									
Croton pycnadenius Müll. Arg.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton scabiosus Bedd.	OW	no	Bisexual	1	no	yes	yes	yes	no	no
Croton schiedeanus Schltdl.	Eluteria	no	Bisexual	1	yes	yes	yes	yes	yes	no
Croton schultzii Benth.	OW	no	Bisexual	1	no	yes	yes	yes	no	yes
Croton sclerocalyx (Didr.) Müll. Arg.	Geiseleria	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton sincorensis Mart.	Geiseleria	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton subferrugineus Müll. Arg.	Adenophylli	no	Bisexual	5	no	yes	yes	yes	no	no
Croton sucrensis Steyerm.	Lasiogyne	no	Bisexual	5	no	yes	yes	yes	no	no
Croton tetradenius Baill.	Geiseleria	no	Bisexual	1	no	yes	yes	yes	no	yes
Croton teucridium Baill.	Geiseleria	no	Bisexual	5	no	yes	yes	yes	no	no
Croton thurifer Kunth	Adenophylli	no	Bisexual	5	no	yes	yes	yes	no	yes
Croton timandroides (Didr.) Müll. Arg.	Barhamia	no	Bisexual	10	no	no	yes	yes	no	yes
Croton tomentellus F. Muell.	OW-Gymnocroton (40)	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton triangularis Müll. Arg.	Geiseleria	no	Bisexual	1	no	yes	yes	yes	no	no
Croton urticifolius Lam.	Barhamia	no	Bisexual	1	yes	yes	yes	yes	no	no
Croton urucurana Baill.	Cyclostigma	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton virgultosus Müll. Arg.	Geiseleria	no	Bisexual	1	no	yes	yes	yes	yes	no
Croton vulnerarius Baill.	Cyclostigma	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton eichleri Müll. Arg.	Prisci (3)	yes	Bisexual	5	no	yes	yes	yes	no	yes
Acidocroton sp*.* Griseb.	Genus *Acidocroton* (15)	yes	Bisexual	12	no	no	no	yes	no	no
Astraea lobata (L.) Klotzsch	Genus *Astraea*	yes	Bisexual	7	no	yes	yes	yes	no	yes
Brasiliocroton mamoninha P.E. Berry & Cordeiro	Genus *Brasiliocroton* (2)	yes	Bisexual	9	no	yes	no	yes	yes	no
Croton aequatoris Croizat	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton alabamensis E.A. Sm. Ex Chapm.	Alabamenses (1)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton alchorneicarpus Croizat	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Species	Section		Sex							
Croton andinus Müll. Arg.	Pedicellati (20)	yes	Bisexual	2	no	no	no	yes	yes	no
Croton antisyphiliticus Mart.	Geiseleria	yes	Bisexual	5	no	yes	yes	yes	no	yes
Croton arboreus Millsp.	Eluteria (22)	yes	Bisexual	1	no	yes	no	yes	yes	no
Croton argenteus L.	Julocroton (41)	yes	Bisexual	11	no	no	yes	yes	yes	no
Croton argyranthemus Michx.	Argyranthemi (2)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton argyratus Blume	OW-Argyrati (2)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton astroites Aiton	Lasiogyne (45)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton axillaris Müll. Arg.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton beetlei Croizat	Corylocroton	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton betulinus Vahl	Geiseleria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton bispinosus C. Wright	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton bixoides Vahl. = C. micans]	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton blanchetianus Baill.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton bonplandianus Baill.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton brittonianus Carabia	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton cajucara Benth.	Cleodora (18)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton californicus Müll. Arg.	Drepadenium	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton capitatus Michx. *	Heptallon (9)	yes	Bisexual	11	no	no	yes	yes	no	no
Croton cascarilloides Raeusch.	OW	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton caudatus Geiseler.	OW-Caudati (1)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton ceanothifolius Baill.	Lamprocroton (37)	yes	Bisexual	8	no	no	yes	yes	yes	no
Croton celtidifolius Baill.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton chichenensis Lundell	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton chocoanus Croizat	Luntia (19)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton ciliatoglandulifer Ortega	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton conduplicatus Kunth	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton cordiifolius Baill.	Cordiifolii (1)	yes	Unisexual	17	no	yes	yes	yes	yes	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton coriaceus Kunth	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton corinthius Poveda & J.Á. González	Corinthii (1)	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton corylifolius Lam.	Corylocroton	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton crassifolius Geiseler.	OW	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton cuneatus Klotzsch.	Cuneati	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton cupreatus Croizat	Cupreati (1)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton decalobus Müll. Arg.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton decalobus Müll. Arg.	Lasiogyne	no	Bisexual	1	yes	yes	yes	yes	no	no
Croton dioicus Cav.	Drepadenium (6)	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton discolor Willd.	Adenophylli	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton draco Schltdl. & Cham.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton echinocarpus Baill.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton flavens L. [checking ID with Karina, I need to see voucher]	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton floccosus B.A. Sm.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton floribundus Spreng.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton fragrans Kunth	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton fruticulosus Torr.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton fuscescens Spreng.	Julocroton	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton glabellus L. [sic.]	Eluteria	yes	Bisexual	1	yes	yes	yes	yes	no	no
Croton glandulosepalus Millsp.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton glandulosus L.	Geiseleria	yes	Bisexual	6	no	yes	yes	yes	yes	no
Croton gossypiifolius Vahl.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton goudotii Baill.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton gracilipes Baill.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton gratissimus Burch.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton guianensis Aubl.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton guildinguii Griseb.	Geiseleria	yes	Bisexual	12	no	no	no	yes	no	no
Croton helicoideus Müll. Arg. (=C. micansw.)	Lasiogyne	yes	Bisexual	1			yes	yes	no	no
Croton heliotropiifolius Kunth	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	no	no
Croton hibiscifolius Kunth ex Spreng.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
*Croton hircinus*Vent.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton hirtus L`Hér.	Geiseleria	yes	Bisexual	6	no	yes	yes	yes	yes	no
Croton hoffmannii Müll. Arg.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton huberi Steyerm.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton humilis L.	Adenophylli	yes	Bisexual	6	no	yes	yes	yes	yes	no
Croton icche Lundell	Eluteria	yes	Bisexual	1	no	yes	no	yes	yes	no
Croton impressus Urb.	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton insularis Baill.	OW-Insulares (35)	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton jamaicensis B.W. van Ee & P.E. Berry	Eluteria	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton jimenezii Standl. & Valerio	Cyclostigma	yes	Bisexual	1	no	yes	yes	yes	yes	no
Croton jutiapensis Croizat	Geiseleria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton kongensis Gagn.	OW	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton lachnocarpus Benth.	OW	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton laevigatus Vahl.	OW	yes	Bisexual	3	no	yes	no	yes	no	no
Croton lechleri Müll. Arg.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton lindheimerianus Scheele	Heptallon	yes	Bisexual	13	no	no	yes	yes	no	yes
Croton linearis Jacq.	Adenophylli	yes	Bisexual	17	no	yes	yes	yes	yes	no
Croton luetzelburgii Pax & K. Hoffm.	Luetzelburgiorum (1)	yes	Bisexual	2	no	yes	no	yes	no	no
Croton lundellii Standl.	Corylocroton	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton malambo H. Karst.	Cuneati (11)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton matourensis Aubl.	Luntia	yes	Bisexual	7	no	yes	yes	yes	no	yes

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton megistocarpus J. A. González & Poveda	Pachypodi (5)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton micans Sw.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton michauxii G. L. Webster	Crotonopsis (1)	yes	Bisexual	15	yes	yes	no	yes	yes	no
Croton microtiglium Burkill	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton monanthogynus Michx.	Heptallon	yes	Bisexual	12	no	no	no	yes	no	no
Croton myricifolius Griseb.	Eluteria	yes	Bisexual	14	no	yes	no	yes	no	no
Croton niveus Jacq.	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton nubigenus G. Webster	Nubigeni (1)	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton olivaceus Müll. Arg.	Olivacei (1)	yes	Bisexual	1	yes	yes	yes	yes	no	no
Croton organensis Baill.	Cleodora	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton ovalifolius Vahl in H. West.	Barhamia	yes	Bisexual	1	yes	yes	yes	yes	no	no
Croton pachypodus G.L. Webster	Pachypodi	yes	Bisexual	5	yes	yes	yes	yes	yes	no
Croton palanostigma Klotzsch	Luntia	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton pallidulus Baill.	Lamprocroton	yes	Bisexual	11	no	no	yes	yes	no	no
Croton pedicellatus H.B.K. (Det. Croizat)	Pedicellati	yes	Bisexual	2	no	no	no	yes	yes	no
Croton perspeciosus Croizat	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton pilulifer Rusby	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton poecilanthus Urb.	Moacroton (8)	yes	Bisexual	7	yes	yes	yes	yes	no	yes
Croton polyandrus Spreng.	Eutropia (1)	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton punctatus Jacq.	Drepadenium	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton pungens Jacq.	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton reflexifolius Kunth	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton ripensis Kaneh & Hatus	OW-Dockrilliorum	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton roraimensis Croizat	Cuneati	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton rosmarinoides Millsp.	Lasiogyne	yes	Bisexual	11	no	no	yes	yes	no	no
Croton ruizianus Müll. Arg.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton saltensis Griseb.	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton salutaris Casar.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton sampatik Müll. Arg.	Sampatik (4)	yes	Bisexual	7	yes	yes	yes	yes	no	yes
Croton sapiifolius Müll. Arg.	Quadrilobi (1)	yes	Unisexual	16	no	yes	yes	yes	yes	no
Croton scaber Willd.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton schiedeanus Schltdl.	Eluteria	yes	Bisexual	3	no	yes	no	yes	no	no
Croton sellowii Baill.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton setiger Hook.	Eremocarpus (1)	yes	Bisexual	11	no	no	yes	yes	no	no
Croton speciosus Müll. Arg.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton sphaerogynus Baill.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton spruceanus Benth.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton stipulaceus Kunth	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	yes	no
Croton suberosus Kunth	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton thomasii Riina & P.E. Berry	Prisci	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton tiglium L.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton tricolor Klotzsch ex Baill.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton triqueter Lam.	Julocroton	yes	Bisexual	1	no	yes	yes	yes	yes	no
Croton velutinus Baill.	Barhamia	yes	Bisexual	1	no	no	yes	yes	no	no
Croton verreauxii Baill.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton xalapensis Kunth	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton yavitensis Croizat	Cuneati	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton yucatanensis Lundell	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton abutiloides Kunth	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
*Croton rivinifolius*Kunth	Adenophylli	no	Unisexual	17	no	yes	yes	yes	yes	no
Croton rusbyi Britton ex Rusby	Cyclostigma	no	Bisexual	9	no	yes	no	yes	no	no
Croton scouleri Hook. f.	Adenophylli	no	Unisexual	17	no	yes	yes	yes	yes	no
Croton splendidus Mart. ex Colla	Lamprocroton	no	Bisexual	14	no	yes	no	yes	yes	no

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Species	Clade	Phylogeny	N°flowers.inflores	N°Female_flowers	N°Male_flowers	Proportion.FEMALE.MALE	Stamens.range	Stamens.average	Male_investment
Astraea comosa (Müll. Arg.) B. W. Van Ee	Genus *Astraea* (14)	no	160	20	140	0,14	10	15	2100
Astraea divaricata Klotzsch	Genus *Astraea*	no	90	10	80	0,13	30 to 35	33	2640
Croton acronychioides F. Muell.	OW (450)	no	90	10	80	0,13	11	11	880
Croton adamantinus Müll. Arg.	Geiseleria (82)	no	130	30	100	0,30	12	12	1200
Croton adipatus Kunth	Adenophylli (223)	no	115	115	115	1,00	12	12	1380
Croton alloeophyllus Urb.	Adenophylli	no	300	30	270	0,11	10	10	2700
Croton amentiformis Riina	Cyclostigma (46)	no	600	20	580	0,03	12	12	6960
Croton angustatus Urb.	Adenophylli	no	300	30	270	0,11	12	12	3240
Croton arnhemicus Müll. Arg.	OW-Arnhemici (5)	no	65	5	60	0,08	20	20	1200
Croton atrorufus Müll. Arg.	Barhamia	no	180	80	100	0,80	20	20	2000
Croton azuensis Urb.	Adenophylli	no	300	30	270	0,11	12	12	3240
Croton betaceus Baill.	Adenophylli	no	65	15	50	0,30	12	12	600
Croton billbergianus Müll. Arg.	Cleodora	no	95	15	80	0,19	15 to 17	16	1280
Croton caldensis Müll. Arg.	Cyclostigma	no	180	80	100	0,80	15	15	1500
Croton campestris A. St. -Hil.	Adenophylli	no	80	10	70	0,14	15 to 17	16	1120
Croton capitis-york Airy Shaw	OW	no	140	20	120	0,17	13	13	1560
Croton caracasanus Pittier	Corylocroton (11)	no	400	100	300	0,33	11 or 12	12	3600
Croton chaetocalyx Müll. Arg.	Barhamia (84)	no	50	10	40	0,25	10	10	400
Croton chaetophorus Müll. Arg.	Barhamia	no	45	15	30	0,50	10	10	300
Croton choristadenius K. Schum.	OW	no	190	30	160	0,19	10	10	1600
Croton ciliatoglandulosus Steud (= C. ciliatoglandulifer)	Adenophylli	no	300	30	270	0,11	10	10	2700
Croton cinerellus Müll.	Lamprocroton	no	200	50	150	0,33	8 to 10	9	1350

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Species	Section								
Arg.									
Croton compressus Lam.	Lasiogyne	no	200	40	160	0,25	12	12	1920
Croton confertus Bak.	OW	no	200	50	150	0,33	15	15	2250
Croton cortesianus Kunth	Adenophylli	no	300	30	270	0,11	10 to 15	13	3510
	Heptallon	no	Xeric	Shrub	3	15 to 20	18	2700	
*Croton costaricensis*Pax (= C. ortholobus)	Geiseleria	no	300	30	270	0,11	10 to 15	13	3510
Croton costatus Kunth	Luntia	no	80	20	60	0,33	10 to 16	13	780
Croton curyphyllus L.	OW	no	80	10	70	0,14	22	22	1540
Croton decipiens Baill.	Barhamia	no	600	100	500	0,20	15	15	7500
Croton dockrilli Airy Shaw	OW-Dockrilliorum (4)	no	190	30	160	0,19	8 to 10	9	1440
Croton eluteria (L) W. Wright	Eluteria	no	180	80	100	0,80	14	14	1400
Croton fishlockii Britton	Adenophylli	no	240	60	180	0,33	11 or 12	12	2160
Croton frieseanus Müll. Arg.	Adenophylli	no	115	115	115	1,00	12	12	1380
Croton garckeanus Baill.	Barhamia	no	200	50	150	0,33	15	15	2250
Croton glutinosus Müll. Arg.	Barhamia	no	150	50	100	0,50	8 to 10	9	900
Croton goyazensis Müll. Arg.	Geiseleria	no	60	10	50	0,20	12	12	600
Croton hainanensis Men.	OW	no	240	40	200	0,20	15 to 17	16	3200
Croton ichthygaster L.B. Sm. & Downs	Adenophylli	no	200	50	150	0,33	20	20	3000
Croton javarisensis Secco	Cleodora	no	270	50	220	0,23	15	15	3300
*Croton julopsidium*Baill.	Lamprocroton	no	230	50	180	0,28	11	11	1980
Croton laetifolius Baill.	OW	no	80	20	60	0,33	10	10	600
Croton laetifolius Baill.	OW	no	130	80	100	0,80	10	10	1000
Croton lehmannii Pax	Adenophylli	no	80	10	70	0,14	12	12	840
Croton lucidus L.	Adenophylli	no	85	15	70	0,21	12	12	840
Croton lundianus (Didr.) Müll. Arg.	Geiseleria	no	150	50	100	0,50	8 to 10	9	900

