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Evolution of floral diversity in the genus *Croton* and related genera (Crotonoideae, Euphorbiaceae)

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Abstract

Croton, a mega-diverse genus with about 1,200 species, has great variation in floral and inflorescence structures. Flowers of *Croton* are unisexual with great dimorphism between male and female flowers. Previous studies revealed ambiguous interpretations of some characters between two flower sexes, e.g., female filamentous structures vs. male petals, and the nectary in male and female flowers. Great diversity in the androecium was reported but there no detailed investigation has ever been conducted. Knowledge from relatives of *Croton* in the tribe Crotoneae may help our understanding of floral morphology of *Croton*. However, their floral morphology is poorly known. Extensive examination of the inflorescence and floral morphology of all genera in the tribe will provide useful characters for taxonomic purpose. Moreover, our study will contribute to better understand the complex floral morphology in the whole family Euphorbiaceae which is under-explored. To explore developmental processes underlying androecial diversity in *Croton* and tribe Crotoneae, flower buds from about 100 species of *Croton* and related genera were collected from the wild, cultivated collections or extracted from herbarium sheets. Many techniques were used in the present study, e.g., light microscopy, thin section, scanning electron microscopy and micro-computer tomography (μCT). Morphological data were then mapped on the phylogenetic tree to study character evolution.

The tribe Crotoneae is found to be heterogeneous with a combination of many characters. Stamen form, structure and arrangement are different in each genus. However, the synapomorphies of the whole tribe were identified, e.g., the presence of only male flowers on the distal part of the inflorescence, more than 10 stamens and reduced petals in female flowers. Within the tribe Crotoneae, two clades were recognised, i.e., a *Sagotia-Sandwithia* clade and a *Acidocroton-Astraea-Brasiliocroton-Croton* clade. In the *Sagotia-Sandwithia* clade, shared characters are a botryoid inflorescence, petals larger than sepals at anthesis and outermost stamens alternate with petals. In the *Acidocroton-Astraea-Brasiliocroton-Croton* clade, synapomorphies are the presence of thyrses or racemes, stellate trichomes and stellate-derived trichomes, e.g., lepidote and dendritic types, and the outermost stamen

opposite petals. Inflorescence and floral morphologies of the tribe Crotoneae and other related groups in the Euphorbiaceae were also discussed.

The question of common origin (homology) between organs in male and female flowers of *Croton* is a controversial subject. We examined floral development in unusual *Croton* species with fully developed petals in female flowers, i.e., *C. alabamensis* and *C. schiedeanus*, compared with *C. chilensis* which has filamentous structures in female flowers. Floral ontogeny reveals that filamentous structures should be considered a reductive form of petals. Moreover, we also question the interpretation of the nectary as of staminodial origin since its variable number and position suggest that it may have a receptacular origin. Interestingly, male floral morphology and development are highly diverse with the presence of a concave receptacle (hypanthium) in *C. alabamensis*, while other species of *Croton* have a convex receptacle. Stamens of *Croton* also show centrifugal development, which is highly unique among angiosperms because this condition is normally found in flowers with very high stamen number.

There are many enigmatic groups within *Croton* with extreme variation of floral morphology. Many of these were thought to be distinct genera before the molecular phylogeny classification. Our study reveals great developmental diversity among them. In *C. celtidifolius* with very high stamen number (>100), stamen development is chaotic due to the breaking down of general whorl development. Several groups of *Croton* have a reduction of stamen number. In section *Moacroton* subgenus *Quadrilobi*, two to six stamens are present with the outermost stamen whorl alternate with petals, suggesting the loss of the centrifugal whorl. In *Croton* subgenus *Geiseleria*, several species, i.e., *C. monanthogynus*, *C. michauxii*, *C. setiger* and *C. trinitatis*, have a reduction of stamen number to three to eight evolving in parallel. They also lose the centrifugal stamen development, but the outermost stamen whorl is opposite petals differently from the condition in section *Moacroton*. Female flowers from some species also show variation in floral form. *C. monanthogynus* has a bicarpellate ovary contrary to the generally tricarpellate ovary in the genus and also in the family. Ovaries of *C. setiger* and *C. michauxii* have an unusual ovary with one

carpel linked with a reduction of other floral organs. However, their detailed morphologies are different, suggesting parallel evolution combined with their distant position on the phylogeny.

Our results reveal that the floral morphology in *Croton* and related genera is highly diverse with complex floral architectures. We use the relatively new technique of computer tomography to study rare and delicate samples. Synapomorphies of the tribe and its subclades and taxonomic informative characters were found which would improve identification of these plants in the field. We found that a combination of morphology, ontogeny and phylogeny facilitate the interpretation of origin and homology of several floral characters, e.g., inflorescence, male petals and female filamentous structures, floral nectary and androecial morphology, within *Croton* and the tribe Crotoneae. We hope the present study will be an important step towards better understanding floral morphology in the family Euphorbiaceae.

Lay summary

Croton is a genus of flowering plant with high species number and great variation of floral structure. *Croton* produces male and female flowers separately on the same plant. Previous studies found that male and female flowers are strongly different, for example, male flowers have petals while female flower have minute filamentous structures. Variation among male and female flowers is also described. So, we aim to the flower and inflorescence from relatives of *Croton* and also within the genus *Croton* to gain knowledge about mechanisms behind their variation. The present study examines *Croton* and related genera to understand the evolutionary development mechanism behind the diversity of flowers in this group. We use several techniques to help observe floral structure of these delicate plants, for example scanning electron microscopy with great magnification to observe small samples and micro-computed tomography to conduct non-damaging study to some rare or delicate samples. The second chapter explores the diversity and evolution of inflorescences and flowers from related genera of *Croton*. Informative characters useful for field work are identified. Architecture of stamen, pollen producing organ, are highly variable. Several stamen arrangement patterns were found in different relatives of *Croton*. The third chapter deals with the question why male and female flowers of *Croton* look different with a focus on petals. Comparative studies between male and female flowers are carried out. It is found that time and space availability during development are important factors to make male and female flower different. Interestingly an inside out stamen developmental pattern, which is unusual character, is found in *Croton*. The fourth chapter explores flowers of several unusual *Croton*. Mechanisms behind a very low or very high stamen number are found. Female flowers also show variation in the fruit producing organ, the ovary. Therefore, our work explores structures of male and female flowers of *Croton* and relatives. We hope that our knowledge will contribute to a better understanding of flower forms in other plants.

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Chapter 1: General introduction

1.1 background

Croton L., the second biggest genus in family Euphorbiaceae with more than 1,200 species, is distributed in tropical and subtropical regions worldwide with the centre of diversity in Brazil followed by the Caribbean and Madagascar (Webster, 1993; Govaerts et al., 2000; Berry et al., 2005b; van Ee and Berry, 2010a). The genus is used by people around the world for various reasons, e.g., traditional medicine, ornamentals, animal hunting and even weaponry (Schultes, 1987). A wide range of bioactive compounds were extracted from *Croton* and many of them were demonstrated to have medicinal properties (Salatino et al., 2007; Xu et al., 2018). Inflorescence of *Croton* are thyrsoid/racemose, usually with male and female flowers on the same inflorescence. A male flower consists of sepals, petals, nectaries and stamens, while a female flower has sepals, filamentous structures, nectaries and an ovary. The strong dimorphism between male and female flowers is the centre of many discussions about the homology and evolution of floral organs (e.g., Michaelis, 1924; Gandhi and Thomas, 1983; De-Paula et al., 2011; Gagliardi et al., 2017). Studying the taxonomy of *Croton* is difficult due to its enormous size and high homoplasy. To explain the origin of floral diversity in *Croton*, a comparative study of the related genera in the tribe Crotoneae is required which may reveal ancestral states and/or intermediate forms. The present thesis will explore floral diversity and evolution in *Croton* and related genera which is an essential contribution to understand the evolution of Euphorbiaceae, one of the largest and most diverse group of an angiosperms.