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton megalobotrys Müll. Arg.	OW	no	100	30	70	0,43	14	14	980
Croton muscicapa Müll. Arg.	Barhamia	no	30	10	20	0,50	4	4	80
Croton origanifolius Lam.	Adenophylli	no	250	50	200	0,25	15 to 20	18	3600
Croton phebalioides F. Muell. ex Müll. Arg.	OW	no	100	20	80	0,25	10	10	800
Croton poitaei Urb.	Adenophylli	no	300	30	270	0,11	12	12	3240
Croton polytomus Urb.	Adenophylli	no	250	50	200	0,25	15 to 20	18	3600
Croton pycnadenius Müll. Arg.	Adenophylli	no	150	30	120	0,25	10	10	1200
Croton scabiosus Bedd.	OW	no	60	10	50	0,20	14	14	700
Croton schiedeanus Schltdl.	Eluteria	no	180	80	100	0,80	14	14	1400
Croton schultzii Benth.	OW	no	200	40	160	0,25	12	12	1920
Croton sclerocalyx (Didr.) Müll. Arg.	Geiseleria	no	70	10	60	0,17	12	12	720
Croton sincorensis Mart.	Geiseleria	no	28	8	20	0,40	8 to 10	9	180
Croton subferrugineus Müll. Arg.	Adenophylli	no	90	20	70	0,29	12	12	840
Croton sucrensis Steyerm.	Lasiogyne	no	60	10	70	0,14	10	10	700
Croton tetradenius Baill.	Geiseleria	no	220	60	160	0,38	12	12	1920
Croton teucridium Baill.	Geiseleria	no	95	15	80	0,19	12	12	960
Croton thurifer Kunth	Adenophylli	no	150	30	120	0,25	11	11	1320
Croton timandroides (Didr.) Müll. Arg.	Barhamia	no	100	20	80	0,25	90 to 100	95	7600
Croton tomentellus F. Muell.	OW-Gymnocroton (40)	no	140	20	120	0,17	6	6	720
Croton triangularis Müll. Arg.	Geiseleria	no	95	15	80	0,19	12	12	960
Croton urticifolius Lam.	Barhamia	no	180	40	140	0,29	11	11	1540
Croton urucurana Baill.	Cyclostigma	no	240	40	200	0,20	15 to 17	16	3200
Croton virgultosus Müll. Arg.	Geiseleria	no	80	10	70	0,14	10	10	700
Croton vulnerarius Baill.	Cyclostigma	no	95	15	80	0,19	15	15	1200

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton eichleri Müll. Arg.	Prisci (3)	yes	130	10	120	0,08	15	15	1800
Acidocroton sp. Griseb.	Genus *Acidocroton* (15)	yes	25	2	23	0,09	12	12	276
Astraea lobata (L.) Klotzsch	Genus *Astraea*	yes	100	20	80	0,25	10 to 15	13	1040
Brasiliocroton mamoninha P.E. Berry & Cordeiro	Genus *Brasiliocroton* (2)	yes	100	70	30	2,33	10 to 12	11	330
Croton aequatoris Croizat	Adenophylli	yes	250	30	220	0,14	15 to 20	18	3960
Croton alabamensis E.A. Sm. Ex Chapm.	Alabamenses (1)	yes	120	20	100	0,20	15	15	1500
Croton alchorneicarpus Croizat	Cyclostigma	yes	500	70	430	0,16	12	12	5160
Croton andinus Müll. Arg.	Pedicellati (20)	yes	35	15	20	0,75	6 to 8	7	140
Croton antisyphiliticus Mart.	Geiseleria	yes	200	30	170	0,18	12	12	2040
Croton arboreus Millsp.	Eluteria (22)	yes	200	50	150	0,33	10	12	1800
Croton argenteus L.	Julocroton (41)	yes	40	5	35	0,14	10 or 11	11	385
Croton argyranthemus Michx.	Argyranthemi (2)	yes	70	20	50	0,40	12	12	600
Croton argyratus Blume	OW-Argyrati (2)	yes	170	30	140	0,21	10	10	1400
Croton astroites Aiton	Lasiogyne (45)	yes	300	30	270	0,11	15 to 20	18	4860
Croton axillaris Müll. Arg.	Lasiogyne	yes	400	40	360	0,11	12	12	4320
Croton beetlei Croizat	Corylocroton	yes	200	20	180	0,11	13 to 15	14	2520
Croton betulinus Vahl	Geiseleria	yes	70	5	65	0,08	12	12	780
Croton bispinosus C. Wright	Eluteria	yes	100	20	80	0,25	6	6	480
Croton bixoides Vahl. = C. micans]	Lasiogyne	yes	300	30	270	0,11	12	12	3240
Croton blanchetianus Baill.	Lasiogyne	yes	200	50	150	0,33	15	15	2250
Croton bonplandianus Baill.	Adenophylli	yes	200	40	160	0,25	15	15	2400
Croton brittonianus Carabia	Eluteria	yes	100	20	80	0,25	6	6	480
Croton cajucara Benth.	Cleodora (18)	yes	240	40	200	0,20	15 to 17	16	3200
Croton californicus Müll.	Drepadenium	yes	120	120	120	1,00	12	12	1440

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Arg.

Croton capitatus Michx. *	Heptallon (9)	yes	40	10	30	0,33	10	10	300
Croton cascarilloides Raeusch.	OW	yes	70	10	60	0,17	13	13	780
Croton caudatus Geiseler.	OW-Caudati (1)	yes	170	30	140	0,21	25	25	3500
Croton ceanothifolius Baill.	Lamprocroton (37)	yes	40	10	30	0,33	12	12	360
Croton celtidifolius Baill.	Cyclostigma	yes	210	30	180	0,17	60 to 70	65	11700
Croton chichenensis Lundell	Adenophylli	yes	200	50	150	0,33	15	15	2250
Croton chocoanus Croizat	Luntia (19)	yes	240	40	200	0,20	10	10	2000
Croton ciliatoglandulifer Ortega	Adenophylli	yes	150	30	120	0,25	30 to 35	33	3960
Croton conduplicatus Kunth	Adenophylli	yes	270	70	200	0,35	17	17	3400
Croton cordiifolius Baill.	Cordiifolii (1)	yes	170	170	170	1,00	12	12	2040
Croton coriaceus Kunth	Cyclostigma	yes	100	20	80	0,25	25 to 30	28	2240
Croton corinthius Poveda & J.Á. González	Corinthii (1)	yes	180	40	140	0,29	15 to 17	16	2240
Croton corylifolius Lam.	Corylocroton	yes	170	30	140	0,21	15 to 17	16	2240
Croton crassifolius Geiseler.	OW	yes	60	10	50	0,20	30	30	1500
Croton cuneatus Klotzsch.	Cuneati	yes	390	50	340	0,15	15	15	5100
Croton cupreatus Croizat	Cupreati (1)	yes	135	15	120	0,13	10	10	1200
Croton decalobus Müll. Arg.	Lasiogyne	yes	300	30	270	0,11	12	12	3240
Croton decalobus Müll. Arg.	Lasiogyne	no	180	80	100	0,80	15	15	1500
Croton dioicus Cav.	Drepadenium (6)	yes	40	40	40	1,00	10	10	400
Croton discolor Willd.	Adenophylli	yes	115	115	115	1,00	15 to 20	18	2070
Croton draco Schltdl. & Cham.	Cyclostigma	yes	420	100	320	0,31	15	15	4800
Croton echinocarpus Baill.	Cyclostigma	yes	500	70	430	0,16	12	12	5160
Croton flavens L. [checking ID with Karina, I need to see voucher]	Adenophylli	yes	80	10	70	0,14	12	12	840

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Species	Section								
Croton floccosus B.A. Sm.	Cyclostigma	yes	500	70	430	0,16	12	12	5160
Croton floribundus Spreng.	Lasiogyne	yes	340	60	280	0,21	12	12	3360
Croton fragrans Kunth	Cleodora	yes	240	40	200	0,20	15 to 17	16	3200
Croton fruticulosus Torr.	Adenophylli	yes	50	10	40	0,25	11	11	440
Croton fuscescens Spreng.	Julocroton	yes	240	60	180	0,33	11 or 12	12	2160
Croton glabellus L. [sic.]	Eluteria	yes	160	20	140	0,14	15 to 17	16	2240
Croton glandulosepalus Millsp.	Barhamia	yes	250	50	200	0,25	11	11	2200
Croton glandulosus L.	Geiseleria	yes	80	10	70	0,14	10 or 11	11	770
Croton gossypiifolius Vahl.	Cyclostigma	yes	590	140	450	0,31	20	20	9000
Croton goudotii Baill.	OW	yes	200	40	160	0,25	15 to 20	18	2880
Croton gracilipes Baill.	Adenophylli	yes	200	40	160	0,25	15	15	2400
Croton gratissimus Burch.	OW	yes	350	50	300	0,17	15 to 20	18	5400
Croton guianensis Aubl.	Barhamia	yes	250	50	200	0,25	11	11	2200
Croton guildinguii Griseb.	Geiseleria	yes	25	5	20	0,25	12	12	240
Croton helicoideus Müll. Arg. (=C. micansw.)	Lasiogyne	yes	120	20	100	0,20	10 to 13	12	1200
Croton heliotropiifolius Kunth	Adenophylli	yes	360	80	280	0,29	14	14	3920
Croton hibiscifolius Kunth ex Spreng.	Cyclostigma	yes	500	70	430	0,16	20	20	8600
Croton hircinus Vent.	Barhamia	yes	160	20	140	0,14	11	11	1540
Croton hirtus L` Hér.	Geiseleria	yes	48	8	40	0,20	20 to 25	23	920
Croton hoffmannii Müll. Arg.	Cleodora	yes	85	15	70	0,21	13	13	910
Croton huberi Steyerm.	Cyclostigma	yes	500	70	430	0,16	20	20	8600
Croton humilis L.	Adenophylli	yes	48	8	40	0,20	10 to 13	12	480
Croton icche Lundell	Eluteria	yes	200	80	120	0,67	6 to 8	7	840
Croton impressus Urb.	Adenophylli	yes	200	20	180	0,11	15 to 20	18	3240
Croton insularis Baill.	OW-Insulares (35)	yes	170	30	140	0,21	16	16	2240

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton jamaicensis B.W. van Ee & P.E. Berry	Eluteria	yes	85	15	70	0,21	5 to 6	6	420
Croton jimenezii Standl. & Valerio	Cyclostigma	yes	300	150	150	1,00	20	20	3000
Croton jutiapensis Croizat	Geiseleria	yes	100	15	85	0,18	12	12	1020
Croton kongensis Gagn.	OW	yes	160	20	140	0,14	10	10	1400
Croton lachnocarpus Benth.	OW	yes	250	50	200	0,25	8	8	1600
Croton laevigatus Vahl.	OW	yes	180	20	160	0,13	12	12	1920
Croton lechleri Müll. Arg.	Cyclostigma	yes	1400	200	1200	0,17	12	12	14400
Croton lindheimerianus Scheele	Heptallon	yes	25	5	20	0,25	10	10	200
Croton linearis Jacq.	Adenophylli	yes	150	150	150	1,00	15 to 20	18	2700
Croton luetzelburgii Pax & K. Hoffm.	Luetzelburgiorum (1)	yes	105	5	100	0,05	13 to 15	14	1400
Croton lundellii Standl.	Corylocroton	yes	170	30	140	0,21	13 to 15	14	1960
Croton malambo H. Karst.	Cuneati (11)	yes	250	30	220	0,14	15	15	3300
Croton matourensis Aubl.	Luntia	yes	240	40	200	0,20	10	10	2000
Croton megistocarpus J. A. González & Poveda	Pachypodi (5)	yes	170	30	140	0,21	14	14	1960
Croton micans Sw.	Lasiogyne	yes	200	40	160	0,25	12	12	1920
Croton michauxii G. L. Webster	Crotonopsis (1)	yes	35	15	20	0,75	5	5	100
Croton microtiglium Burkill	OW	yes	170	30	140	0,21	12	12	1680
Croton monanthogynus Michx.	Heptallon	yes	25	5	20	0,25	6	6	120
Croton myricifolius Griseb.	Eluteria	yes	100	1	99	0,01	6	6	594
Croton niveus Jacq.	Eluteria	yes	300	40	260	0,15	12	12	3120
Croton nubigenus G. Webster	Nubigeni (1)	yes	180	40	140	0,29	13 to 15	14	1960
Croton olivaceus Müll. Arg.	Olivacei (1)	yes	230	50	180	0,28	20	20	3600
Croton organensis Baill.	Cleodora	yes	180	50	130	0,38	15	15	1950
Croton ovalifolius Vahl in H. West.	Barhamia	yes	80	20	60	0,33	12	12	720

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton pachypodus G.L. Webster	Pachypodi	yes	180	40	140	0,29	14	14	1960
Croton palanostigma Klotzsch	Luntia	yes	340	40	300	0,13	11	11	3300
Croton pallidulus Baill.	Lamprocroton	yes	30	10	30	0,33	10	10	300
Croton pedicellatus H.B.K. (Det. Croizat)	Pedicellati	yes	25	10	15	0,67	8	8	120
Croton perspeciosus Croizat	Cyclostigma	yes	500	70	430	0,16	20	20	8600
Croton pilulifer Rusby	Cyclostigma	yes	500	70	430	0,16	50 to 60	55	23650
Croton poecilanthus Urb.	Moacroton (8)	yes	140	20	120	0,17	20	20	2400
Croton polyandrus Spreng.	Eutropia (1)	yes	160	20	140	0,14	13 to 15	14	1960
Croton punctatus Jacq.	Drepadenium	yes	30	30	30	1,00	10	10	300
Croton pungens Jacq.	Adenophylli	yes	250	50	200	0,25	15 to 20	18	3600
Croton reflexifolius Kunth	Eluteria	yes	100	20	80	0,25	12	12	960
Croton ripensis Kaneh & Hatus	OW-Dockrilliorum	yes	170	50	120	0,42	8 to 10	9	1080
Croton roraimensis Croizat	Cuneati	yes	390	50	340	0,15	15	15	5100
Croton rosmarinoides Millsp.	Lasiogyne	yes	24	4	20	0,20	10	10	200
Croton ruizianus Müll. Arg.	Adenophylli	yes	200	40	160	0,25	15 to 20	18	2880
Croton saltensis Griseb.	Adenophylli	yes	300	40	260	0,15	15 to 20	18	4680
Croton salutaris Casar.	Cleodora	yes	320	70	250	0,28	15 to 20	18	4500
Croton sampatik Müll. Arg.	Sampatik (4)	yes	400	80	320	0,25	8 to 10	9	2880
Croton sapiifolius Müll. Arg.	Quadrilobi (1)	yes	100	100	100	1,00	10 to 15	13	1300
Croton scaber Willd.	Lasiogyne	yes	150	20	130	0,15	10	10	1300
Croton schiedeanus Schltdl.	Eluteria	yes	210	50	160	0,31	6	6	960
Croton sellowii Baill.	Barhamia	yes	150	50	100	0,50	10	10	1000
Croton setiger Hook.	Eremocarpus (1)	yes	45	5	40	0,13	6	6	240
Croton speciosus Müll. Arg.	Cyclostigma	yes	500	70	430	0,16	140 to 160	150	64500

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton sphaerogynus Baill.	Cleodora	yes	320	70	250	0,28	15 to 20	18	4500
Croton spruceanus Benth.	Cleodora	yes	320	70	250	0,28	15 to 20	18	4500
Croton stipulaceus Kunth	Adenophylli	yes	360	100	260	0,38	16	16	4160
Croton suberosus Kunth	Adenophylli	yes	170	70	100	0,70	13	13	1300
Croton thomasii Riina & P.E. Berry	Prisci	yes	135	15	120	0,13	15 or 16	16	1920
Croton tiglium L.	OW	yes	170	20	150	0,13	14 to 16	15	2250
Croton tricolor Klotzsch ex Baill.	Lasiogyne	yes	150	30	120	0,25	15	15	1800
Croton triqueter Lam.	Julocroton	yes	150	50	100	0,50	11 or 12	12	1200
Croton velutinus Baill.	Barhamia	yes	60	10	50	0,20	10	10	500
Croton verreauxii Baill.	OW	yes	170	30	140	0,21	12	12	1680
Croton xalapensis Kunth	Adenophylli	yes	150	30	120	0,25	20 to 25	23	2760
Croton yavitensis Croizat	Cuneati	yes	390	50	340	0,15	15	15	5100
Croton yucatanensis Lundell	Lasiogyne	yes	300	50	250	0,20	10	10	2500
Croton abutiloides Kunth	Adenophylli	yes	350	40	310	0,13	15 to 20	18	5580
Croton rivinifolius Kunth	Adenophylli	no	115	115	115	1,00	12	12	1380
Croton rusbyi Britton ex Rusby	Cyclostigma	no	200	50	150	0,33	15	15	2250
Croton scouleri Hook. f.	Adenophylli	no	115	115	115	1,00	12	12	1380
Croton splendidus Mart. ex Colla	Lamprocroton	no	400	100	300	0,33	11 or 12	12	3600

Figure 3. General morphology of *Croton* inflorescences. **A**. Thyrse. **B**. Axillary branches. **C**. Terminal branches.

Flower development in species of *Croton* (Euphorbiaceae) and its implications for floral morphological diversity in the genus

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Abstract. The Euphorbiaceae are notable for floral diversity and evolutionary complexity. *Croton* is the second largest genus in the family and exhibits particular diversity in its flowers. The aim of this study was to investigate the floral ontogeny and structure of three *Croton* species with distinct morphologies, with a focus on testing the hypothesis that the filaments of female flowers, which have received different interpretations in the literature and are currently described as reduced petals, are staminodes and part of a vestigial androecium. With the ontogenetic study we can understand the origin of the organs and associate these with flower evolution in the genus. Flowers in several stages of development were analysed using light microscopy and scanning electron microscopy. In the early stage of development, the sepals are the first structures to be formed, although they do not continue to grow in female *Croton fuscescens* Spreng. flowers. Petals are absent in female flowers, with filamentous, petaloid structures, interpreted here as staminodes, alternating with the sepals in *Croton lundianus* (Didr.) Müll. Arg. In *Croton sphaerogynus* Baill., the staminodes are located between the nectary lobes. The stamens exhibit centripetal development in the flower bud stage, and the carpels are post-genitally connate, with differences in style branching. Besides the ontogenetic interpretation for the filamentous structures, the genus shows transitional structures that we consider evolutionary reductions. Our results can explain how developmental alterations have influenced the suppression and modification of floral organs in the genus.