1.2 The tribe Crotoneae

1.2.1 Taxonomic classification

In pre-molecular classifications, *Croton* was usually grouped with its own satellite taxa, e.g., *Eremocarpus* Benth., *Crotonopsis* Michx., *Julocroton* Mart. and

Cubacroton Alain. (Müller, 1866, 1873; Bentham and Hooker, 1880). In some classifications, the genus *Micrandra* Benth. is grouped with *Croton* as well, based on shared inflexed stamens in bud (Müller, 1866, 1873), but this idea was discarded by later taxonomists (Bentham and Hooker, 1880; Hutchinson, 1969; Webster, 1975, 1994b). Webster (1994b) later merged all small genera with inflexed stamens into *Croton* and also included *Mildbraedia* Pax., *Paracroton* Miq. (under the synonym *Fahrenheitia* Rchb. F. & Zoll.) and *Cubacroton* Croizat. into the tribe. Later classification by Radcliffe-smith (2001) accepted the inclusion of the three genera but rejected the lumping of *Eremocarpus*, *Crotonopsis* and *Julocroton* into *Croton*. However, he synonymised *Cubacroton* with *Croton* without explanation (Radcliffe-Smith, 2001). In 2005, phylogenetic studies revealed that *Milbraedia*, *Paracroton* and *Micrandra* are distantly related to *Croton*, but *Sagotia* Baill., *Sandwithia* Lanj., *Acidocroton* Griseb. (including *Ophellantha* Standl.) and a newly describe genus *Brasiliocroton* P.E. Berry & Cordeiro are closer to *Croton* establishing the modern concept of the tribe Crotoneae (Berry et al., 2005a; b; Wurdack et al., 2005). Phylogenetic studies also confirm the position of *Eremocarpus*, *Crotonopsis*, *Julocroton* and *Cubacroton* as a subgroup within *Croton*. However, the phylogenetic studies also revealed that *Croton* is polyphyletic because one of its sections is closer to *Acidocroton*, while the rest of the genus is closer to *Brasiliocroton*. Therefore, a genus *Astraea* is resurrected (Wurdack et al., 2005). Close relationship of *Croton* and *Brasiliocroton* and segregation of *Astraea* are supported by later phylogenetic studies (van Ee et al., 2011; Riina et al., 2014; van Welzen et al., 2020) and also with some morphological characters (de Sá-Haiad et al., 2009; De-Paula et al., 2011; Riina et al., 2014; Vitarelli et al., 2015). However, this relationship is challenged by a new phylogenetic study which found *Croton* forming a grade with *Astraea* and *Acidocroton* (Silva et al., 2020). At the moment, no morphological link between *Astraea* and *Acidocroton* together is known.

1.2.2 Inflorescence and floral diversity

From the literature, inflorescences of genera in the tribe Crotoneae are highly diverse and described as cymose, racemose, clustered, racemoid-paniculate, thyrsoid-paniculate, glomerular, racemoid and paniculate (Webster, 2014). *Croton* and *Astraea* are known to have similar thyrsoid inflorescences with condensed cymules (Silva et al., 2020). However, *Brasilicroton*, the closest relative of *Croton*, has paniculate inflorescences with elongate branching. Moreover, glomerules of flowers are reported in the genus *Acidocroton*, the sister group of *Astraea*. Apart of the Crotoneae, a cymose inflorescence is common in the family Euphorbiaceae (Webster, 2014). This type of inflorescence is described in the Jatropheae, the sister tribe of the Crotoneae in the clade C1 (Wurdack et al., 2005; van Welzen et al., 2020). Connection between these two tribes may be waiting to be explored. Therefore, a comparative inflorescence morphological study may help us explain the diversity of inflorescences in the tribe and eliminate the confusion in terminology used by different authors.

Common floral characters of the tribe are the presence of unisexual flowers (monoecy), reduction of petals in pistillate flowers and presence of a nectary in both flower sexes (Webster, 2014). The presence of reduced petals in the form of filamentous structures or their complete loss is considered to be a synapomorphy of the tribe, contrary to the sister tribe Jatropheae with fully developed petals (Silva et al., 2020). The presence of petals at least in male flowers is a synapomorphy of the core-crotonoideae, in which the tribe Crotoneae is nested, but rare among other Euphorbiaceae (Wurdack et al., 2005). Therefore, examination of their floral morphology may give a hint about petal origins in the core-crotonoideae. Male flowers in the tribe Crotoneae are highly diverse. Webster (2014) reported a range of stamen numbers in the tribe from three to 400. *Croton* and *Astraea* also share the presence of unusual inflexed stamens in bud with unknown origin or functions. The arrangement of stamens with the outermost whorl opposite to petals was known to occur in *Croton, Astraea* and *Jatropha* from the sister tribe (Baillon, 1858; Marchand, 1860; Venkata-Rao and Ramalakshmi, 1968; Gandhi and Thomas, 1983; Singh, 2005; Liu et al., 2008, 2015; De-Paula et al., 2011) but less is known about stamen arrangement in other genera in the Crotoneae. A nectary is found in both male and female flowers (except

Sagotia) (Webster, 2014). In *Croton*, the nectary was interpreted as derived from the receptacle (Freitas et al., 2001; Caruzo and Cordeiro, 2007) or of staminode origin (Michaelis, 1924; De-Paula et al., 2011). A recent developmental study in *Croton* found that the nectary develops late in both male and female flowers and is rarely replaced by a stamen in female flowers, which gives support to the staminode- origin hypothesis (De-Paula et al., 2011). However, there is no information about nectary organisation and origin in other genera in the Crotoneae.

1.3 The genus *Croton*

1.3.1 Taxonomic classification

A taxonomic study of *Croton* is made difficult by its enormous size and also many homoplasies (Webster, 1993; Berry et al., 2005b). The genus *Croton* was first described by Linnaeus in 1753. The type of the genus is *C. tiglium* L. Pre-molecular classifications of the genus are highly variable. Several early authors have described multiple genera (e.g. Klotzsch, 1841; Baillon, 1858) which were later lumped together by Müller (1866, 1873), except for *Crotonopsis*, *Eremocarpus*, and *Julocroton*. Nearly a century later, *Moacroton* was partitioned from *Croton* by Croizat (1945) and *Cubacroton* was described by Alain (1960). Earlier works of Webster recognised these groups as separate genera as well (Webster, 1967, 1975). He later grouped most of them into *Croton* in his sectional classification of *Croton* (except *Moacroton* and *Cubacroton*) (Webster, 1992, 1993). However, the sectional classification of Webster (1993) is biased toward neotropical species and only few representative species were listed in each genus. A decade later, Radcliffe-Smith (2001) published his conservative Euphorbiaceae classification and recognised those segregated genera of *Croton* again. In 2005, several molecular phylogenetic studies found that *Croton* is polyphyletic with the section *Astraea* closer to the genus *Acidocroton* (include *Ophellantha*) while the rest of the genus (including *Moacroton*) is closer to the newly discovered genus *Brasiliocroton* instead (Berry et al., 2005a; b; Wurdack et al., 2005).

In the present circumscription, four subgenera of *Croton* are recognized based on the molecular phylogeny, i.e., subg. *Quadrilobi* (Mull. Arg.) Pax in Engl.& Prantl, subg. *Croton* L., subg. *Adenophylli* (Griseb.) Riina, B.W. van Ee & P.E. Berry and subg. *Geiseleria* A. Gray (van Ee et al., 2011). Subgenus *Croton* is distributed in the old world (Africa, Madagascar, Asia and Australia) while three other subgenera are present in the new world (North and South America) (Berry et al., 2005; van Ee et al., 2011; Haber et al., 2017). Several sections from Webster's classification are found to be highly polyphyletic (Berry et al., 2005b; Riina et al., 2009, 2010a); therefore, reclassification were carried out resulting in 31 New World sections and six Australian sections (van Ee et al., 2011, 2015). However, distinguishing each subgenus and section with morphological characters is found to be difficult because of homoplasy (van Ee et al., 2011). Floral microstructure is a valuable source for taxonomically informative characters. However, previously microstructure studies in *Croton* focused on the foliar microstructure (mainly trichomes) (Webster et al., 1996; de Sá-Haiad et al., 2009; Senakun and Chantaranothai, 2010; Liu et al., 2013; Vitarelli et al., 2015, 2016). Due to the large size and uncertain internal relationships, extensive investigations of floral organs diversity and evolution within the whole genus context have never been conducted before. Combination of the present Crotoneae phylogenies together with detailed floral anatomical, morphology and developmental information are critical to understand homology and floral organ evolution in *Croton.*

1.3.2 Floral diversity

Flowers of *Croton* are unisexual and both sexes occur on the same inflorescence with female flower on the lower part and male flowers on the upper part. Female flowers bloom before male flowers. Male flowers have a bipartite perianth with sepals and petals while in female flowers only the sepals are visible while there are filamentous structures present in the same position as petals in male flowers instead. In male flowers of *Croton*, stamens are inflexed in bud, except for a few taxa (van Ee et al., 2008, 2011; Webster, 2014), and the outermost whorl is arranged opposite to petals (Baillon, 1858; Gandhi and Thomas, 1983; De-Paula et al., 2011; Gagliardi et al., 2017). The androecium has much variation in stamen number, including number of whorls and presence of one or three central stamens (Gandhi and

Thomas, 1983; Riina et al., 2009; Ronse De Craene, 2010). The variable number of stamen whorls occurs occasionally in other members of Euphorbiaceae (Ronse De Craene, 2010), but appears unusual among Rosids. It is unclear what developmental mechanism regulates the pattern of stamen increases and the variation in number and this should be investigated. The present study will investigate the structure and arrangement of stamens within the context of phylogenetic relationships of the genus and related genera.

Pistillate flowers also show variation in shape and size of the perianth with sepals ranging from equal to unequal or even zygomorphic in some group (De-Paula et al., 2011; van Ee et al., 2011). The petals also vary from reduced filamentous-like structures to fully developed petals in some taxa (Webster, 1993; van Ee et al., 2008; De-Paula et al., 2011). Inside the corolla, there is a whorl of nectaries similar to male flowers, but with obscure origin. The centre of the female flowers is occupied by an ovary generally composed of three carpels with the exception of uni- or bi- carpellate ovaries in some taxa (Webster, 1993; van Ee and Berry, 2009a; van Ee et al., 2011). Style branching patterns are highly diverse, ranging from two to many (Webster, 1993; van Ee et al., 2011). Because of this great diversity of floral organs in *Croton*, a wide range of species with different morphologies will be compared in the present anatomical and morphological studies to improve understanding of floral structures in the genus.

1.4 Aims of the study and thesis structure

The present study aims to outline the diversity in inflorescences and flowers within the genus *Croton* and related genera. Patterns of floral and inflorescence evolution in *Croton* and tribe Crotoneae will be generated from comparative morphological data. We also aim to identify characters with taxonomical significance of *Croton* and relatives to support the molecular classification. Moreover, floral developmental and anatomical structures of *Croton* and related genera will be studied to understand complex floral diversity of *Croton*. Three chapters conduct examination in different perspectives.

Why other genera in the Crotoneae look different from *Croton*? (Chapter 2)

The second chapter will explore the inflorescence and floral morphology in relatives of *Croton*, the tribe Crotoneae. Several techniques were used to study these plants from different aspects, e.g., scanning electron microscopy, thin sectioning, micro-computed tomography and phylogenetic, to gain knowledge from these plants which are poorly known to science.

What is the mechanism behind floral dimorphism in *Croton*? (Chapter 3)

This chapter emphasises the differences in petals development between male and female flowers. However, developmental differences between the same flower type is our focus as well. Comparative study in species with different morphology will help us to understand developmental mechanisms behind species diversification.

What is the developmental basis behind some unusual *Croton*? (Chapter 4)

The last research chapter will conduct comparative development and morphology in several highly specialised *Croton* species, e.g., species with very low stamen number, species with very high stamen number, species which lost their petals, species that shift to bilateral symmetry, and species with unusual carpel number. We would like to explore what mechanisms make them different and which features link them with typical *Croton*.

The general concluding chapter summarize previous chapters and also points towards future work.

Chapter2: Diversity and evolution of floral and inflorescence structures in the tribe Crotoneae Dumort. (Crotonoideae, Euphorbiaceae)

Data contribution: For the micro-computed tomography, sample preparations were done by the author. The scanning of samples and image stack construction were conducted by Dr Alexander Ziegler and Dr Julius Jeiter (Universität Bonn, Germany). Later visualisation and analyses were carried out by the author. Other examination and analyses were done by the author.

2.1 Chapter summary

Phylogenetic studies in the early 21st century suggest grouping six heterogeneous genera, i.e., *Sagotia*, *Sandwithia*, *Acidocroton*, *Astraea*, *Brasiliocroton* and *Croton*, together in the tribe Crotoneae. Apart of *Croton*, inflorescences and flowers from other genera were not included in any comparative morphological study. In the present study, inflorescences and flowers from representative species from all six genera were included in our examination. The aims of this study are to find taxonomically informative characters and also understand evolution within the tribe. We use a combination of methods, e.g., light microscopy, scanning electron microscopy (SEM), resin sectioning and micro-computed topography (μCT) to conduct the study. Inflorescences in the tribe are highly diverse but could be classified in three groups, racemes, thyrses and panicles with variation in the presence of the terminal flower. Comparison with other related group suggested that determinate thyrses are likely to be an ancestral character. Floral trichomes are also diverse in the tribe and evolution from simple trichomes to stellate trichomes is discussed. Flowers of all genera in the tribe are unisexual with strong dimorphism between male and female flowers. The petals are present in male flowers while petals in female flowers are reduced or lost. Since the presence of petals in flowers is uncommon among Euphorbiaceae, three hypotheses about the origin of petals in the core-Crotonoideae

are discussed. Different forms of nectaries are found in the tribe ranging from separate lobed, a ring to honeycomb-like structures covering the receptacle. The most diverse floral structure in the tribe is the stamen. Each genus is found to have a unique way to pack their stamens in the limited space of the bud. The number of stamens in the tribe ranges from one to more than 100 but the presence of more than 10 stamens is a common character among all genera. Stamen architecture in each genus is unique and could be derived from an ancestor with labile stamen development pattern. Floral traits related to pollination are also discussed. The present study identifies more synapomorphies that successfully explain the evolutionary developmental mechanism behind the divergent floral morphology in the tribe.