Additional keywords: Crotonoideae, evolution, ontogeny, perianth, petals, staminodes.

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Introduction

Among the angiosperms, families such as the Euphorbiaceae are notable for their floral and inflorescence diversity. Many types of cymose inflorescences are found in this family, and some of them are unique and morphologically different from other groups, such as the pseudanthia of *Dalechampia* and *Euphorbia*.

Croton, which is the second largest genus of Euphorbiaceae, includes between 1200 and 1300 species of herbs, shrubs, trees, and lianas; it is pantropical, with ~300 species in Brazil (Berry *et al*. 2005; Cordeiro *et al*. 2016). Its inflorescences are terminal thyrses that have proximal cymules with either female or both female and male flowers, and distal cymules usually with only male flowers.

Croton has female and male flowers with particular differences, especially concerning the presence or absence of perianth whorls (Webster 1967). *Croton* female flowers have the combination of lobed, entire, pinatissected calyx, usually imbricate or valvate and petals are usually reduced or absent; filaments are usually common, though their interpretation is debatable, sometimes

described as petals or glands (De-Paula *et al*. 2011; Caruzo and Cordeiro 2013). The male flowers have valvate calyx, as many petals as there are sepals and stamens with no filaments (Webster 1967).

Caruzo *et al*. (2011) studied the evolution of characters and the phylogeny of *Croton* section *Cleodora* (Klotzsch) Baill., a group widely spread through America but which has not been revised recently. Caruzo *et al*. (2011) described the pistillate flowers of this group as generally apetalous, or with greatly reduced petals and rarely conspicuous petals. They argued that when filaments or glandular structures are present in the position of petals, these structures should be referred to as reduced petals. They also noted that the presence of filaments is shared by a majority of New World *Croton* species, where two-thirds of the species occur (Van Ee *et al*. 2011).

De-Paula *et al*. (2011) analysed the flowers of two genera of the tribe Crotoneae, *Croton* and *Astraea* and interpreted the male flowers as having five sepals and five petals and the female ones as having five sepals and five filamentous structures, which are unvascularised and interpreted as reduced petals. Nectaries

were interpreted as staminodial nectaries due to their external position in relation to the first staminal whorl.

The identification of petals in these flowers requires a deep study on the origin of the organs. In particular, the term 'petals' is imprecise and has been applied to a diverse range of showy, non-homologous structures in the second whorl of a perianth. Several degrees and different forms of perianth allied to the numerous cases of perianth organs loss and gain could result in the misinterpretation of perianth parts (Ronse De Craene and Brockington 2013). These represent cases where morphological structures derived from different whorls may develop different functions, a phenomenon generally called heterotopy or transference of function (Baum and Donoghue 2002).

Considering the floral variations described for *Croton*, especially with respect to sepals, petals and filaments, the study of flower development allied to evolutionary perspectives is important to understand heterotopy and transference of function. Ontogenetic studies also help understand differences in floral shape, particularly explaining the differences in primordia development and meristem activity (cell division, expansion and differentiation patterns).

The aim of this study is to test the hypothesis that the filaments of female flowers currently described as reduced petals are staminodes, and like the nectaries considered staminodes by De-Paula *et al*. (2011), these filaments are also part of a vestigial androecium. In order to test the hypothesis above we chose three species: *Croton sphaerogynus* (*Croton* sect. *Cleodora* (Klotzsch) Baill.), *Croton fuscescens* (*Croton* sect. *Julocroton* (Mart.) G.L. Webster) and *Croton lundianus* (*Croton* sect. *Geiseleria* (A. Gray) Baill.). These species were chosen because they show contrasting flower morphologies, especially concerning the number of whorls of the perianth, the shape of the sepals, petals and filaments, as well as the presence or absence of these structures. The chosen species represent much of the floral morphological variation found in *Croton*, they belong to different sections and were comparatively investigated in relation to the development and structure of the flowers, correlating our data with the available phylogeny in an evolutionary context.

Materials and methods

Inflorescences and flowers in several stages of development were collected in São Paulo at the Instituto de Botânica and in the city of Itanhaém. Voucher specimens were deposited in the Herbarium of the Universidade de São Paulo (SPF): *Croton fuscescens* Spreng. (*Gagliardi and Demarco 9* [SPF]), *Croton sphaerogynus* Baill. (*Gagliardi and Demarco 10* [SPF]), *Croton lundianus* (Didr.) Müll. Arg. (*Gagliardi and Demarco 11* [SPF]).

Flower meristems, flower buds, and pre-anthetic, anthetic, and post-anthetic flowers were isolated, fixed under vacuum in FAA (formalin, acetic acid, 50% ethyl alcohol) for 24 h (Johansen 1940) and in NBF (neutral buffered formalin) for 48 h (Lillie 1965), and then stored in 70% ethyl alcohol.

The material was dehydrated in a butyl series (Johansen 1940), embedded in Paraplast, and transversely and longitudinally sectioned every 10–12 μm in a rotary microtome (Microm HM340E). The sections were stained with astra blue and safranin (Gerlach 1984) and the blades mounted in synthetic resin. Photomicrographs were taken using a light microscope (Leica DMLB).

For the ontogenetic study, micromorphological analyses were performed using the material fixed in FAA. After isolation of the floral parts, the material was dehydrated in an ethanol series, critical point dried with CO_2 (Balzers CPD 030), mounted on aluminium stubs, and sputter coated with gold (Balzers SCD 050). Observations were then made and images taken using a scanning electron microscope (Zeiss DSM 940) with a digital camera attachment.

Results

Morphology of the inflorescences and flowers

The inflorescences of all three studied species are thyrses, with cymules of female and male flowers differently arranged (Fig. 1a–c). In *C. sphaerogynus*, the flowers are continuously distributed along the axis of the thyrse, and the proximal cymules have both female and male flowers and the distal ones have exclusively male flowers (Fig. 1a). In *C. fuscescens*, the flowers are also continuous, with proximal cymules containing only female flowers and distal ones male flowers (Fig. 1b). In *C. lundianus*, the cymules containing female flowers are separated from the ones containing male flowers through a sterile area on the main axis of the thyrse (Fig. 1c).

The female flowers are apparently monochlamydeous in the three species (Fig. 1e, f), but *C. lundianus* presents an extra whorl composed of petaloid, white, and slightly expanded filamentous structures alternating with the sepals (Fig. 1f). The female flowers are hexamerous in *C. lundianus* and pentamerous in *C. sphaerogynus* and *C. fuscescens.*

The male flowers (Fig. 1d) are all pentamerous, but the androecium varies in the number of stamens: eleven stamens in *C. fuscescens*, fifteen in *C. sphaerogynus*, and 10 in *C. lundianus*.

Development of the inflorescences and cymules

The cymules containing female flowers are the first to initiate development in the inflorescences of all three species, and the terminal flower is the first to initiate differentiation in each cymule. This characterises the development as basipetal for the cymule and acropetal for the main axis (Fig. 2a).

Development of female flowers

Calyx

The first whorl to start differentiation is the calyx (Fig. 2b, c), and the sepals develop in an anticlockwise direction (Fig. 2b). The species have sepals with similar size and shape, except for *C. fuscescens*, which has larger, anterior sepals first differentiated, followed by small sepals (Fig. 2c).

When the elongation is almost complete, it can be observed in *C. sphaerogynus* and *C. lundianus* that the sepals develop continuously and all at the same rate (Fig. 2d), different from *C. fuscescens* (Fig. 2e). During the development of the calyx in *C. fuscescens*, the different rate of sepal elongation is notable and there is a delay in the elongation of the two posterior sepals allied to a lacination of the three anterior ones, resulting in a calyx
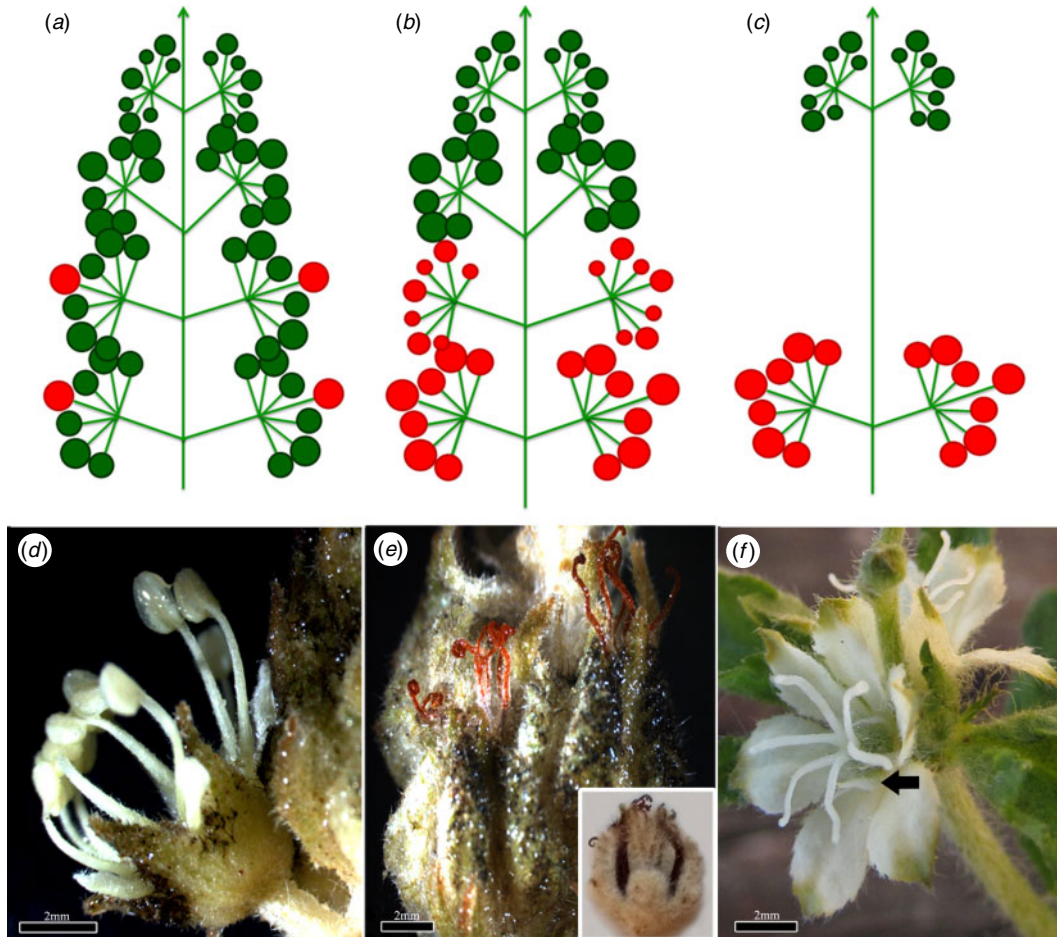
Fig. 1. Inflorescences and flowers of *Croton*. (*a*) Inflorescences of *Croton sphaerogynus*, (*b*) inflorescence of *Croton fuscescens*, (*c*) inflorescence of *Croton lundianus*, (*d*) staminate flower of *C. fuscescens*, (*e*) proximal region of the inflorescence of *C. fuscescens*, detail of the pistillate flower, and (*f*) pistillate flower of *C. lundianus*, note the petaloid structures (arrow).

with two reduced sepals and three that are longer and laciniate (Fig. 2*e*, *f*).

Filamentous-petaloid whorl

The development of filamentous, petaloid structures alternating with the sepals was observed in *C. sphaerogynus* and in *C. lundianus* (Fig. 2*g*, *k*). Following the elongation of the calyx, these filamentous are alternate with the sepals (Fig. 2*g*), and in *C. lundianus* the petaloid structures become more elongated and exhibit two distinct regions: a long basal portion, and a slightly flattened apical region (Fig. 2*h*). With the elongation of the sepals, the petaloid structures become spatulate, with a thin elongation at the apex in *C. lundianus* (Fig. 2*i*), but short and pointed in *C. sphaerogynus* (Fig. 2*k*).

Nectaries

During the elongation of the organs in the three species studied, the adaxial-basal meristematic cells adjacent to the sepals start differentiating into nectaries (Fig. 2*i–l*). The development of these glands around the ovary is followed by elongation. In *C. sphaerogynus*, the nectaries become lobed and develop around the ovary with filamentous structures between the lobes (Fig. 2*k*); in *C. lundianus*, the nectaries are segmented into small, rounded structures, associated with the sepals (Fig. 2*i*). *C. fuscescens*, by contrast, exhibits deeply 3-lobed nectaries that develop only opposite and adjacent to the three large sepals, thereby surrounding part of the ovary (Fig. 2*j, l*). When the flowers are completely developed, the nectaries become larger and secretory (Fig. 2*i–l*).

Gynoecium

After the elongation of the calyx, the meristematic cells internal to the calyx start developing into the carpels. These are initially separate and then become fused during the course of development (post-genital fusion) in all three species (Fig. 2*m, n*), with evident ovule primordia inside the young ovaries (Fig. 2*n*). The elongation of the carpels is followed by the formation of the styles (Fig. 2*j, k, o, p*). A complete gynoecium is observed at this stage, with a hairy stigma in all the species, glandular and nonglandular trichomes (Fig. 2*o*); the styles are in division and are quite elongated in *C. fuscescens* and in *C. lundianus* (Fig. 2*o, p*), and also curved in the latter species
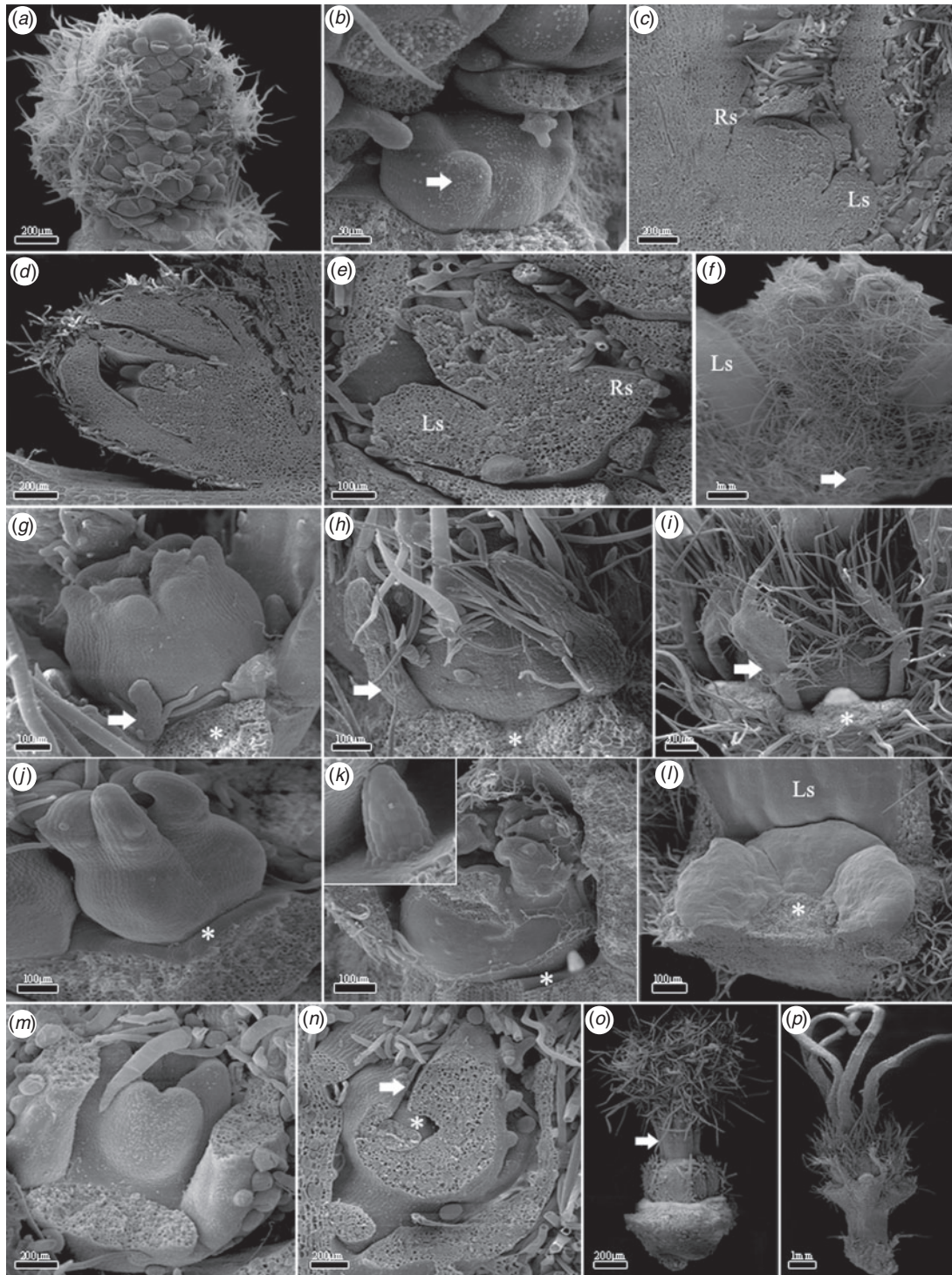
Fig. 2. Ontogeny of inflorescences and female flowers. (*a–c, e, f, j, l, m–o*) Young inflorescence of *Croton fuscescens*, (*b*) reproductive bud with sepals' primordia (*arrow*), (*c*) reproductive bud with sepals in development, long sepals and reduced sepals, (*d, k*) flower bud of *Croton sphaerogynus* with elongated sepals, (*e*) flower bud with sepals in different rhythm of elongation, (*f*) detail of the abaxial long sepals and reduced sepals (arrow), (*g–i, p*) flower bud of *Croton lundianus* with sepals removed (asterisk), note the petaloid structures (arrow), (*h*) anthetic flower with sepals removed (asterisk) and petaloid structures in elongation process (arrow), (*i*) mature flower with sepals removed (asterisk), elongated petaloid structures (arrow) and nectary (highlighted) associated with the removed sepal, (*j*) gynoecium with nectary (asterisk) in the basis of the ovary, (*k*) gynoecium with nectary (asterisk) in the basis of the ovary and filament structure (highlighted and in detail), (*l*) detail of the horse-shaped nectary around the ovary, which was removed (asterisk), (*m*) flower buds with carpels in early stage of development, (*n*) detail of the gynoecium with carpels almost fused (arrow) and ovule primordia (asterisk), (*o*) anthetic flower with elongation of styles (arrow) and ramification of stigmatic region, and (*p*) mature flower with elongated styles. Abbreviations: Ls, long sepal; Rs, reduced sepal.