2.2 Introduction

Croton is the most well-known genus in the tribe Crotoneae with high species number, great morphological diversity and worldwide tropical and subtropical distribution (Govaerts et al., 2000; Berry et al., 2005b; van Ee et al., 2011). The genus was included in several morphological studies which revealed its floral diversity and complex character evolution (Baillon, 1858; Marchand, 1860; Michaelis, 1924; Nair and Abraham, 1962; Venkata-Rao and Ramalakshmi, 1968; Gandhi and Thomas, 1983; De-Paula et al., 2011; Gagliardi et al., 2017). However, little is known about floral morphology in relatives of *Croton* in the tribe Crotoneae. Several molecular phylogenetic studies from the last decade contributed in establishing the modern concept of tribal and generic delimitation which are valuable for the study of floral character evolution (Berry et al., 2005; Wurdack et al., 2005; Sun et al., 2016). The tribe Crotoneae in the present concept comprises six genera, i.e., *Acidocroton* Griseb. (included *Ophellantha* Standl.), *Astraea* Klotzsch, *Brasiliocroton* P.E. Berry & Cordeiro, *Croton* L., *Sagotia* Baill. and *Sandwithia* Lanj. (Fig. 1), grouped together based on molecular phylogenetic data (Fig. 1; 2) (Berry et al., 2005; Wurdack et al., 2005). All genera are distributed in North and South America while many *Croton* and a few *Astraea* occur in Africa, Asia and Australia (Webster, 2014; van Ee et al., 2015; Haber et al., 2017; Silva et al., 2019). Common floral characters of all tribe members are the presence of unisexual flowers (monoecy), reduction of petals in

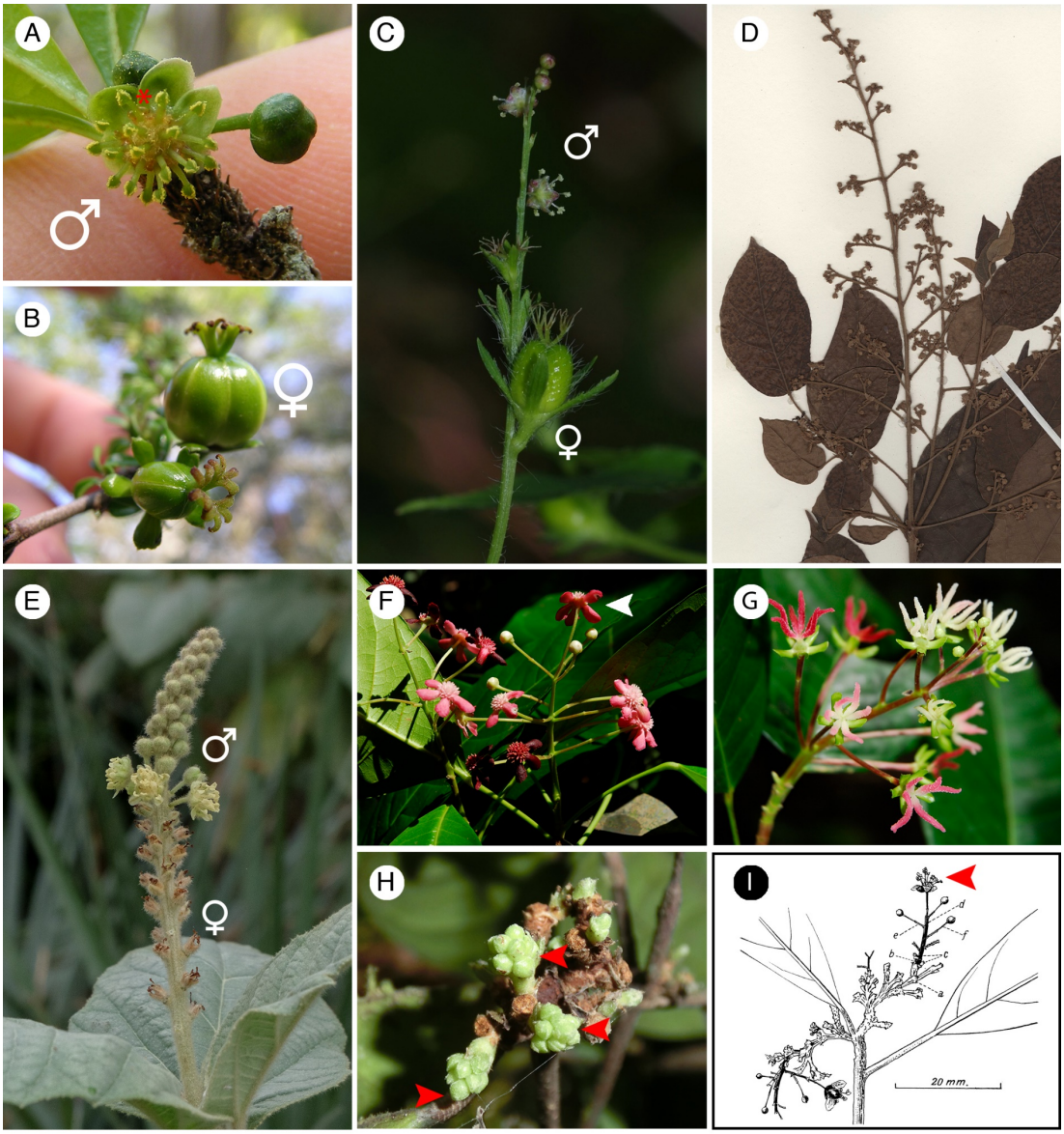

Figure 1. Inflorescences and flowers of all genera in the tribe Crotoneae. (A, B) *Acidocroton*; (C) *Astraea*; (D) *Brasiliocroton*; (E) *Croton*; (F, G) *Sagotia*; (H, I) *Sandwithia*. (A) Male flowers of *Ac. verrucosus* at anthesis. Droplets of possible nectar are visible on the pilose area on the stamen bases (asterisk). (Use of image courtesy of Alan Frank) (B) Two female flowers (possible young fruits) of *Ac. oligostemon*. Tetrafid stigmas are visible. (Photographed by Benjamin van Ee. Use of image courtesy of Ricarda Riina) (C) An inflorescence of *As. lobata*. The inflorescence type is raceme with single female flowers and male flowers as subunits. (Use of image courtesy of Hervé Galliffet) (D) A cluster of highly branched paniculated inflorescences of *B. mamoninha* (E) An inflorescence of *C. chilensis*. The inflorescence is protogynous with female flowers (on proximal part) become mature before male flowers (on distal part). (Photography by the authors) (F) Male inflorescence of *Sag. racemosa*. Young flowers have white petals while more mature flowers have pink to red petals. Note, a terminal male flower that bloom earlier than lower flowers (arrowhead).

Figure 1 continued. (G) A bisexual inflorescence of *Sag. racemosa*. The colour of stigma changes from white to red during maturity. (Image F and G. Photographed by Paul E. Berry. Use of image courtesy of Ricarda Riina) (H) A cluster of young inflorescences of *San. guyanensis*. New inflorescences born on the remnant of old inflorescences. Note there is a terminal flower in each inflorescence (arrowhead). (Use of image courtesy of Marc Litaudon and Simon Remy) (I) An illustration of male inflorescences in the original description of the *San. guyanensis* (Lanjouw, 1932). The illustration shows the terminal flower (arrowhead) and also joint on each pedicel. (Use of image courtesy of the Royal Botanic Garden Kew, UK)

pistillate flowers and presence of a nectary in both flower sexes (Webster, 2014). The pre-molecular phylogeny of tribe Crotoneae was different from the modern concept. Two genera, viz., *Mildbraedia* Pax. and *Paracroton* Miq. (under the synonym *Fahrenheitia* Rchb. F. & Zoll.), were previously included in the tribe (Webster, 1994; Radcliffe-Smith, 2001) but later it was found that their position is distant from the Crotoneae on the phylogeny (Berry et al., 2005; Wurdack et al., 2005) resulting in both genera being assigned to their own tribe named Paracrotoninae (Webster, 2014). Apart from *Croton* and *Astraea*, the other four genera have never been associated with the tribe Crotoneae before. Previously, *Acidocroton* and *Sagotia* were assigned to tribe Cordiaeae, whilst *Sandwithia* was placed in tribe Aleuritidae (Webster, 1975, 1994b; Radcliffe-Smith, 2001). Later, *Sagotia* and *Sandwithia* were found to be closely related genera based on molecular phylogenetic studies (Berry et al., 2005; Wurdack et al., 2005), supporting the previously suggested close relationship by J. Lanjouw (1932) and R. de S. Secco (1987, 1988). There are many cases of misidentification between these two genera. Recently, *Sandwithia* became a monotypic genus because one of the two species was transferred to *Sagotia* (Secco et al., 2019). *Ophellantha* was recognized as a genus in some classifications (Webster, 1975; Radcliffe-Smith, 2001). It was lowered to a section of *Acidocroton* by Webster (1994b), later supported by its position as a sister clade to *Acidocroton* (Berry et al., 2005). However, the two groups are geographically far apart, i.e., sect. *Acidocroton* is distributed in islands of the Greater Antilles while sect. *Ophellantha* occurs from Mexico to Colombia (Webster, 1994b; Radcliffe-Smith, 2001; Webster, 2014). Among all genera, *Croton* is the biggest genus estimated to have more than 1,200 species, while the combined species from all other genera are less than 40 (Govaerts et al., 2000; Berry et al., 2005b; van Ee et al., 2011). Numerous species were previously recognised as separated

genera, i.e., *Colobocarpos* Esser & Welzen, *Crotonopsis* Michx., *Cubacroton* Alain., *Eremocarpus* Benth., *Julocroton* Mart. and *Moacroton* Croizat, (Webster, 1975; Radcliffe-Smith, 2001) but they are found to be aberrant variants of *Croton*, as supported by both morphology and molecular data (Webster, 1994b; Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2011; Webster, 2014). Despite a concrete generic concept from molecular studies, there is no morphological synapomorphy available for each clade or the whole tribe.

The relationship of *Acidocroton, Astraea*, *Brasiliocroton* and *Croton* within the tribe is complicated. Formerly, the genus *Astraea* was treated as a section of *Croton* since both taxa share 'inflexed anthers in the bud' (Webster, 1993; Berry et al., 2005b). However, the newly discovered genus *Brasiliocroton,* which has erect stamens in bud, is found to be the closest relative of *Croton* (Fig. 2A, B) (Berry et al., 2005a, b; Wurdack et al., 2005; Riina et al., 2014; Sun et al., 2016) or it forms a grade with *Croton* and the *Acidocroton-Astraea* clade (Fig. 2C) (Silva, 2018). Floral micro-morphological, anatomical investigations, and leaf morphology corroborated the segregation of *Astraea* from *Croton* (Berry et al., 2005a; De-Paula et al., 2011; Webster, 2014). In addition, several vegetative characters, e.g., tree habit, petiolar glands and transitional forms of stellate to lepidote indumentum, support the close relationship of *Croton-Brasiliocroton* (de Sá-Haiad et al., 2009; Riina et al., 2014). However, there is no morphological support for a sister relationship between *Acidocroton* and *Astraea*. *Acidocroton* also has simple trichomes which are distinct from the other three genera having stellate or stellate-derived trichomes (Webster et al., 1996; Webster, 2014). Both *Brasiliocroton* and *Acidocroton* have never been included in any detailed investigation of inflorescence and flowers before. There is a need for such detailed morphological investigation which will also contribute to a better understanding of flower morphology of *Astraea* and *Croton*, and also the tribe Crotoneae.

The diversity and evolution of the inflorescence in the tribe Crotoneae has never been discussed before. Many inflorescence types, e.g., glomerulae, panicles, racemes and thyrses, were reported in the tribe (Webster, 2014). Webster (1994a,

2014) suggested that dichasial cymes found in *Jatropha* L. and *Cnidoscolus* Pohl are the basic type of the Euphorbiaceae. However, a previous inflorescence morphological study in *Jatropha* suggested that paniculate inflorescences might be ancestral to cymes (Dehgan and Webster, 1979). Moreover, the mentioned work did not discuss evolution of inflorescences in a cladistics framework. Molecular phylogenetic studies revealed

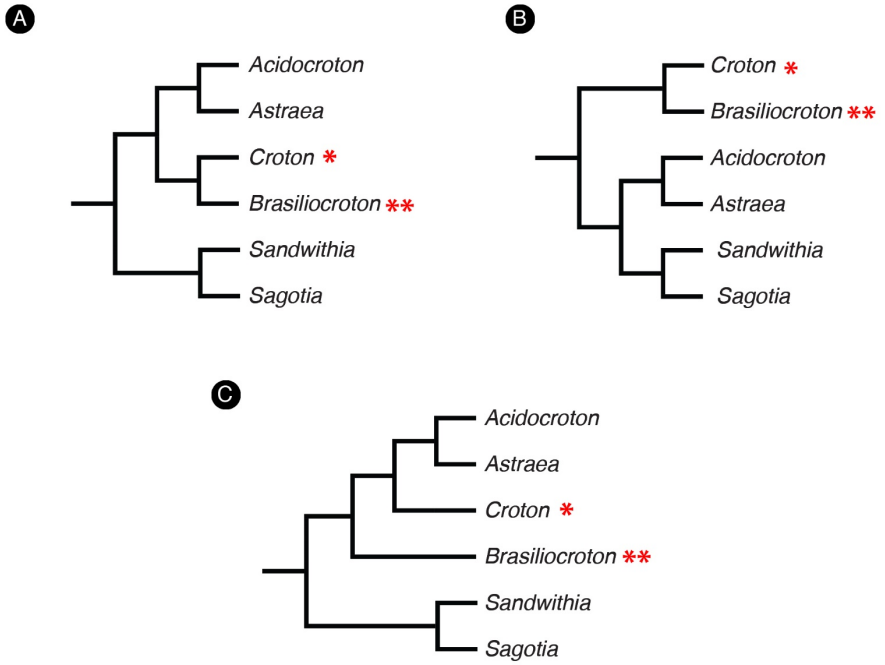

Figure 2. Three different hypotheses of intergeneric relationship within the tribe Crotoneae obtained from different publications. (A) *Brasiliocroton-Croton* sister to *Acidocroton-Astraea* hypothesis (Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2011; van Welzen et al., 2020). (B) *Acidocroton-Astraea* sister to *Sagotia-Sandwithia* hypothesis (Sun et al., 2016). (C) *Brasiliocroton*, *Croton, Acidocroton, Astraea* grade hypothesis (Silva, 2018). Asterisk and double asterisks signs show position of *Croton* and *Brasiliocroton* respectively on each topology.

that the tribe Jatropheae (comprises *Jatropha*, *Joannesia* Vell. and *Vaupesia* R.E.Schult.) is sister to tribe Crotoneae (Wurdack et al., 2005). Therefore, it would be interesting to know whether there are any connections between different types of inflorescence among these two tribes. Within the context of a recent phylogeny, an extensive comparative inflorescence morphological study between tribe Crotoneae and tribe Jatropheae may reveal evolutionary processes behind inflorescence diversity in the tribe Crotoneae and other Euphorbiaceous taxa.