(Fig. 2*p*), with a globose, hairy ovary. With elongation and complete development of the gynoecium, the styles become long and hairy, bifid in *C. lundianus* and *C. fuscescens* (Fig. 2*o*) and multifid in *C. sphaerogynus*. They have further curved branches in *C. fuscescens* and *C. sphaerogynus*, in contrast to *C. lundianus*, in which the stigmas become more elongate and less curved (Fig. 2*p*).

Anatomy of female flowers

Calyx

The sepals present a uniseriate epidermis composed of round-shaped cells in all the studied species (Fig. 3*a*) and slightly elongated cells in *C. sphaerogynus* and in the small sepals of *C. fuscescens* (Fig. 3*b*). In some regions of the abaxial epidermis
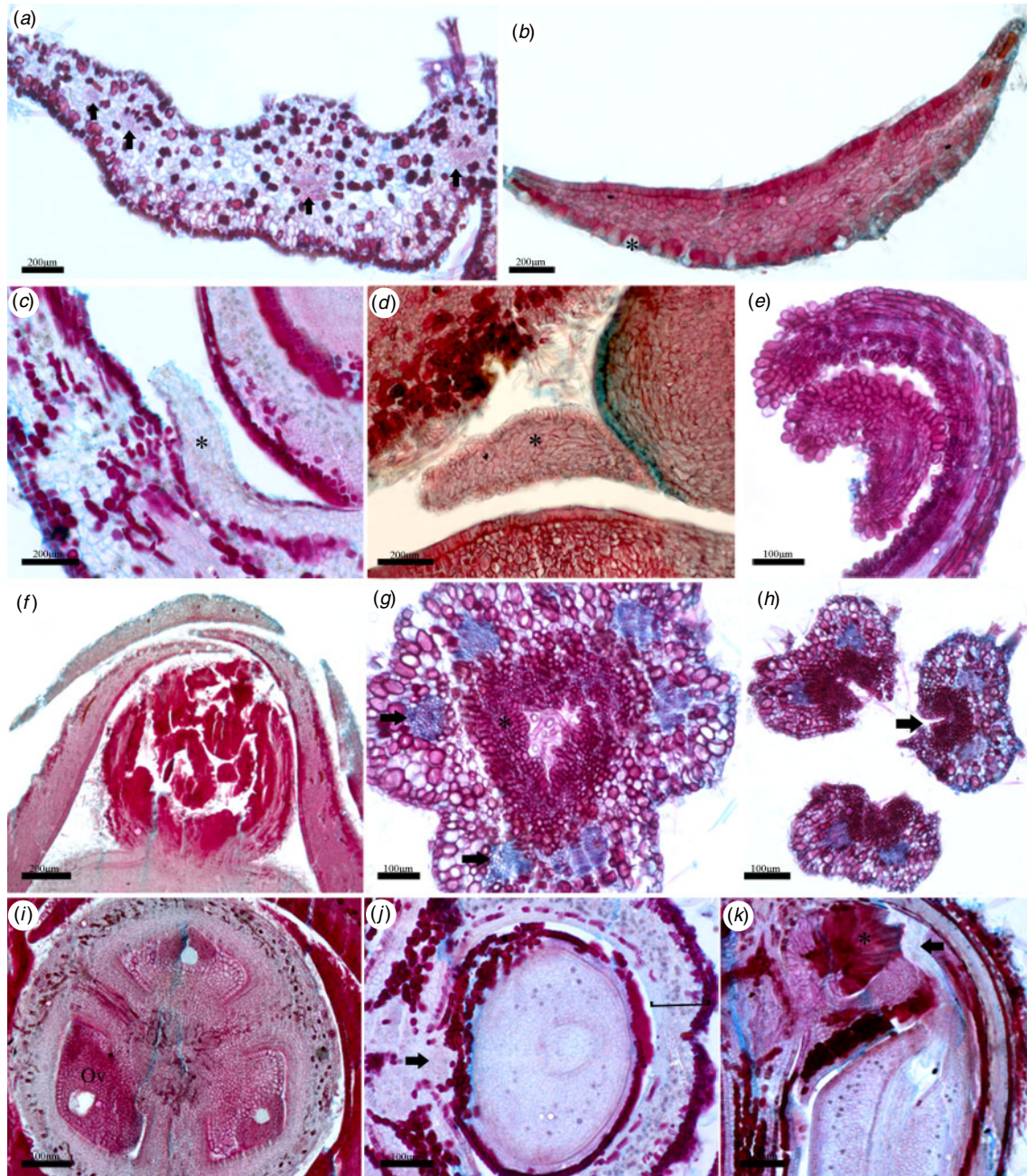

Fig. 3. Anatomy of pistillate flowers. (*a, e, g, h*) Transverse section of *Croton fuscescens* sepal with vascular traits (arrows), (*b, d, f, i*) transverse section of *Croton sphaerogynus* sepal with mucilage cells (asterisk), (*c, j, k*) longitudinal section of petaloid structure of *Croton lundianus* (asterisk), (*d*) longitudinal section of filament structure of *C. sphaerogynus* (asterisk), (*e*) longitudinal section of style and stigma, (*f*) longitudinal section of the curved and ramifies styles, (*g*) transverse section of style, note the stylar canal (asterisk) and vasculature of the ramified stylar branches (arrows), (*h*) division process (arrow) of the styles/stigmas, (*i*) transverse section of the ovary, (*j*) details of the ovary and ovule, note the ovary wall (bar) and vasculature (arrow), and (*k*) ovule with nucellar beak (arrow) and placentary obturator (asterisk). Abbreviation: Ov, ovule.

of *C. sphaerogynus* there are large and round crystal idioblasts and mucilage cells (Fig. 3b). The mesophyll is homogeneous, with chlorophyllous parenchyma, and the vasculature is formed by one to three collateral vascular bundles or even more, as in *C. fuscescens* (Fig. 3a).

Petaloid whorl and filamentous structures

The petaloid structures of *C. lundianus* and the filamentous structures of *C. sphaerogynus* are anatomically similar to the sepals, although the mesophyll is thinner, with fundamental parenchyma and no vasculature (Fig. 3c, d).

Gynoecium

The gynoecium has long stigmas with a uniseriate and papillose epidermis in all three species (Fig. 3e); stigmas are slightly elongated in *C. sphaerogynus* (Fig. 3f) and more pronounced in *C. fuscescens* and *C. lundianus* (Fig. 3e). The style branches are hollow in all three species and consist of a uniseriate epidermis of small, cubic cells on both faces, and a central region with a stylar canal composed of secretory palisade cells (Fig. 3g, h) that are present through the whole style branch, from the stigma to the ovary. In the apical region of the ovary, the styles are united in a short extension that divides into three in the larger distal portion (Fig. 3h); each one of these parts divides again into two stigmata, creating a total of six branches. In *C. sphaerogynus* the styles are more curved than those in the other species and crystal idioblasts and mucilage cells are widely present in the style cells (Fig. 3g).

The ovary of all three species is tricarpellate and trilocular, with one ovule in each locule (Fig. 3i). It presents a uniseriate outer epidermis composed of small, isodiametric cells in *C. fuscescens*, a biseriate epidermis with cubic cells in *C. lundianus* (Fig. 3j), and a uniseriate epidermis with slightly elongated cells in *C. sphaerogynus* (Fig. 3i). Nonglandular trichomes are found in all three species, and crystal idioblasts are present in *C. sphaerogynus* (Fig. 3i). The mesophyll is homogeneous, with dorsal and ventral collateral vascular bundles (Fig. 3j). The inner epidermis is composed of elongated cells, uniseriate in *C. sphaerogynus* and *C. lundianus*, and biseriate in *C. fuscescens* (Fig. 3i, j).

The ovules are bitegmic (Fig. 3j) and anatropous, with a thicker (3–5 cell layers) outer integument and a thinner (2–3) inner integument. The micropyle is composed of both integuments; there is also a placentary obturator and nucellar beak present in all three species (Fig. 3k).

Development of male flowers

The cymules containing male flowers show later development compared with the ones with female flowers (Figs 2a, 4a).

Calyx

The sepals are the first structures to be formed, with five protuberances in anticlockwise direction (Fig. 4b). After differentiation and the beginning of elongation, the sepals undergo post-genital fusion (Fig. 4c). The elongation of the sepals is followed by the formation of trichomes on their surface, covering the stamens (Fig. 4c).

Corolla

Following the development of the sepals and alternating with them, the free primordia of petals begin their differentiation in all three species (Fig. 4d). The petals exhibit slow and delayed elongation (Fig. 4d, e), and whereas the calyx is quite elongated, the corolla is still reduced. With the elongation of the perianth, the petals reach their final shape and size.

Androecium

Internal to the calyx and corolla primordia, a group of meristematic cells begins to differentiate and some stamen primordia may be observed in a centripetal formation in the marginal, outer region of the receptacle (Fig. 4e, f). Followed by cell divisions, in bud stage, the primordia of the stamens show elongation of the filaments and formation of the anthers, which are rounded before thecal differentiation. The anthers are quite immature with early differentiation of their walls (Fig. 4f). As they elongate, from bud stage to pre-anthesis, the filaments begin to curve and the anthers become completely formed, with pollen grains that are initiating their own development (Fig. 4g). With elongation, the stamens become more curved, with longer connectives that expand over the anthers (Fig. 4h, i). The anthers present only two layers of cells in their walls (epidermis and endothecium), with the secretory tapetum consumed for the production of the pollen grains, which are now completely formed (Fig. 4l). In the post-anthetic stage, the stamens show longer filaments, and the anthers are characterised by flattening of the thecae (Fig. 4j, k).

Nectaries

During the elongation of the perianth, the nectaries around the androecium differentiate (Fig. 4g). These glands go through the elongation process and develop as five small and cubic structures closely associated and almost adnate to the stamens (Fig. 4g). When the flowers are completely developed, the nectaries become fully differentiated and secretory (Fig. 4k).

Anatomy of male flowers
Calyx and corolla

The sepals and petals of the male flowers in all three species present a uniseriate epidermis, composed of round-shaped cells with abundant nonglandular trichomes only on the abaxial surface (Fig. 5a). The mesophyll is homogeneous with phenolic idioblasts and crystalliferous idioblasts containing druses. The vasculature is formed by collateral vascular bundles, with one medium vascular bundle and one or two lateral bundles in the sepals (Fig. 5a). The petals have a similar anatomical structure, with one collateral vascular bundle; the abaxial epidermis of *C. sphaerogynus* is exceptionally composed of large, rounded, crystal idioblasts and mucilage cells (Fig. 5b). On the apex of the sepals and petals of all three species, there are nonglandular trichomes (Fig. 5c) and in *C. fuscescens* there are long nonglandular trichomes at the base of the petals also (Fig. 5f). These trichomes intercalate to each other and keep the calyx and corolla closely attached and associated with each other.
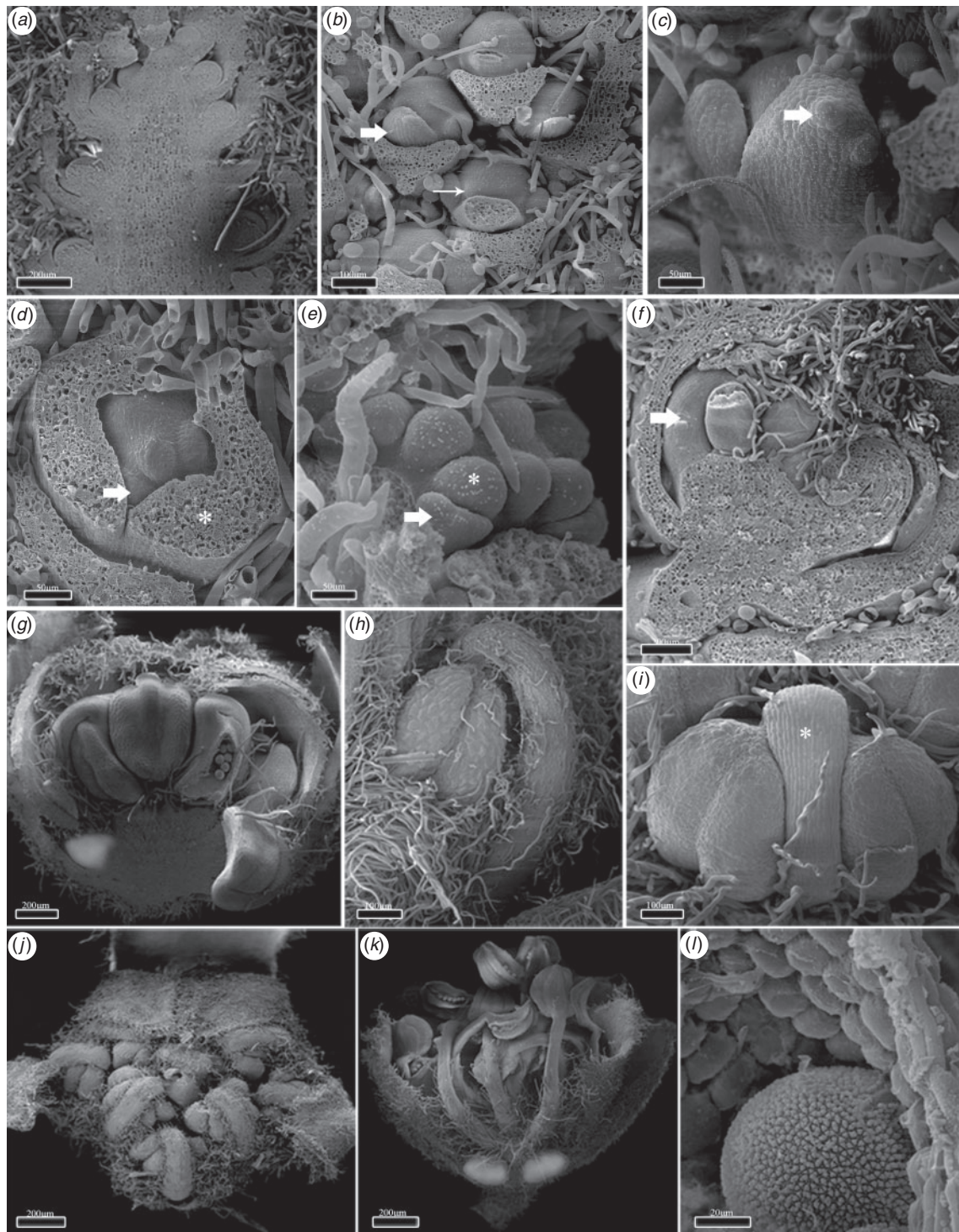
Fig. 4. Ontogeny of staminate flowers. (*a–f, h, j*) Young inflorescence of *Croton fuscescens*, (*b*) flower buds with sepals (thick arrow) and petals formation (middle arrow), (*c*) flower bud with sepals' elongation and initial trichomes (arrow), (*d*) flower bud with sepals removed (asterisk) and petal in early stage of development (arrow), (*e*) flower bud with petals starting elongation (arrow) and stamens initiating development (asterisk), (*f*) longitudinal section of flower bud with stamen filaments in elongation process (arrow), (*g, k*) longitudinal section of *Croton sphaerogynus* flower with the stamens and anthers in differentiation, note the nectary (highlighted), (*h*) detail of the elongated and curved stamen, (*i*) detail of the long connective (asterisk) which involves the anthers, (*j*) anthetic-flower with long and developed whorls, (*k*) post-anthetic flower with the maximum elongation of the whorls and mature stamens, note the developed nectaries (highlighted), and (*l*) detail of a developed anther with pollen grains of *Croton lundianus*.
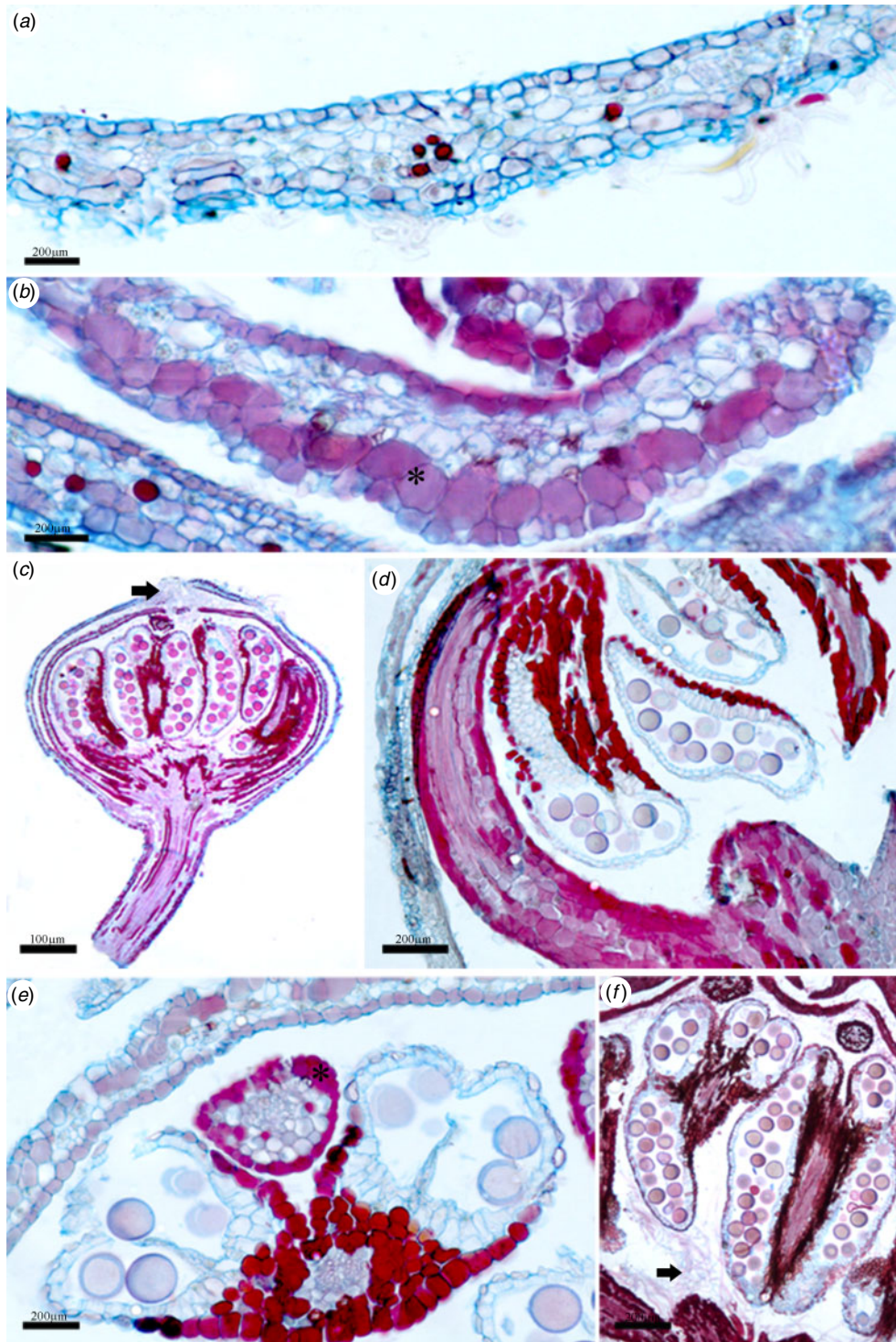
Fig. 5.   Anatomy of staminate flowers. (*a*) Sepal of *Croton fuscescens*, (*b, d, e*) petal of *Croton sphaerogynus*, note the mucilage cells (asterisk), (*c, f*) anthetic-flower of *Croton lundianus* with developed stamens, though sepals and petals still united by trichomes (arrow), (*d*) developed stamen with long and curved filament, (*e*) detail of developed anther, note the connective with idioblast cells (asterisk), and (*f*) detail of the trichomes on the base of petals and stamens (arrow).

Androecium

The filaments exhibit a uniseriate epidermis and are composed of parenchyma cells (Fig. 5*d*). The connective is long and presents an apical region composed of cells with a secretory aspect (Fig. 5*e, e*). The anther is tetrasporangiate with longitudinal dehiscence of the pollen grains (Fig. 5*e*). The anther walls

present epidermal cells that are tangentially elongated, an endothecium with palisade cells and wall thickenings forming trabeculae; the secretory tapetum is totally consumed during the production of the pollen grains, which are organised in monads (Fig. 5*e*, *f*).

Discussion

Perianth, petaloid whorl, and transitional structures

The results obtained in this study for the development of *Croton* flowers corroborate our hypothesis that the filaments in the female flowers of *C. lundianus* are staminodes, thus part of a vestigial androecium, such as the nectaries studied by De-Paula *et al*. (2011).

Croton flowers have similarities in the initiation developmental step and differences in origin of the whorls and in elongation. In the initiation, the first structures to develop in all the species were sepals, followed by the petals and androecium in male flowers, staminodes and gynoecium in the female flowers. The differences are focussed on the elongation developmental step, which we assume to be the responsible for the high morphological floral diversity in *Croton*, especially the elongation of the sepals and filaments of the female flowers. In *C. sphaerogynus* and *C. lundianus* the five sepals elongate in the same way and rhythm, while in *C. fuscescens* the three exposed sepals become thicker faster and irregularly elongated, resulting in a deeply laciniate calyx, with large three sepals and two smallest ones. In the other species, the sepals are not as thick and large as in *C. fuscescens*, although they are present around the whole flower, thereby providing protection to the fertile whorls.

Croton male flowers have a whorl of petals that alternate with the sepals. The female flowers are all monochlamydeous but sometimes show an extra whorl of filaments or petaloid structures, which were initially, described as reduced petals (De-Paula *et al*. 2011). Although, the elongation of the petaloid structures of *C. lundianus* is different from a petal development and characterises a stamen with two different regions, one basal and more elongated, such as a stamen filament and an apical portion slightly flattened, such as an anther. The flattening in the apical portion characterises these structures as staminodes that did not complete anther development. In the mature stage, they exhibit an elongated and thin connective region and a more flattened antheroid.

Staminodes bearing antheroids are relatively uncommon, but widely distributed taxonomically (Walker-Larsen and Harder 2000). Rijpkema *et al*. (2006) studied the ontogeny of *Petunia* flowers and suggested that the modification of petals into staminodes could be associated with a mutation of BLIND-BL gene, as *bl* mutants displayed a homeotic conversion of the corolla into antheroid structures in the second whorl. The development of *Croton* staminodes observed here corroborates our hypothesis that petals in the core eudicots have a staminodial nature as previously suggested by Ronse De Craene (2007).

In taxonomical studies, *C. lundianus* has been described as a monochlamydeous flower with reduced petals (Lima and Pirani 2003; Caruzo and Cordeiro 2007; Silva *et al*. 2010), but according to our ontogeny results, *C. lundianus* should be interpreted as a monochlamydeous flower with a whorl of

staminodes. In *C. sphaerogynus* filamentous structures were also observed between the nectary lobes, and based on their position (opposite to the sepals) these structures are described and interpreted here for the first time as staminodes. The same interpretation was used for De-Paula *et al*. (2011), who have described nectaries as staminodes based on the position of these structures opposite to the sepals.

Besides the elongation developmental step which explains the high morphological diversity in *Croton*, the presence of staminodes in female flowers can be interpreted as transference of function and heterochrony cases. The staminodes of *C. lundianus* provide an example of transference of function, in which the staminodes assumed the role of petals. This is similar to the case of the coloured staminodes of *Jacquinia macrocarpa* (Theophrastaceae), which are morphologically very similar to the petals, but represent an aborted stamen whorl (Ronse De Craene 2003). The same author studied Papaveraceae flowers and concluded that the organ identity can switch at the boundary of petals and stamens, thereby culminating in the transition of petals into stamens and *vice versa*.

Heterochrony is observed in the development of *C. sphaerogynus* staminodes, a process defined by Baum and Donoghue (2002) as a temporal developmental change (a phenotypic modification of pre-existing structures). This phenotypic modification is the loss of function of filamentous structures, observed through the initial development of the stamens filament, which showed an early and premature maturation of the tissues, resulting in no further development of the stamens, thereby anthers and pollen grains were not developed.

Contrary to our conclusion, De-Paula *et al*. (2011) suggested that these filamentous/petaloid structures of *Croton* may be interpreted as reduced and transformed petals; further ontogenetic and vasculature analysis is necessary to draw firm conclusions on the origin of these structures.

Based on our observations and the available literature, Crotonoideae flowers exhibit sterile whorls and fertile whorls in the composition of their flowers, though with different patterns of development including transitional structures, such as the staminodes, that give rise to different floral morphologies. Staminodes are considered transitory structures, and according to Walker-Larsen and Harder (2000) and Ronse De Craene and Smets (2001), these structures point to an evolutionary change, either the loss or modification of a whole whorl of petals, such as in *C. lundianus* in which the petals were modified in staminodes, though keeping the attraction role. *C. sphaerogynus* presented the partial reduction of stamens within a whorl, which was also observed in other angiosperms, such as in Geraniaceae, Primulaceae, Myrtaceae, Scrophulariaceae and Verbenaceae (Ronse De Craene and Smets 2001).

Evolutionary interpretation of the floral developmental patterns

The species analysed belong to different sections of *Croton*. *Croton fuscescens* belongs to *Croton* sect. *Julocroton* (Mart.) G.L. Webster; *C. sphaerogynus* is included in *Croton* sect. *Cleodora* (Klotzsch) Baill.; and *C. lundianus* belongs to *Croton* sect. *Geiseleria* (A. Gray) Baill. (Van Ee *et al*. 2011). *Croton* sect.

Julocroton is the most recent section when compared with *Cleodora* and *Geiseleria*, and based on the results discussed above we could assume that the irregular sepal development in the female flowers of *C. fuscescens* explains the zygomorphic morphology of the flower, which results from the different sized, laciniate sepals. The zygomorphy and the laciniate calyx would be considered an apomorphic character.

Croton sphaerogynus belongs to *Croton* sect. *Cleodora* (Klotzsch) Baill, a phylogenetically oldest lineage section when compared with *Geiseleria* and *Julocroton* (Van Ee *et al.* 2011). *Croton* sect. *Geiseleria* (A. Gray) Baill. (Van Ee *et al.* 2011), in which *C. lundianus* is included, is an intermediate lineage section when compared with *Cleodora* and *Julocroton*. The petaloid structures, interpreted here as staminodes, and the filamentous structures in *C. sphaerogynus*, also interpreted as staminodes, are assumed to be transitional structures in the evolution of *Croton* flowers. These staminodes are absent in *C. fuscescens* (sect. *Julocroton*), which represents the most recent lineage species here, and thus the presence of staminodes could be expected to be a conserved characteristic in *C. lundianus* sect. *Geiseleria* and in *C. sphaerogynus* sect. *Cleodora*.

Other species of Euphorbiaceae show perianth initiation similar to what we observed in *C. sphaerogynus* and *C. lundianus*. Liu *et al.* (2008) analysed the ontogeny of *Jatropha* flowers, which also develop simultaneous and continuous sepals and petals. In other species of *Croton* and *Astraea*, such as *Croton glandulosus*, *Croton piptocalyx*, *Croton urucurana* and *Astraea lobata*, the pattern of sepal initiation is similar, although in *Croton triqueter* the sepals show unidirectional development (De-Paula *et al.* 2011), similar to the pattern we observed in *C. fuscescens* and both species belong to *Croton* sect. *Julocroton*.

In contrast to our observations, some Euphorbiaceae flowers exhibit vestigial or even no perianth formation (Prenner and Rudall 2007; Narbona *et al.* 2008; Cacho *et al.* 2010; Prenner *et al.* 2011). Gagliardi (2014) studied the pseudanthia of Peraceae and Euphorbiaceae (Acalyphoideae and Euphorbioideae), and the Acalyphoideae inflorescences showed female and male flowers with developed sepals, whereas the perianth is entirely lacking in Euphorbioideae.

The loss of petals in female flowers, as documented here in *C. fuscescens* and *C. sphaerogynus*, and the modification of staminodes into petaloid structures, as in *C. lundianus*, have occurred numerous times in flower evolution and may characterise large groups, such as the Ranunculales, Fagales and Cyperaceae (Endress 2011*a*). The developmental study of *Croton* flowers can help us to understand floral evolution in the genus and in Euphorbiaceae, since *Croton* is an intermediate flower morphotype in the whorl reduction issue in comparison to many other Euphorbiaceae. In addition to the filamentous structures (staminodes) assuming the role of petals, *Croton* flowers maintain all the whorls present during ontogeny, unlike what has been described for *Calycopeplus*, *Euphorbia* and *Dalechampia* (Prenner and Rudall 2007; Gagliardi 2014).

With respect to the fertile whorls, the studied species are similar in the connation of the carpels, which has also been reported in other Crotonoideae species (De-Paula *et al.* 2011; Gagliardi 2014), and which may be considered a common

ontogenetic characteristic for these phylogenetically associated flowers. The gynoecia of the species studied exhibit long and secretory stigmata, transmitting tissues, and anatropous ovules with nucellar beaks and placentary obturators. The long stigma is important to attract pollinators and maximise pollen grain deposition. All the characteristics above, except for the long stigma, have been frequently reported in the Euphorbiaceae (Tokuoka and Tobe 1995, 1998, 2002, 2003, 2006; Tokuoka 2007; Souza *et al.* 2010; Gagliardi *et al.* 2012, 2014; Gagliardi 2014).

The development of the stamens in all the species studied here was found to be centripetal, and according to Rudall (2007), this may be considered a basal characteristic of the eudicot clade, one also described by Johri *et al.* (1992) when understanding the evolutionary history of angiosperms.

Anatomy of flowers

The sepals and the petals of the species studied show indumentum in the abaxial epidermis with a larger number of trichomes in the apex that closely associate the calyx and corolla together through their intertwining. According to Weberling (1989), this may be interpreted as a special type of gamosepaly, described as capillinection, in which a close intertwining of trichomes maintains the adhesion of the perianth. This mechanism keeps the fertile whorls protected.

Crystal druses and phenolic idioblasts occur in the epidermis and mesophyll of the sepals, petals and anthers, which is associated with a special mechanism of protection against herbivores, dehydration and calcium control (Fahn 1979, 2000).

The gynoecium is quite similar in all *Croton* flowers and its structure seems to be a pattern for other flowers in Euphorbiaceae (Haber 1925; Souza *et al.* 2010; De-Paula and Sajo 2011; Gagliardi 2014; Martins *et al.* 2016).

The ovule characteristics of *Croton* flowers, such as curvature (antitropous), number of integuments (bitegmic), the thickness of integuments (thicker outer integument and a thinner inner integument), and the presence of nucellus are common in apparently all Euphorbiaceae (Endress 2011*a*) and in some Malpighiaceae as well.

The androecium exhibits a tetrasporangiate anther with an epidermis and endothecium similar to those described for other *Croton* species (De-Paula and Sajo 2011). The curvature and elongation of the filaments and the position of the anther are also similar to other *Croton*. This is an important character to differentiate *Croton* from *Brasiliocroton*, which shows erect filaments when in flower bud (Berry *et al.* 2005).

The structural characteristics described here have not been previously reported for the studied species and may be able to play an important role in the classification of *Croton* species and in the clarification of the differences among them.

Conclusions

The flowers of *Croton* studied here represent the different morphological patterns found in the genus and we corroborated the two hypotheses which motivated this study. The morphologically different flowers of *Croton* result from different developmental steps, especially the first steps, which include sepals initiation and elongation, emphasised by the

laciniation process of *C. fuscescens*, considered an apomorphic character for the genus. Based on our ontogenetic analysis, the filaments of female flowers usually described as reduced petals are interpreted here as staminodes, first described for *C. sphaerogynus*. These structures represent cases of transference of function and heterochrony and are considered a conserved characteristic.