Chapter 2: Crotoneae

Floral morphology in the tribe Crotoneae show great dimorphism between the two flower sexes. From previous studies, the morphology of female flowers in this tribe is relatively conserved with the presence of filamentous structures instead of petals, a tricarpellate ovary, axile placentation, anatropous ovules and bifid styles (Marchand, 1860; Michaelis, 1924; Secco, 1987; De-Paula et al., 2011; Webster, 2014), which are also common character within the Euphorbiaceae (Sutter and Endress, 1995; Webster, 2014). Silva and colleagues (2020) identified the presence of reduced petals in female as a synapomorphy of the tribe. Filamentous structures were interpreted to have a staminodial origin (Gagliardi et al., 2017) or an origin from reduced petals (Nair and Abraham, 1962; Webster, 1993; Radcliffe-Smith, 2001; De-Paula et al., 2011). Nevertheless, fully developed petals in female flowers could be found in the sister tribe Jatropheae, *Sandwithia,* and some *Croton* (van Ee et al., 2011; Webster, 2014). Interestingly, an illustration and description of a female flowers of *Ac. adelioides* Griseb. indicates the presence of several filamentous-like structures surrounding an ovary with unknown origin which requires further investigation (description in Grisebach, 1859) (Fig. 104H, page 316 -Fawcett and Rendle, 1920). Contrary to female flowers, the morphology of male flowers is much more diverse especially for the androecium. The stamen number in the tribe is reported to range from three to 400 (Webster, 2014). Moreover, each genus has a unique shape and structure of stamens (Berry et al., 2005b; Wurdack et al., 2005; Webster, 2014). The origin of inflexed anthers in bud present in *Astraea* and *Croton* is still unknown. The arrangement of stamens is also interesting, with the presence of the outermost stamen whorl opposite petals reported in *Croton* and *Astraea* (Baillon, 1858; Gandhi and Thomas, 1983; De-Paula et al., 2011). Outermost antepetalous stamens are also found in *Jatropha* (Singh, 2005; Liu et al., 2008, 2015) and possibly in other genera of the Jatropheae. However, there is no information about stamen arrangement in other genera in the Crotoneae. A nectary is found in both male and female flowers from all genera of the Crotoneae except in *Sagotia* (Webster, 2014). The origin of this structure is also problematic. In *Astraea* and *Croton*, the nectary has been interpreted as of staminodial origin (De-Paula et al., 2011; Gagliardi et al., 2017) or of receptacular origin (Caruzo and Cordeiro, 2007). However, in analogy to petals and filamentous structures, the nature of the nectary has never been investigated in other genera in the

Crotoneae before. Interestingly, there are obconical-shaped structures present between the stamens in *Ac. verrucosus* Urb. (Fig. 104B, D, page 316 - Fawcett and Rendle, 1920) which is unique among other genera and requires further study. Therefore, an extensive detailed floral morphology examination will expand our knowledge about floral morphology, evolution and systematics in the tribe Crotoneae

It is challenging to understand the morphological relationships among all six genera as the tribe consists of a combination of different morphologies. Analysing the inflorescence and floral morphology of Crotoneae in a phylogenetic framework is essential to understand their evolution and diversity. Our aims are to explain the origin of floral and inflorescence diversity within the tribe and find characters with taxonomical significance for each taxon in the tribe to clarify the evolution and diversity in this heterogeneous group.

2.3 Materials and Methods

2.3.1 Materials collection and preparation

At least one species from each genus in Crotoneae was included in this study (Table 1). Samples of *As. lobata* and *As. surinamensis* were collected in the field, fixed in an FAA solution and then transferred into 50% or 70% ethanol for long-term storage. Other samples with acceptable condition were extracted from herbarium sheets, except *As. comosa* which is a dried non-pressed sample. All dried and herbarium samples were put in a solution of 6:1 10 % Aerosol-OT aqueous solution/acetone for 12-48 hours depending on each sample then washed with distilled water and stored in 70% alcohol to soften and regain the 3D structure (Peterson et al., 1978; Erbar, 1995).

Table 1. Source information of samples of Crotoneae taxa included in morphological and anatomical examination this study

Genus/ Abbreviation	Species	Condition/ Source	Collection number
Acidocroton **Griseb. (*Ac.*)**	*Ac. gentryi* Fern. Alonso & R. Jaram.	Herbarium/ MA	Fernandez 8143
		Herbarium/ MA	Jaramillo 8289
	Ac. oligostemon Urb.	Herbarium/ BM	F. Rugel 355
			BM001123040
		Herbarium/ NY	NL Britton, EG Britton, JF Cowell 12635
	Ac. spinosus (Standl.) G.L.Webster (= *Ophellantha spinosa* Standl.)	Herbarium/ XAL	Nee 24762(XAL)
		Herbarium/ MICH	G.L. Webster 17886
Astraea **Klotzsch (*As.*)**	*As. comosa* (Müll.Arg.) B.W.van Ee.	Dry (treated with Aerosol/Acetone)	Riina 1369
	As. lobate (L.) Klotzsch.	Spirited/ wild collection	Riina 1890
	As. surinamensis (Miq.) O.L.M. Silva & Cordeiro	Spirited/ wild collection	Riina 1924
Brasiliocroton **P.E. Berry & Cordeiro (*B.*)**	*B. mamoninha* P.E.Berry & Cordeiro	Herbarium/ K	De Souza, V. 266
		Herbarium/ MICH	D.A. Folli 2127
		Herbarium/ NY	MM Arbo, AMM Carvalho, MS Ferrucci 7819
	B. muricatus Riina & Cordeiro	Herbarium/ HUEFS	D.S. Carneiro-Torres 1233
			HUEFS 206088
		Herbarium/ WY, HUES B	R.S. Moreira 13
Croton **L. (*C.*)**	*C. alabamensis* var. *alabamensis* E.A.Sm. ex Chapm.	Spirited/ wild collection	A. Wilkins
		Spirited/ cultivated plant	Pratt & Ferrly PFA8
	C. bonplandianus Baill.	Spirited/ Kanchanaburi, Thailand	PT001, PT006

	C. chilensis Müll.Arg.	Spirited/ cultivated plant (RBGE Accession number 20090241A)	Baines, R. Gardner, M., Hechenleitner, P. Morter, C. Rae, d. 53
	C. floribundus Spreng.	Spirited/ wild collection	R.F. Santos, M.B.R. Caruzo & K.B. Gagliardi 03 (SP)
	C. fluviatilis Esser	Spirited/ Wang Nam Yen Botanical garden, Thailand	PT004
	C. maestrensis (Alain) B.W.van Ee & P.E.Berry	Herbarium/ NY	Jorge Guiferrez, Paul E Berry, B van Ee, B Jestrow (HAJB 81958)
	C. monanthogynus Michx.	Spirited/ cultivated plant at RBGE	LBJWC-48 (Lady Bird Johnson wild flower centre, Texus USA)
	C. nubigenus G.L.Webster	Herbarium/ BM	G. Davidse, R. Zúniga & P.R. Honse 34508 BM000038774
	C. polyandrus Spreng.	Spirited/ wild collection	M.B.R. Caruzo, R.F. Santos, A.A.C. Sousa & L. Daneu 203 (SP)
	C. rusbyi Britton ex Rusby	Herbarium/ MICH	N. Chapi 226
	C. sampatik Müll.Arg.	Herbarium/ K	Vicentini, A. & Pereira, E 735
	C. tiglium L.	Spirited/ Suanluang Rama IX botanical garden, Thailand	PT007
	C. trinitatis Millsp.	Spirited/ wild collection	Riina 1920
	C. urucurana Baill.	Spirited/ wild collection	Riina 1903
		Spirited/ wild collection	Riina 1317
***Sagotia* Baill. (*Sag.*)**	*Sag. brachysepala* (Müll.Arg.) Secco (under name *Sag. racemosa* subsp. *brachysepala* Müll.Arg.)	Herbarium/ K	Beddington 31
		Herbarium/ K	Jansen-Jacobs, M.J. et al. 3039