The flowers of *Croton* exhibit intermediate conditions with respect to whorl reduction when compared with many other Euphorbiaceae; this is due to the modifications in their floral developmental patterns. These evolutionary modifications gave rise to an extensive diversity of floral morphologies in the large genus *Croton*, though future floral development studies including different species are important to verify the application of the patterns described here or also report unknown developmental alterations. Considering that *Croton* is one of the largest genus among angiosperms, studies concerning floral vasculature and molecular analysis are also essential to understand the floral evolution of the genus.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Species	Vouchers
Croton cascarilloides Raeusch.	Beechey;
Croton crassifolius Geiseler.	Kurz, S. 2485
Croton kongensis Gagn.	Balansa, B. 4533
Croton lachnocarpus Benth.	Champion, J.G. 473
Croton laevigatus Vahl.	
Croton tiglium L.	Wallich 7722
Croton goudotii Baill.	R. CAPURON 27073
Croton gratissimus Burch.	Burchell, W.J. 2154
Croton confertus Bak.	Bent, J.T. 231
Croton curyphyllus L.	
Croton hainanensis Men.	
Croton laetifolius Baill.	
Croton scabiosus Bedd.	Beddome, R.H.
Croton megalobotrys Müll. Arg.	MRS. H. M. Richards 13369
Croton argyratus Blume	Schultz, F. 609
Croton schultzii Benth.	
Croton argyranthemus Michx.	Berlandier, J.L. 1554
Croton arnhemicus Müll. Arg.	Dallachy, J.
Croton insularis Baill.	Caldwell, E.
Croton microtiglium Burkill	Crosby, C.S. 150
Croton verreauxii Baill.	
Croton phebalioides F. Muell. ex Müll. Arg.	
Croton capitis-york Airy Shaw	
Croton acronychioides F. Muell.	Dallachy, J.
Croton ripensis Kaneh & Hatus	
Croton tomentellus F. Muell.	Mueller, F.J.H.von.
Croton choristadenius K. Schum.	
Croton dockrilli Airy Shaw	
Croton hirtus L` Hér.	Hinton, G.B. 4552
Croton glandulosus L.	Lindheimer, F. J. 691
Croton antisyphiliticus Mart.	Harley, R.M.; Giulietti, A.M.; Stannard, B.L.; Hind, D.J.N.; Kameyama, C.; Prado, J.; Rudall, P.J.; Simão-Bianchini, R.; Taylor, N.P.; Zappi, D.C. 24843
Croton guildinguii Griseb.	
Croton jutiapensis Croizat	P. C. Standley 74971
Croton betulinus Vahl	Ledru, A. P.
Croton lundianus (Didr.) Müll. Arg.	Elias Contreras 8680
Croton sincorensis Mart.	
Croton virgultosus Müll. Arg.	
Croton adamantinus Müll. Arg.	Sasaki, D.; Camargo, A.C.; Rosa, S.A.; Piva, J.H. 1196
Croton sclerocalyx (Didr.) Müll. Arg.	Riedel 1122
Croton goyazensis Müll. Arg.	Riedel
Croton tetradenius Baill.	
Croton triangularis Müll. Arg.	
Croton teucridium Baill.	

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Croton costaricensis Pax (= C. Ortholobus)	Kuntze, O. 2237
Croton punctatus Jacq.	Pringle, C.G. 6355
Croton dioicus Cav.	Hartweg 83
Croton californicus Müll. Arg.	Hinds
Croton lindheimerianus Scheele	Wright, C. 641
*Croton capitatus Michx.	Peter, R.
Croton monanthogynus Michx.	Pringle, C.G. 11164
Croton humilis L.	
Croton fruticulosus Torr.	Pringle, C.G.
Croton stipulaceus Kunth	
Croton suberosus Kunth	Hinton, G.B. 10853
Croton heliotropiifolius Kunth	Gardner 1400
Croton conduplicatus Kunth	
Croton flavens L.	Macnab, G.
Croton gracilipes Baill.	M. Serrano; S. Churchill; J. Vilalobos; D. Villarroel
Croton bonplandianus Baill.	Omondi W.; Kiamba J & Obunyali C & Odunga S
Croton xalapensis Kunth	Percy H. Gentle 2218
Croton discolor Willd.	Pollard, B.J. 1272
Croton pungens Jacq.	Fendler, A. 1213
Croton ruizianus Müll. Arg.	Paul C. Hutchison & J. Kenneth Wright 7148
Croton abutiloides Kunth	Felix Woytkowski 7789
Croton linearis Jacq.	Hamilton, M.A. et al. 671
Croton aequatoris Croizat	Eggers, H.F.A. von 15489
Croton saltensis Griseb.	Lorentz, P.G.; Hieronymus, G. 231
Croton impressus Urb.	Urban, I. 3893
Croton ciliatoglandulifer Ortega	Pringle, C.G. 1914
Croton bixoides Vahl	
Croton chichenensis Lundell	
Croton scouleri Hook. f.	
Croton rivinifolius Kunth	
Croton fishlockii Britton	Fishlock, W.C. 311
Croton alloeophyllus Urb.	
Croton angustatus Urb.	
Croton azuensis Urb.	
Croton poitaei Urb.	
Croton polytomus Urb.	
Croton origanifolius Lam.	Wright, C. 564
Croton subferrugineus Müll. Arg.	Pohl 1622
Croton campestris A. St. - Hil.	Harley, R.M.; Bromley, G.L.; Carvalho, A.M.; Nunes, J.M.S.; Hage, J.L.; Santos, E.B. 22376
Croton betaceus Baill.	Gardner 1840?
Croton pycnadenius Müll. Arg.	Burchell 8988
Croton thurifer Kunth	
Croton frieseanus Müll. Arg.	
Croton lehmannii Pax	Lehmann, F.C. 4821
Croton adipatus Kunth	
Croton lucidus L.	Emery C. Leonard & Genevieve M. Leonard

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Species	Voucher
Croton ciliatoglandulosus Ortega	
Croton cortesianus Kunth	Berlandier 2244 = 824
Croton schiedeanus Schltdl.	Ibarra Manríquez, G.; Sinaca C., S. 1361
Croton jamaicensis B.W. van Ee & P.E. Berry	
Croton myricifolius Griseb.	E. L. Ekman.
Croton brittonianus Carabia	C. Wright
Croton bispinosus C. Wright	Wright, C.
Croton niveus Jacq.	A. C. Smith 10179
Croton icche Lundell	
Croton arboreus Millsp.	
Croton reflexifolius Kunth	Triana, J. 3644
Croton eluteria (L) W. Wright	Wright, C. 1971
Croton schiedeanus Schltdl.	Ibarra Manríquez, G.; Sinaca C., S. 1361
Croton "glabellus" L. [sic.]	
Croton decalobus Müll. Arg.	
Croton micans Sw.	Blanchet 3655
Croton floribundus Spreng.	Chagas e Silva, F. 1751
Croton tricolor Klotzsch ex Baill.	Sellow
Croton blanchetianus Baill.	Blanchet 3094
Croton rosmarinoides Millsp.	Wright, C. 1968
Croton astroites Aiton	Walsh, J.J.
Croton scaber Willd.	Fendler, A. 1234
Croton decalobus Müll. Arg.	
Croton axillaris Müll. Arg.	
Croton yucatanensis Lundell	Lundell, C.L.; Lundell, A.A. 7400
Croton compressus Lam.	
Croton sucrensis Steyerm.	
Croton hoffmannii Müll. Arg.	
Croton sphaerogynus Baill.	Riedel
Croton salutaris Casar.	
Croton spruceanus Benth.	Spruce, R. 2205
Croton cajucara Benth.	Spruce, R. 528
Croton organensis Baill.	
Croton fragrans Kunth	Purdie
Croton billbergianus Müll. Arg.	Purdie 118
Croton javarisensis Secco	
Croton draco Schltdl. & Cham.	Chavarria, M.M.; Solis, A. 882
Croton coriaceus Kunth	
Croton gossypiifolius Vahl.	Holton, I.F. 868
Croton celtidifolius Baill.	Gardner 618
Croton jimenezii Standl. & Valerio	
Croton hibiscifolius Kunth ex Spreng.	Timothy Plowman 1935
Croton huberi Steyerm.	Fendler, A. 1221
Croton perspeciosus Croizat	C. Vargas 8549
Croton pilulifer Rusby	O. W. Stutter 11
Croton lechleri Müll. Arg.	Wilson Quizhpe; V. Granda; D. Veintimilla; H. Salas & P. Wampash 1501

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Croton speciosus Müll. Arg.	Linden, I. 34
Croton floccosus B.A. Sm.	van der Werff, H.; Gray, B.; Fuentes, P. 13379
Croton alchorneicarpus Croizat	Kuhlmann, M.
Croton echinocarpus Baill.	non especified
Croton rusbyi Britton ex Rusby	Rusby, H.H. 1224
Croton amentiformis Riina	
Croton ichthygaster L.B. Sm. & Downs	Hatschbach, G. 14976
Croton caldensis Müll. Arg.	Regnell, A.F. III 1080
Croton vulnerarius Baill.	Glaziou, A.F.M. 4916
Croton urucurana Baill.	
Croton pedicellatus H.B.K. (Det. Croizat)	Gardner 2308
Croton andinus Müll. Arg.	
Croton ovalifolius Vahl in H. West.	
Croton sellowii Baill.	J. S. Blanchet 1803
Croton velutinus Baill.	Blanchet 3660
Croton hircinus Vent.	
Croton guianensis Aubl.	Aublet, J.B.C.F.
Croton glandulosepalus Millsp.	
Croton atrorufus Müll. Arg.	Pohl 1636
Croton timandroides (Didr.) Müll. Arg.	Martius 958
Croton decipiens Baill.	
Croton garckeanus Baill.	F. Sellow 2363
Croton chaetophorus Müll. Arg.	
Croton muscicapa Müll. Arg.	
Croton glutinosus Müll. Arg.	
Croton urticifolius Lam.	Burchell 705
Croton chaetocalyx Müll. Arg.	Burchell 6496
Croton palanostigma Klotzsch	Zappi, D.C.; Sasaki, D.; Milliken, W.; Biggs, N.; Silveira, E.A.; Philippsen, M.; Bessa, M.A.; Piva, J.H. 873
Croton matourensis Aubl.	Brito, J.M.; Ribeiro, J.E.L.S.; Pereira, E. da C. 16
Croton chocoanus Croizat	Killip, E. P. 35482
Croton costatus Kunth	
Croton cuneatus Klotzsch.	Schomburgk
Croton yavitensis Croizat	L. O. Williams 1942
Croton roraimensis Croizat	A. S. Pinkus 122
Croton malambo H. Karst.	Karsten,H.
Croton olivaceus Müll. Arg.	
Croton sampatik Müll. Arg.	
Croton cordiifolius Baill.	Blanchet 3719
Croton triqueter Lam.	
Croton argenteus L.	
Croton fuscescens Spreng.	
Croton setiger Hook.	
Croton michauxii G. L. Webster	
Croton poecilanthus Urb.	Urban, I. 1172
Croton alabamensis E.A. Sm. Ex Chapm.	Mohr, C.
Croton corylifolius Lam.	Wright, C. 566

Table. Studied species in *Chapter 3* and vouchers. *The missing vouchers will be annotated for publication

Croton lundellii Standl.	
Croton beetlei Croizat	Elbert L.; Little Jr. 6712
Croton caracasauus Pittier	
Croton corymbulosus Engelm	
Croton nubigenus G. Webster	A. Grijalva 313
Croton corinthius Poveda & J.Á. González	
Croton cupreatus Croizat	
Croton eichleri Müll. Arg.	
Croton thomasii Riina & P.E. Berry	
Croton luetzelburgii Pax & K. Hoffm.	
Croton polyandrus Spreng.	
Croton ceanothifolius Baill.	Riedel 391/407
Croton pallidulus Baill.	
Croton splendidus Mart. ex Colla	
*Croton julopsidium*Baill.	
Croton cinerellus Müll. Arg.	Riedel
Croton pachypodus G.L. Webster	Cid Ferreira, C.A. 1076
Croton megistocarpus J. A. González & Poveda	Morales, Juan Francisco 3915
Croton sapiifolius Müll. Arg.	
Croton caudatus Geiseler.	Harmand, F.J.
Brasiliocroton mamoninha P.E. Berry & Cordeiro	Souza, V. 266
Astraea lobata (L.) Klotzsch	Harley, R.M. 25311
Astraea comosa (Müll. Arg.) B. W. Van Ee	Glaziou, A.F.M. 15390
Astraea divaricata Klotzsch	Sellow
Acidocroton sp*.* Griseb.	Ekman, E.L. 16896

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Species	Clade	Phylo	Infloresc	Pattern	Ramif.	Elong.	F.flowers.concent	M.cymes	F.cymes	M/F. cymes
Astraea comosa (Müll. Arg.) B. W. Van Ee	Genus *Astraea* (14)	no	Bisexual	7	no	yes	no	yes	no	no
Astraea divaricata Klotzsch	Genus *Astraea*	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton acronychioides F. Muell.	OW (450)	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton adamantinus Müll. Arg.	Geiseleria (82)	no	Bisexual	10	No	yes	yes	yes	no	no
Croton adipatus Kunth	Adenophylli (223)	no	Bisexual	1	No	yes	yes	yes	yes	no
Croton alloeophyllus Urb.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton amentiformis Riina	Cyclostigma (46)	no	Bisexual	1	No	no	yes	yes	no	no
Croton angustatus Urb.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton arnhemicus Müll. Arg.	OW-Arnhemici (5)	no	Bisexual	7	No	yes	no	yes	no	no
Croton atrorufus Müll. Arg.	Barhamia	no	Bisexual	10	No	yes	yes	yes	yes	no
Croton azuensis Urb.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton betaceus Baill.	Adenophylli	no	Bisexual	7	No	yes	yes	yes	no	no
Croton billbergianus Müll. Arg.	Cleodora	no	Bisexual	7	No	yes	yes	yes	no	no
Croton caldensis Müll. Arg.	Cyclostigma	no	Bisexual	5	No	yes	yes	yes	yes	no
Croton campestris A. St. - Hil.	Adenophylli	no	Bisexual	1	No	yes	yes	yes	yes	no
Croton capitis-york Airy Shaw	OW	no	Bisexual	7	No	yes	yes	yes	no	yes
Croton caracasanus Pittier	Corylocroton (11)	no	Bisexual	5	No	yes	no	yes	yes	no
Croton chaetocalyx Müll. Arg.	Barhamia (84)	no	Bisexual	7	No	yes	yes	yes	yes	no
Croton chaetophorus Müll. Arg.	Barhamia	no	Bisexual	6	No	yes	yes	yes	yes	no
Croton choristadenius K. Schum.	OW	no	Bisexual	7	No	yes	yes	yes	no	yes
Croton ciliatoglandulosus Steud (= C. ciliatoglandulifer)	Adenophylli	no	Bisexual	1	No	yes	yes	yes	no	no
Croton cinerellus Müll. Arg.	Lamprocroton	no	Bisexual	11	No	no	yes	yes	no	yes
Croton compressus Lam.	Lasiogyne	no	Bisexual	1	No	yes	yes	yes	no	no
Croton confertus Bak.	OW	no	Bisexual	1	No	yes	yes	yes	yes	no
Croton cortesianus Kunth	Adenophylli	no	Bisexual	16	No	yes	yes	yes	no	no
*Croton costaricensis*Pax (= C.	Geiseleria	no	Bisexual	1	No	yes	yes	yes	no	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

ortholobus)

Species	Group		Sexuality	N						
Croton costatus Kunth	Luntia	no	Bisexual	3	no	yes	yes	yes	no	no
Croton curyphyllus L.	OW	no	Bisexual	7	no	yes	yes	yes	no	no
Croton decipiens Baill.	Barhamia	no	Bisexual	5	yes	yes	yes	yes	no	yes
Croton dockrilli Airy Shaw	OW-Dockrilliorum (4)	no	Bisexual	5	no	yes	yes	yes	no	yes
Croton eluteria (L) W. Wright	Eluteria	no	Bisexual	1	yes	yes	yes	yes	yes	no
Croton fishlockii Britton	Adenophylli	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton frieseanus Müll. Arg.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	yes	no
Croton garckeanus Baill.	Barhamia	no	Bisexual	1	yes	yes	no	yes	no	no
Croton glutinosus Müll. Arg.	Barhamia	no	Bisexual	3	no	yes	yes	yes	yes	no
Croton goyazensis Müll. Arg.	Geiseleria	no	Bisexual	7	no	yes	yes	yes	yes	no
Croton hainanensis Men.	OW	no	Bisexual	1	no	yes	yes	yes	no	yes
Croton ichthygaster L.B. Sm. & Downs	Adenophylli	no	Bisexual	5	no	yes	yes	yes	yes	no
Croton javarisensis Secco	Cleodora	no	Bisexual	7	no	yes	yes	yes	no	no
Croton julopsidium Baill.	Lamprocroton	no	Bisexual	10	yes	yes	yes	yes	no	no
Croton laetifolius Baill.	OW	no	Bisexual	5	no	yes	yes	yes	no	no
Croton laetifolius Baill.	OW	no	Bisexual	1	no	yes	yes	yes	yes	no
Croton lehmannii Pax	Adenophylli	no	Bisexual	5	no	yes	yes	yes	yes	no
Croton lucidus L.	Adenophylli	no	Bisexual	10	no	yes	yes	yes	no	yes
Croton lundianus (Didr.) Müll. Arg.	Geiseleria	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton megalobotrys Müll. Arg.	OW	no	Bisexual	7	no	yes	yes	yes	no	no
Croton muscicapa Müll. Arg.	Barhamia	no	Bisexual	1	no	yes	yes	yes	yes	no
Croton origanifolius Lam.	Adenophylli	no	Bisexual	5	no	yes	yes	yes	no	yes
Croton phebalioides F. Muell. ex Müll. Arg.	OW	no	Bisexual	7	no	yes	yes	yes	no	no
Croton poitaei Urb.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	no	no
Croton polytomus Urb.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	no	yes

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton pycnadenius Müll. Arg.	Adenophylli	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton scabiosus Bedd.	OW	no	Bisexual	1	no	yes	yes	yes	no	no
Croton schiedeanus Schltdl.	Eluteria	no	Bisexual	1	yes	yes	yes	yes	yes	no
Croton schultzii Benth.	OW	no	Bisexual	1	no	yes	yes	yes	no	yes
Croton sclerocalyx (Didr.) Müll. Arg.	Geiseleria	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton sincorensis Mart.	Geiseleria	no	Bisexual	6	no	yes	yes	yes	yes	no
Croton subferrugineus Müll. Arg.	Adenophylli	no	Bisexual	5	no	yes	yes	yes	no	no
Croton sucrensis Steyerm.	Lasiogyne	no	Bisexual	5	no	yes	yes	yes	no	no
Croton tetradenius Baill.	Geiseleria	no	Bisexual	1	no	yes	yes	yes	no	yes
Croton teucridium Baill.	Geiseleria	no	Bisexual	5	no	yes	yes	yes	no	no
Croton thurifer Kunth	Adenophylli	no	Bisexual	5	no	yes	yes	yes	no	yes
Croton timandroides (Didr.) Müll. Arg.	Barhamia	no	Bisexual	10	no	no	yes	yes	no	yes
Croton tomentellus F. Muell.	OW-Gymnocroton (40)	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton triangularis Müll. Arg.	Geiseleria	no	Bisexual	1	no	yes	yes	yes	no	no
Croton urticifolius Lam.	Barhamia	no	Bisexual	1	yes	yes	yes	yes	no	no
Croton urucurana Baill.	Cyclostigma	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton virgultosus Müll. Arg.	Geiseleria	no	Bisexual	1	no	yes	yes	yes	yes	no
Croton vulnerarius Baill.	Cyclostigma	no	Bisexual	7	no	yes	yes	yes	no	yes
Croton eichleri Müll. Arg.	Prisci (3)	yes	Bisexual	5	no	yes	yes	yes	no	yes
Acidocroton sp. Griseb.	Genus *Acidocroton* (15)	yes	Bisexual	12	no	no	no	yes	no	no
Astraea lobata (L.) Klotzsch	Genus *Astraea*	yes	Bisexual	7	no	yes	yes	yes	no	yes
Brasiliocroton mamoninha P.E. Berry & Cordeiro	Genus *Brasiliocroton* (2)	yes	Bisexual	9	no	yes	no	yes	yes	no
Croton aequatoris Croizat	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton alabamensis E.A. Sm. Ex Chapm.	Alabamenses (1)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton alchorneicarpus Croizat	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton andinus Müll. Arg.	Pedicellati (20)	yes	Bisexual	2	no	no	no	yes	yes	no
Croton antisyphiliticus Mart.	Geiseleria	yes	Bisexual	5	no	yes	yes	yes	no	yes
Croton arboreus Millsp.	Eluteria (22)	yes	Bisexual	1	no	yes	no	yes	yes	no
Croton argenteus L.	Julocroton (41)	yes	Bisexual	11	no	no	yes	yes	yes	no
Croton argyranthemus Michx.	Argyranthemi (2)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton argyratus Blume	OW-Argyrati (2)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton astroites Aiton	Lasiogyne (45)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton axillaris Müll. Arg.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton beetlei Croizat	Corylocroton	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton betulinus Vahl	Geiseleria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton bispinosus C. Wright	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton bixoides Vahl. = C. micans]	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton blanchetianus Baill.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton bonplandianus Baill.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton brittonianus Carabia	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton cajucara Benth.	Cleodora (18)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton californicus Müll. Arg.	Drepadenium	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton capitatus Michx. *	Heptallon (9)	yes	Bisexual	11	no	no	yes	yes	no	no
Croton cascarilloides Raeusch.	OW	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton caudatus Geiseler.	OW-Caudati (1)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton ceanothifolius Baill.	Lamprocroton (37)	yes	Bisexual	8	no	no	yes	yes	yes	no
Croton celtidifolius Baill.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton chichenensis Lundell	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton chocoanus Croizat	Luntia (19)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton ciliatoglandulifer Ortega	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton conduplicatus Kunth	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton cordiifolius Baill.	Cordiifolii (1)	yes	Unisexual	17	no	yes	yes	yes	yes	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton coriaceus Kunth	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton corinthius Poveda & J.Á. González	Corinthii (1)	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton corylifolius Lam.	Corylocroton	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton crassifolius Geiseler.	OW	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton cuneatus Klotzsch.	Cuneati	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton cupreatus Croizat	Cupreati (1)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton decalobus Müll. Arg.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton decalobus Müll. Arg.	Lasiogyne	no	Bisexual	1	yes	yes	yes	yes	no	no
Croton dioicus Cav.	Drepadenium (6)	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton discolor Willd.	Adenophylli	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton draco Schltdl. & Cham.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton echinocarpus Baill.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton flavens L. [checking ID with Karina, I need to see voucher]	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton floccosus B.A. Sm.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton floribundus Spreng.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton fragrans Kunth	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton fruticulosus Torr.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton fuscescens Spreng.	Julocroton	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton glabellus L. [sic.]	Eluteria	yes	Bisexual	1	yes	yes	yes	yes	no	no
Croton glandulosepalus Millsp.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton glandulosus L.	Geiseleria	yes	Bisexual	6	no	yes	yes	yes	yes	no
Croton gossypiifolius Vahl.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton goudotii Baill.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton gracilipes Baill.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton gratissimus Burch.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton guianensis Aubl.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Species	Group									
Croton guildinguii Griseb.	Geiseleria	yes	Bisexual	12	no	no	no	yes	no	no
Croton helicoideus Müll. Arg. (=C. micansw.)	Lasiogyne	yes	Bisexual	1			yes	yes	no	no
Croton heliotropiifolius Kunth	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	no	no
Croton hibiscifolius Kunth ex Spreng.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
*Croton hircinus*Vent.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton hirtus L` Hér.	Geiseleria	yes	Bisexual	6	no	yes	yes	yes	yes	no
Croton hoffmannii Müll. Arg.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton huberi Steyerm.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton humilis L.	Adenophylli	yes	Bisexual	6	no	yes	yes	yes	yes	no
Croton icche Lundell	Eluteria	yes	Bisexual	1	no	yes	no	yes	yes	no
Croton impressus Urb.	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton insularis Baill.	OW-Insulares (35)	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton jamaicensis B.W. van Ee & P.E. Berry	Eluteria	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton jimenezii Standl. & Valerio	Cyclostigma	yes	Bisexual	1	no	yes	yes	yes	yes	no
Croton jutiapensis Croizat	Geiseleria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton kongensis Gagn.	OW	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton lachnocarpus Benth.	OW	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton laevigatus Vahl.	OW	yes	Bisexual	3	no	yes	no	yes	no	no
Croton lechleri Müll. Arg.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton lindheimerianus Scheele	Heptallon	yes	Bisexual	13	no	no	yes	yes	no	yes
Croton linearis Jacq.	Adenophylli	yes	Bisexual	17	no	yes	yes	yes	yes	no
Croton luetzelburgii Pax & K. Hoffm.	Luetzelburgiorum (1)	yes	Bisexual	2	no	yes	no	yes	no	no
Croton lundellii Standl.	Corylocroton	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton malambo H. Karst.	Cuneati (11)	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton matourensis Aubl.	Luntia	yes	Bisexual	7	no	yes	yes	yes	no	yes

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Species	Group		Sexuality							
Croton megistocarpus J. A. González & Poveda	Pachypodi (5)	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton micans Sw.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton michauxii G. L. Webster	Crotonopsis (1)	yes	Bisexual	15	yes	yes	no	yes	yes	no
Croton microtiglium Burkill	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton monanthogynus Michx.	Heptallon	yes	Bisexual	12	no	no	no	yes	no	no
Croton myricifolius Griseb.	Eluteria	yes	Bisexual	14	no	yes	no	yes	no	no
Croton niveus Jacq.	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton nubigenus G. Webster	Nubigeni (1)	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton olivaceus Müll. Arg.	Olivacei (1)	yes	Bisexual	1	yes	yes	yes	yes	no	no
Croton organensis Baill.	Cleodora	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton ovalifolius Vahl in H. West.	Barhamia	yes	Bisexual	1	yes	yes	yes	yes	no	no
Croton pachypodus G.L. Webster	Pachypodi	yes	Bisexual	5	yes	yes	yes	yes	yes	no
Croton palanostigma Klotzsch	Luntia	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton pallidulus Baill.	Lamprocroton	yes	Bisexual	11	no	no	yes	yes	no	no
Croton pedicellatus H.B.K. (Det. Croizat)	Pedicellati	yes	Bisexual	2	no	no	no	yes	yes	no
Croton perspeciosus Croizat	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton pilulifer Rusby	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton poecilanthus Urb.	Moacroton (8)	yes	Bisexual	7	yes	yes	yes	yes	no	yes
Croton polyandrus Spreng.	Eutropia (1)	yes	Bisexual	8	no	yes	yes	yes	no	yes
Croton punctatus Jacq.	Drepadenium	yes	Unisexual	17	no	yes	yes	yes	yes	no
Croton pungens Jacq.	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton reflexifolius Kunth	Eluteria	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton ripensis Kaneh & Hatus	OW-Dockrilliorum	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton roraimensis Croizat	Cuneati	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton rosmarinoides Millsp.	Lasiogyne	yes	Bisexual	11	no	no	yes	yes	no	no
Croton ruizianus Müll. Arg.	Adenophylli	yes	Bisexual	1	no	yes	yes	yes	no	no

Table 2. Studied species of *Chapter 3* and Inflorescence traits

Croton saltensis Griseb.	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton salutaris Casar.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton sampatik Müll. Arg.	Sampatik (4)	yes	Bisexual	7	yes	yes	yes	yes	no	yes
Croton sapiifolius Müll. Arg.	Quadrilobi (1)	yes	Unisexual	16	no	yes	yes	yes	yes	no
Croton scaber Willd.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton schiedeanus Schltdl.	Eluteria	yes	Bisexual	3	no	yes	no	yes	no	no
Croton sellowii Baill.	Barhamia	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton setiger Hook.	Eremocarpus (1)	yes	Bisexual	11	no	no	yes	yes	no	no
Croton speciosus Müll. Arg.	Cyclostigma	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton sphaerogynus Baill.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton spruceanus Benth.	Cleodora	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton stipulaceus Kunth	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	yes	no
Croton suberosus Kunth	Adenophylli	yes	Bisexual	5	no	yes	yes	yes	yes	no
Croton thomasii Riina & P.E. Berry	Prisci	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton tiglium L.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton tricolor Klotzsch ex Baill.	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton triqueter Lam.	Julocroton	yes	Bisexual	1	no	yes	yes	yes	yes	no
Croton velutinus Baill.	Barhamia	yes	Bisexual	1	no	no	yes	yes	no	no
Croton verreauxii Baill.	OW	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton xalapensis Kunth	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton yavitensis Croizat	Cuneati	yes	Bisexual	7	no	yes	yes	yes	no	yes
Croton yucatanensis Lundell	Lasiogyne	yes	Bisexual	1	no	yes	yes	yes	no	no
Croton abutiloides Kunth	Adenophylli	yes	Bisexual	7	no	yes	yes	yes	no	yes
*Croton rivinifolius*Kunth	Adenophylli	no	Unisexual	17	no	yes	yes	yes	yes	no
Croton rusbyi Britton ex Rusby	Cyclostigma	no	Bisexual	9	no	yes	no	yes	no	no
Croton scouleri Hook. f.	Adenophylli	no	Unisexual	17	no	yes	yes	yes	yes	no
Croton splendidus Mart. ex Colla	Lamprocroton	no	Bisexual	14	no	yes	no	yes	yes	no

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Species	Clade	Phylogeny	N°flowers.inflores	N°Female_flowers	N°Male_flowers	Proportion.FEMALE.MALE	Stamens.range	Stamens.average	Male_investment
Astraea comosa (Müll. Arg.) B. W. Van Ee	Genus *Astraea* (14)	no	160	20	140	0,14	10	15	2100
Astraea divaricata Klotzsch	Genus *Astraea*	no	90	10	80	0,13	30 to 35	33	2640
Croton acronychioides F. Muell.	OW (450)	no	90	10	80	0,13	11	11	880
Croton adamantinus Müll. Arg.	Geiseleria (82)	no	130	30	100	0,30	12	12	1200
Croton adipatus Kunth	Adenophylli (223)	no	115	115	115	1,00	12	12	1380
Croton alloeophyllus Urb.	Adenophylli	no	300	30	270	0,11	10	10	2700
Croton amentiformis Riina	Cyclostigma (46)	no	600	20	580	0,03	12	12	6960
Croton angustatus Urb.	Adenophylli	no	300	30	270	0,11	12	12	3240
Croton arnhemicus Müll. Arg.	OW-Arnhemici (5)	no	65	5	60	0,08	20	20	1200
Croton atrorufus Müll. Arg.	Barhamia	no	180	80	100	0,80	20	20	2000
Croton azuensis Urb.	Adenophylli	no	300	30	270	0,11	12	12	3240
Croton betaceus Baill.	Adenophylli	no	65	15	50	0,30	12	12	600
Croton billbergianus Müll. Arg.	Cleodora	no	95	15	80	0,19	15 to 17	16	1280
Croton caldensis Müll. Arg.	Cyclostigma	no	180	80	100	0,80	15	15	1500
Croton campestris A. St. -Hil.	Adenophylli	no	80	10	70	0,14	15 to 17	16	1120
Croton capitis-york Airy Shaw	OW	no	140	20	120	0,17	13	13	1560
Croton caracasanus Pittier	Corylocroton (11)	no	400	100	300	0,33	11 or 12	12	3600
Croton chaetocalyx Müll. Arg.	Barhamia (84)	no	50	10	40	0,25	10	10	400
Croton chaetophorus Müll. Arg.	Barhamia	no	45	15	30	0,50	10	10	300
Croton choristadenius K. Schum.	OW	no	190	30	160	0,19	10	10	1600
Croton ciliatoglandulosus Steud (= C. ciliatoglandulifer)	Adenophylli	no	300	30	270	0,11	10	10	2700
Croton cinerellus Müll.	Lamprocroton	no	200	50	150	0,33	8 to 10	9	1350

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Arg.											
Croton compressus Lam.	Lasiogyne	no			200	40	160	0,25	12	12	1920
Croton confertus Bak.	OW	no			200	50	150	0,33	15	15	2250
Croton cortesianus Kunth	Adenophylli	no			300	30	270	0,11	10 to 15	13	3510
	Heptallon	no	Xeric	Shrub	3	15 to 20	18				2700
*Croton costaricensis*Pax (= C. ortholobus)	Geiseleria	no			300	30	270	0,11	10 to 15	13	3510
Croton costatus Kunth	Luntia	no			80	20	60	0,33	10 to 16	13	780
Croton curyphyllus L.	OW	no			80	10	70	0,14	22	22	1540
Croton decipiens Baill.	Barhamia	no			600	100	500	0,20	15	15	7500
Croton dockrilli Airy Shaw	OW-Dockrilliorum (4)	no			190	30	160	0,19	8 to 10	9	1440
Croton eluteria (L) W. Wright	Eluteria	no			180	80	100	0,80	14	14	1400
Croton fishlockii Britton	Adenophylli	no			240	60	180	0,33	11 or 12	12	2160
Croton frieseanus Müll. Arg.	Adenophylli	no			115	115	115	1,00	12	12	1380
Croton garckeanus Baill.	Barhamia	no			200	50	150	0,33	15	15	2250
Croton glutinosus Müll. Arg.	Barhamia	no			150	50	100	0,50	8 to 10	9	900
Croton goyazensis Müll. Arg.	Geiseleria	no			60	10	50	0,20	12	12	600
Croton hainanensis Men.	OW	no			240	40	200	0,20	15 to 17	16	3200
Croton ichthygaster L.B. Sm. & Downs	Adenophylli	no			200	50	150	0,33	20	20	3000
Croton javarisensis Secco	Cleodora	no			270	50	220	0,23	15	15	3300
*Croton julopsidium*Baill.	Lamprocroton	no			230	50	180	0,28	11	11	1980
Croton laetifolius Baill.	OW	no			80	20	60	0,33	10	10	600
Croton laetifolius Baill.	OW	no			130	80	100	0,80	10	10	1000
Croton lehmannii Pax	Adenophylli	no			80	10	70	0,14	12	12	840
Croton lucidus L.	Adenophylli	no			85	15	70	0,21	12	12	840
Croton lundianus (Didr.) Müll. Arg.	Geiseleria	no			150	50	100	0,50	8 to 10	9	900