	Sag. brachysepala (Müll.Arg.) Secco	Herbarium/ MICH	T.W. Heukel 3117
		Herbarium/ MICH	L. Ferreira 7915
	Sag. racemosa Baill.	Herbarium/ K	Jansen-Jacobs, M.J. et al. 6127
		Herbarium/ K	Jansen-Jacobs, M.J. et al. 6157
		Herbarium/ MER	Bruijn 1461
		Herbarium/ MICH	H.S. Irwin 55188
***Sandwithia* Lanj. (*San.*)**	*San. guyanensis* Lanj.	Herbarium/ K	Unknown 7774
		Herbarium/ K	Unknown 2731
		Herbarium/ K	Maguire & Cowan R.S. 39343
		Herbarium/ MICH	C.A. Ferreira 5998

2.3.2 General morphological study

Morphology of flower and inflorescence was observed under dissecting light microscopy (LM) (Zeiss Stemi 2000-C) and photographed with the AxioCam MRc 5 (Zeiss). We recorded inflorescence structure and floral biology, e.g., scent and colour, from herbarium sheet from some herbaria, i.e., BM, E, K. We also obtained some data from description in protologues, JSTOR type herbarium and online herbarium, e.g., E, K and NY.

2.3.3 Resin sectioning and light microscopy (LM)

Samples readily stored in 70% ethanol were dehydrated in an 70%-90%-100% ethanol series for one hour each. Next, samples were transferred into series of 1:3, 2:2 and 3:1 ratio infiltration medium (Technovit® 7100 and hardener I)/ 100% ethanol solution for one hour each. Next, samples were soaked in 100% infiltration medium solution at least three days. Later, samples were embedded in moulds filled with 12:1 infiltration medium with hardener II solution. Sample orientation is adjusted by tweezer while liquid is solidifying. About an hour the resin will become solid. Moulds were put into an oven at 40ºC for at least one hour to complete the polymerisation of the resin. Sectioning was carried out in a Leica RM2235 rotary microtome to produce

sections of 5-10 µm thickness. Sections were put on a slide then water was dropped on them to stretch out the resin. Then slides were placed in an oven at 80°C to evaporate the water before transferring them to the hot plate at 80°C to stick the resin slides to the glass. To avoid the folding of the section's margins in the next staining processes, slides were left on a hot plate for two hours or more. Samples were stained in 0.05% Toluidine blue in aqueous solution for 15-30 seconds then washed with distilled water. After that, samples were dehydrated through an ethanol series from 70% ethanol for three minutes, to 90% ethanol for three minutes and to 100% ethanol for two minutes, then soaked in Histoclear (Agar scientific) two times for three and four minutes. Histoclear was evaporated by placing slides in a fume cupboard for several hours. DPX was used as mounting agent carefully covered by a cover glass to avoid bubble formation. Slides were observed under a light microscope (ZEISS Axioskop) and photographed by AxioCam MRc5.

2.3.4 Scanning electron microscopy (SEM)

Samples were dehydrated through a 70%-95%-100% alcohol series then transferred to 100% acetone in two times. Next, samples were soaked in liquid CO_2, then temperature and pressure were set to reach the critical point of CO_2 (approximately 1000 psi and 30º C). After that, samples were dissected and arranged on aluminium stubs and sputter coated with platinum by Emitech K575X Sputter Coater Machine. Later, coated samples were observed with LEO Supra 55VP Scanning Electron Microscope.

2.3.5 Micro-computed tomography (µCT)

Spirited young inflorescence, flower bud or anthetic flower (Table 2) stored in 70% ethanol were soaked in a contrasting solution of 1% phosphotungstic acid (PTA) dissolved in 70% ethanol for at least two weeks (Staedler et al., 2013). The contrasting solution was changed every two days. Before scanning, delicate or rare samples were transferred into plastic tubes filled with an ethanol solution. More tough samples were, dehydrated in serial concentration of ethanol solution (70%, 85%, 96% and 99%) and

100% acetone solution containing 1% PTA, critical point dried, then put on sample holders. Prepared samples were scanned in the SkyScan 1272 Micro-CT ultrahigh resolution desktop 3D scanner (Bruker Co., Billerica, MA, US) with Hamamatsu L11871 20 X-ray source (Hamamatsu Photonics, Japan) following the settings in the table 2. Images were captured with an xiRAY16 camera (Ximea GmbH, Münster, Germany) inside the 3D scanner. Image sequences from scanning were constructed by the InstaRecon Engine v.2 (InstaRecon, Champaign, IL, USA).

Resolution, contrast, brightness and orientation of image sequences were edited by basic tools in the image processing package software Fiji (ImageJ2.0.0-rc-69/1.52p) (Schindelin et al., 2012). Image sequences were transformed into 3D objects which later visualized, manipulated and captured in the AMIRA® 5.4.1 and AVIZO® 9 (FEI Visualization Sciences, France). Transversal and longitudinal sections of samples were manually created using slice function. The surface of interesting organs was semi-automatically labelled with the histogram option in the segmentation module of the AMIRA®/AVIZO® program. Surfaces were rendered with constrain smoothing and compatify enabled in the surface generation tool.

Table 2. High resolution micro-computed tomography scan settings.

Species/ voucher/ sample	Acceleration voltage (kV)	Source current (µA)	Exposure time (ms) (averaged frame)	Pictures per sample	Camera binning	Pixel size (µm)
Ac. gentryi (Fernandez 8143) male flower	45	165	1166 (6)	627	2x2	2.5
(Fernandez 8143) female flower	45	165	755 (6)	633	2x2	4.0
Ac. oligostemon (NL Britton, EG Britton, JF Cowell 12635) male flower	45	165	1166 (6)	627	2x2	2.5
(NL Britton, EG Britton, JF Cowell 12635) female flower	45	165	1166 (6)	627	2x2	3.0

Ac. spinosus (G.L. Webster 17886) male flower	45	165	1166 (6)	627	2x2	2.0
As. comosa (Riina 1369) male flower	45	165	1166 (6)	627	2x2	2.0
(Riina 1369) female flower	45	165	1166 (6)	627	2x2	2.5
B. mamoninha (MM Arbo, AMM Carvalho, MS Ferrucci 7819) male flower	45	165	1166 (6)	627	2x2	2.5
(D.A. Folli 2127) young fruit	60	166	1114 (6)	642	2x2	6.0
B. muricatus (R.S. Moreira 13) male flower	45	165	1166 (6)	627	2x2	2.0
(D.S. Carneiro-Torres 1233 HUEFS 206088) female flower	45	165	1166 (6)	627	2x2	2.5
C. alabamensis (A. Wilkins) male flower	45	165	755 (6)	633	2x2	3.5
(A. Wilkins) female flower	45	165	1166 (6)	627	2x2	2.5
C. chilensis (20090241A) male flower	45	165	755 (6)	1200	2x2	3.5
(20090241A) female flower	45	165	1166 (6)	627	2x2	2.5
C. floribundus (R.F. Santos, M.B.R. Caruzo & K.B. Gagliardi 03) male flower	45	165	1166 (6)	627	2x2	2.0
(R.F. Santos, M.B.R. Caruzo & K.B. Gagliardi 03) female flower	45	165	1166 (6)	627	2x2	3.0