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Species	Section								
Croton megalobotrys Müll. Arg.	OW	no	100	30	70	0,43	14	14	980
Croton muscicapa Müll. Arg.	Barhamia	no	30	10	20	0,50	4	4	80
Croton origanifolius Lam.	Adenophylli	no	250	50	200	0,25	15 to 20	18	3600
Croton phebalioides F. Muell. ex Müll. Arg.	OW	no	100	20	80	0,25	10	10	800
Croton poitaei Urb.	Adenophylli	no	300	30	270	0,11	12	12	3240
Croton polytomus Urb.	Adenophylli	no	250	50	200	0,25	15 to 20	18	3600
Croton pycnadenius Müll. Arg.	Adenophylli	no	150	30	120	0,25	10	10	1200
Croton scabiosus Bedd.	OW	no	60	10	50	0,20	14	14	700
Croton schiedeanus Schltdl.	Eluteria	no	180	80	100	0,80	14	14	1400
Croton schultzii Benth.	OW	no	200	40	160	0,25	12	12	1920
Croton sclerocalyx (Didr.) Müll. Arg.	Geiseleria	no	70	10	60	0,17	12	12	720
Croton sincorensis Mart.	Geiseleria	no	28	8	20	0,40	8 to 10	9	180
Croton subferrugineus Müll. Arg.	Adenophylli	no	90	20	70	0,29	12	12	840
Croton sucrensis Steyerm.	Lasiogyne	no	60	10	70	0,14	10	10	700
Croton tetradenius Baill.	Geiseleria	no	220	60	160	0,38	12	12	1920
Croton teucridium Baill.	Geiseleria	no	95	15	80	0,19	12	12	960
Croton thurifer Kunth	Adenophylli	no	150	30	120	0,25	11	11	1320
Croton timandroides (Didr.) Müll. Arg.	Barhamia	no	100	20	80	0,25	90 to 100	95	7600
Croton tomentellus F. Muell.	OW-Gymnocroton (40)	no	140	20	120	0,17	6	6	720
Croton triangularis Müll. Arg.	Geiseleria	no	95	15	80	0,19	12	12	960
Croton urticifolius Lam.	Barhamia	no	180	40	140	0,29	11	11	1540
Croton urucurana Baill.	Cyclostigma	no	240	40	200	0,20	15 to 17	16	3200
Croton virgultosus Müll. Arg.	Geiseleria	no	80	10	70	0,14	10	10	700
Croton vulnerarius Baill.	Cyclostigma	no	95	15	80	0,19	15	15	1200

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Species	Group								
Croton eichleri Müll. Arg.	Prisci (3)	yes	130	10	120	0,08	15	15	1800
Acidocroton sp. Griseb.	Genus *Acidocroton* (15)	yes	25	2	23	0,09	12	12	276
Astraea lobata (L.) Klotzsch	Genus *Astraea*	yes	100	20	80	0,25	10 to 15	13	1040
Brasiliocroton mamoninha P.E. Berry & Cordeiro	Genus *Brasiliocroton* (2)	yes	100	70	30	2,33	10 to 12	11	330
Croton aequatoris Croizat	Adenophylli	yes	250	30	220	0,14	15 to 20	18	3960
Croton alabamensis E.A. Sm. Ex Chapm.	Alabamenses (1)	yes	120	20	100	0,20	15	15	1500
Croton alchorneicarpus Croizat	Cyclostigma	yes	500	70	430	0,16	12	12	5160
Croton andinus Müll. Arg.	Pedicellati (20)	yes	35	15	20	0,75	6 to 8	7	140
Croton antisyphiliticus Mart.	Geiseleria	yes	200	30	170	0,18	12	12	2040
Croton arboreus Millsp.	Eluteria (22)	yes	200	50	150	0,33	10	12	1800
Croton argenteus L.	Julocroton (41)	yes	40	5	35	0,14	10 or 11	11	385
Croton argyranthemus Michx.	Argyranthemi (2)	yes	70	20	50	0,40	12	12	600
Croton argyratus Blume	OW-Argyrati (2)	yes	170	30	140	0,21	10	10	1400
Croton astroites Aiton	Lasiogyne (45)	yes	300	30	270	0,11	15 to 20	18	4860
Croton axillaris Müll. Arg.	Lasiogyne	yes	400	40	360	0,11	12	12	4320
Croton beetlei Croizat	Corylocroton	yes	200	20	180	0,11	13 to 15	14	2520
Croton betulinus Vahl	Geiseleria	yes	70	5	65	0,08	12	12	780
Croton bispinosus C. Wright	Eluteria	yes	100	20	80	0,25	6	6	480
Croton bixoides Vahl. = C. micans]	Lasiogyne	yes	300	30	270	0,11	12	12	3240
Croton blanchetianus Baill.	Lasiogyne	yes	200	50	150	0,33	15	15	2250
Croton bonplandianus Baill.	Adenophylli	yes	200	40	160	0,25	15	15	2400
Croton brittonianus Carabia	Eluteria	yes	100	20	80	0,25	6	6	480
Croton cajucara Benth.	Cleodora (18)	yes	240	40	200	0,20	15 to 17	16	3200
Croton californicus Müll.	Drepadenium	yes	120	120	120	1,00	12	12	1440

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Arg.									
Croton capitatus Michx. *	Heptallon (9)	yes	40	10	30	0,33	10	10	300
Croton cascarilloides Raeusch.	OW	yes	70	10	60	0,17	13	13	780
Croton caudatus Geiseler.	OW-Caudati (1)	yes	170	30	140	0,21	25	25	3500
Croton ceanothifolius Baill.	Lamprocroton (37)	yes	40	10	30	0,33	12	12	360
Croton celtidifolius Baill.	Cyclostigma	yes	210	30	180	0,17	60 to 70	65	11700
Croton chichenensis Lundell	Adenophylli	yes	200	50	150	0,33	15	15	2250
Croton chocoanus Croizat	Luntia (19)	yes	240	40	200	0,20	10	10	2000
Croton ciliatoglandulifer Ortega	Adenophylli	yes	150	30	120	0,25	30 to 35	33	3960
Croton conduplicatus Kunth	Adenophylli	yes	270	70	200	0,35	17	17	3400
Croton cordiifolius Baill.	Cordiifolii (1)	yes	170	170	170	1,00	12	12	2040
Croton coriaceus Kunth	Cyclostigma	yes	100	20	80	0,25	25 to 30	28	2240
Croton corinthius Poveda & J.Á. González	Corinthii (1)	yes	180	40	140	0,29	15 to 17	16	2240
Croton corylifolius Lam.	Corylocroton	yes	170	30	140	0,21	15 to 17	16	2240
Croton crassifolius Geiseler.	OW	yes	60	10	50	0,20	30	30	1500
Croton cuneatus Klotzsch.	Cuneati	yes	390	50	340	0,15	15	15	5100
Croton cupreatus Croizat	Cupreati (1)	yes	135	15	120	0,13	10	10	1200
Croton decalobus Müll. Arg.	Lasiogyne	yes	300	30	270	0,11	12	12	3240
Croton decalobus Müll. Arg.	Lasiogyne	no	180	80	100	0,80	15	15	1500
Croton dioicus Cav.	Drepadenium (6)	yes	40	40	40	1,00	10	10	400
Croton discolor Willd.	Adenophylli	yes	115	115	115	1,00	15 to 20	18	2070
Croton draco Schltdl. & Cham.	Cyclostigma	yes	420	100	320	0,31	15	15	4800
Croton echinocarpus Baill.	Cyclostigma	yes	500	70	430	0,16	12	12	5160
Croton flavens L. [checking ID with Karina, I need to see voucher]	Adenophylli	yes	80	10	70	0,14	12	12	840

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton floccosus B.A. Sm.	Cyclostigma	yes	500	70	430	0,16	12	12	5160
Croton floribundus Spreng.	Lasiogyne	yes	340	60	280	0,21	12	12	3360
Croton fragrans Kunth	Cleodora	yes	240	40	200	0,20	15 to 17	16	3200
Croton fruticulosus Torr.	Adenophylli	yes	50	10	40	0,25	11	11	440
Croton fuscescens Spreng.	Julocroton	yes	240	60	180	0,33	11 or 12	12	2160
Croton glabellus L. [sic.]	Eluteria	yes	160	20	140	0,14	15 to 17	16	2240
Croton glandulosepalus Millsp.	Barhamia	yes	250	50	200	0,25	11	11	2200
Croton glandulosus L.	Geiseleria	yes	80	10	70	0,14	10 or 11	11	770
Croton gossypiifolius Vahl.	Cyclostigma	yes	590	140	450	0,31	20	20	9000
Croton goudotii Baill.	OW	yes	200	40	160	0,25	15 to 20	18	2880
Croton gracilipes Baill.	Adenophylli	yes	200	40	160	0,25	15	15	2400
Croton gratissimus Burch.	OW	yes	350	50	300	0,17	15 to 20	18	5400
Croton guianensis Aubl.	Barhamia	yes	250	50	200	0,25	11	11	2200
Croton guildinguii Griseb.	Geiseleria	yes	25	5	20	0,25	12	12	240
Croton helicoideus Müll. Arg. (=C. micansw.)	Lasiogyne	yes	120	20	100	0,20	10 to 13	12	1200
Croton heliotropiifolius Kunth	Adenophylli	yes	360	80	280	0,29	14	14	3920
Croton hibiscifolius Kunth ex Spreng.	Cyclostigma	yes	500	70	430	0,16	20	20	8600
*Croton hircinus*Vent.	Barhamia	yes	160	20	140	0,14	11	11	1540
Croton hirtus L`Hér.	Geiseleria	yes	48	8	40	0,20	20 to 25	23	920
Croton hoffmannii Müll. Arg.	Cleodora	yes	85	15	70	0,21	13	13	910
Croton huberi Steyerm.	Cyclostigma	yes	500	70	430	0,16	20	20	8600
Croton humilis L.	Adenophylli	yes	48	8	40	0,20	10 to 13	12	480
Croton icche Lundell	Eluteria	yes	200	80	120	0,67	6 to 8	7	840
Croton impressus Urb.	Adenophylli	yes	200	20	180	0,11	15 to 20	18	3240
Croton insularis Baill.	OW-Insulares (35)	yes	170	30	140	0,21	16	16	2240

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Species	Group								
Croton jamaicensis B.W. van Ee & P.E. Berry	Eluteria	yes	85	15	70	0,21	5 to 6	6	420
Croton jimenezii Standl. & Valerio	Cyclostigma	yes	300	150	150	1,00	20	20	3000
Croton jutiapensis Croizat	Geiseleria	yes	100	15	85	0,18	12	12	1020
Croton kongensis Gagn.	OW	yes	160	20	140	0,14	10	10	1400
Croton lachnocarpus Benth.	OW	yes	250	50	200	0,25	8	8	1600
Croton laevigatus Vahl.	OW	yes	180	20	160	0,13	12	12	1920
Croton lechleri Müll. Arg.	Cyclostigma	yes	1400	200	1200	0,17	12	12	14400
Croton lindheimerianus Scheele	Heptallon	yes	25	5	20	0,25	10	10	200
Croton linearis Jacq.	Adenophylli	yes	150	150	150	1,00	15 to 20	18	2700
Croton luetzelburgii Pax & K. Hoffm.	Luetzelburgiorum (1)	yes	105	5	100	0,05	13 to 15	14	1400
Croton lundellii Standl.	Corylocroton	yes	170	30	140	0,21	13 to 15	14	1960
Croton malambo H. Karst.	Cuneati (11)	yes	250	30	220	0,14	15	15	3300
Croton matourensis Aubl.	Luntia	yes	240	40	200	0,20	10	10	2000
Croton megistocarpus J. A. González & Poveda	Pachypodi (5)	yes	170	30	140	0,21	14	14	1960
Croton micans Sw.	Lasiogyne	yes	200	40	160	0,25	12	12	1920
Croton michauxii G. L. Webster	Crotonopsis (1)	yes	35	15	20	0,75	5	5	100
Croton microtiglium Burkill	OW	yes	170	30	140	0,21	12	12	1680
Croton monanthogynus Michx.	Heptallon	yes	25	5	20	0,25	6	6	120
Croton myricifolius Griseb.	Eluteria	yes	100	1	99	0,01	6	6	594
Croton niveus Jacq.	Eluteria	yes	300	40	260	0,15	12	12	3120
Croton nubigenus G. Webster	Nubigeni (1)	yes	180	40	140	0,29	13 to 15	14	1960
Croton olivaceus Müll. Arg.	Olivacei (1)	yes	230	50	180	0,28	20	20	3600
Croton organensis Baill.	Cleodora	yes	180	50	130	0,38	15	15	1950
Croton ovalifolius Vahl in H. West.	Barhamia	yes	80	20	60	0,33	12	12	720

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton pachypodus G.L. Webster	Pachypodi	yes	180	40	140	0,29	14	14	1960
Croton palanostigma Klotzsch	Luntia	yes	340	40	300	0,13	11	11	3300
Croton pallidulus Baill.	Lamprocroton	yes	30	10	30	0,33	10	10	300
Croton pedicellatus H.B.K. (Det. Croizat)	Pedicellati	yes	25	10	15	0,67	8	8	120
Croton perspeciosus Croizat	Cyclostigma	yes	500	70	430	0,16	20	20	8600
Croton pilulifer Rusby	Cyclostigma	yes	500	70	430	0,16	50 to 60	55	23650
Croton poecilanthus Urb.	Moacroton (8)	yes	140	20	120	0,17	20	20	2400
Croton polyandrus Spreng.	Eutropia (1)	yes	160	20	140	0,14	13 to 15	14	1960
Croton punctatus Jacq.	Drepadenium	yes	30	30	30	1,00	10	10	300
Croton pungens Jacq.	Adenophylli	yes	250	50	200	0,25	15 to 20	18	3600
Croton reflexifolius Kunth	Eluteria	yes	100	20	80	0,25	12	12	960
Croton ripensis Kaneh & Hatus	OW-Dockrilliorum	yes	170	50	120	0,42	8 to 10	9	1080
Croton roraimensis Croizat	Cuneati	yes	390	50	340	0,15	15	15	5100
Croton rosmarinoides Millsp.	Lasiogyne	yes	24	4	20	0,20	10	10	200
Croton ruizianus Müll. Arg.	Adenophylli	yes	200	40	160	0,25	15 to 20	18	2880
Croton saltensis Griseb.	Adenophylli	yes	300	40	260	0,15	15 to 20	18	4680
Croton salutaris Casar.	Cleodora	yes	320	70	250	0,28	15 to 20	18	4500
Croton sampatik Müll. Arg.	Sampatik (4)	yes	400	80	320	0,25	8 to 10	9	2880
Croton sapiifolius Müll. Arg.	Quadrilobi (1)	yes	100	100	100	1,00	10 to 15	13	1300
Croton scaber Willd.	Lasiogyne	yes	150	20	130	0,15	10	10	1300
Croton schiedeanus Schltdl.	Eluteria	yes	210	50	160	0,31	6	6	960
Croton sellowii Baill.	Barhamia	yes	150	50	100	0,50	10	10	1000
Croton setiger Hook.	Eremocarpus (1)	yes	45	5	40	0,13	6	6	240
Croton speciosus Müll. Arg.	Cyclostigma	yes	500	70	430	0,16	140 to 160	150	64500

Table 3. Studied species of *Chapter 3* and Inflorescence traits

Croton sphaerogynus Baill.	Cleodora	yes	320	70	250	0,28	15 to 20	18	4500
Croton spruceanus Benth.	Cleodora	yes	320	70	250	0,28	15 to 20	18	4500
Croton stipulaceus Kunth	Adenophylli	yes	360	100	260	0,38	16	16	4160
Croton suberosus Kunth	Adenophylli	yes	170	70	100	0,70	13	13	1300
Croton thomasii Riina & P.E. Berry	Prisci	yes	135	15	120	0,13	15 or 16	16	1920
Croton tiglium L.	OW	yes	170	20	150	0,13	14 to 16	15	2250
Croton tricolor Klotzsch ex Baill.	Lasiogyne	yes	150	30	120	0,25	15	15	1800
Croton triqueter Lam.	Julocroton	yes	150	50	100	0,50	11 or 12	12	1200
Croton velutinus Baill.	Barhamia	yes	60	10	50	0,20	10	10	500
Croton verreauxii Baill.	OW	yes	170	30	140	0,21	12	12	1680
Croton xalapensis Kunth	Adenophylli	yes	150	30	120	0,25	20 to 25	23	2760
Croton yavitensis Croizat	Cuneati	yes	390	50	340	0,15	15	15	5100
Croton yucatanensis Lundell	Lasiogyne	yes	300	50	250	0,20	10	10	2500
Croton abutiloides Kunth	Adenophylli	yes	350	40	310	0,13	15 to 20	18	5580
Croton rivinifolius Kunth	Adenophylli	no	115	115	115	1,00	12	12	1380
Croton rusbyi Britton ex Rusby	Cyclostigma	no	200	50	150	0,33	15	15	2250
Croton scouleri Hook. f.	Adenophylli	no	115	115	115	1,00	12	12	1380
Croton splendidus Mart. ex Colla	Lamprocroton	no	400	100	300	0,33	11 or 12	12	3600