C. maestrensis (Jorge Guiferrez, Paul E Berry, B van Ee, B Jestrow (HAJB 81958)) male flower	45	165	1166 (6)	627	2x2	2.5
C. polyandrus (M.B.R. Caruzo, R.F. Santos, A.A.C. Sousa & L. Daneu 203) male flower	45	165	1166 (6)	627	2x2	2.0
(M.B.R. Caruzo, R.F. Santos, A.A.C. Sousa & L. Daneu 203) female flower	45	165	1166 (6)	627	2x2	3.0
C. sampatik (Vicentini, A. & Pereira, E 735) male flower	45	165	1166 (6)	627	2x2	2.0
Sag. racemosa (Bruijn 1461) young male inflorescence	45	165	755 (6)	633	2x2	3.5
(Jansen-Jacobs, M.J. et al. 6157) male flower	45	165	1166 (6)	627	2x2	2.5
(Jansen-Jacobs, M.J. et al. 6127) female flower	45	165	755 (6)	633	2x2	4.0
San. guyanensis (Unknown 7774) male flower	45	165	1166 (6)	627	2x2	2.5
(Unknown 2731) female flower	45	165	755 (6)	633	2x2	3.5

2.3.6 Phylogenetic reconstruction and analysis

Sequences of the nuclear ribosomal internal transcribed spacer (ITS1-5.8s nrDNA-ITS2) and the chloroplast *trnL-F* spacer and *trnL* intron (*trnL-F*) were selected due to good available data covering all genera of the Crotoneae (especially two species

of *Brasiliocroton*) and all clades of neotropical *Croton* to reconstruct a phylogenetic tree. Sequences from 81 representative species of all genera in the tribe Jatropheae (external outgroup), tribe Crotoneae (internal outgroup) and *Croton* species were download from Genbank (Sayers et al., 2020) (appendix 1). Most of sequences were previously included in phylogenetic publications including *Croton* and related genera (Soontornchainaksaeng et al., 2003; Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2011, 2015; Riina et al., 2014; Haber et al., 2017; Silva et al., 2020; van Welzen et al., 2020). Sequences of *C. rusbyi* were provided by R. Riina (unpublished data).

Sequence alignment of both regions was conducted in Mafft 7 (Multiple Alignment using Fast Fourier Transform) online version with Q-INS-i algorithm considering secondary structure of RNA (Katoh et al., 2019). Other settings were set as default. After that, aligned sequences were manually checked in the AliView 1.26 (Larsson, 2014). The fittest substitution model of each region was chosen in jModeltest 2.1.10 (Darriba et al., 2012) on the CIPRES server (Miller et al., 2010) by choosing with highest Akaike information model (AIC) score. The fittest model for the ITS region is SYM+I+ G, while for *trnL-F* is GTR+G. Sequences from both regions were combined into a single file but further analyses will treat them as two separate partitions. Phylogenetic trees were constructed from aligned sequences with Markov chain Monte Carlo (MCMC) algorithm using MrBayes 3.2.7a programme (Ronquist et al., 2012) on the CIPRES server. Two runs of 10,000,000 generations MCMC analyses were conducted with three heated chain and one cold chain (nchain = 4). Generated trees were sampled every 1,000 generations with 25% of sampled trees were discarded as burn-in. A 50% majority rule consensus tree was generated from the retained trees with posterior probability value as branch support value. Tree visualization was done in Mesquite 3.61 (Maddison and Maddison, 2019). Ancestral state character reconstruction was conducted in Mesquite 3.61 with parsimony (unordered) and likelihood (MK1-1 model) criteria. Character states is shown in matrix (appendix 2).

2.3.7 Figure and diagram construction

Diagrams were drawn in Illustrator® 2019 (Adobe Inc.) Figure plates preparation and adjustment of brightness and contrast were conducted in Photoshop® cc 2019 (Adobe Inc.).

2.4 Results

2.4.1 *Sagotia*

Inflorescence morphology

Two species of *Sagotia*, *i.e.*, *Sag. racemosa* (male and female flowers) and *Sag. brachysepala* (male flowers), were investigated (Table 1). Inflorescences of *Sagotia* are produced apically of the branch and stem (Fig. 3A). In some specimens, several inflorescences cluster together. Inflorescences are generally bisexual but unisexual inflorescences sometimes also occur (Fig. 1B). Flowers are borne helically along the inflorescence axis with female flowers on the proximal part and male flowers on the distal part ending with a terminal male flower so that the inflorescence could be described as botryoid (determinated raceme) (Fig. 1H, I; 3A-E). Cymules also occurred in some inflorescences which could be described as determinate thyrses (Fig. 3F). Flowers from both sexes are subtended by a bract and two bracteoles that fall off during herbarium preparation but could be observed as scars (Fig. 3 A, B, E, G, H). Bracts are located at the attachment of petiole and the main inflorescence axis; however, bracteoles were borne on a pedicel showing recaulescent growth (Fig. 3 A, B, E, G, H). In female flowers, pedicels below the bracteoles are longer than pedicels above the bracteoles, contrary to male flowers (Fig. 3A, G). Bracteoles usually fall off in mature flowers (Fig. 3A, H). All inflorescence and floral parts are covered with simple trichomes (Fig. 3I).

Figure 3. Inflorescence morphology of *Sagotia*. (A-D, F, I) *Sag. racemosa*; (E, G, H) *Sag. brachysepala*. (A) A bisexual inflorescence with female flowers on the proximal part and male flowers on the distal part. Bracteoles are presence on some pedicels. Note, a terminal male flower is mature before lower male flowers. (Use of image courtesy of the Royal Botanic Garden Kew, UK (M.J. Jansen-Jacobs, B.J.H. ter Welle, P.P. Haripersaud, O. Muller, M. van der Zee 6157; K000648324)) (B) Micro-computed tomography image of a young male inflorescence with a terminal male flower. Note pedicel joints are presence in some flowers (arrowhead).

Figure 3 continued. (C, D) Successive micro-computed tomography images of transverse sections of the inflorescence. Flowers are arranged in helical order ending with a terminal flower. (E) The tip of a young inflorescence with a terminal male flower. (F) A cluster of several male inflorescences with presence of cymes. (Use of image courtesy of the Royal Botanic Garden Kew, UK (http://specimens.kew.org/herbarium/K001210057). (G) A bisexual inflorescence. The length of the pedicel below the bracteole in female flowers is longer than in male flowers (arrowhead indicates bracteole position). (H) Several male inflorescences. In younger inflorescence, bract and bracteoles of each flower are visible while they are likely to fall off in older inflorescences. (I) Simple trichomes present in a pedicel. Tf, terminal flower; Yi, younger inflorescence; Oi, older inflorescence. Scale bars: (A, F-H) = 1 cm; (B-D) = 500 μm; (E) = 1,000 μm; (I) = 50 μm.

Male floral morphology

Male floral morphology was observed in two species of *Sagotia* (Table 1). The perianth in male flowers is pentamerous. The perianth consists of two whorls, i.e., a calyx with green colour and corolla with initially white colour that changes to red after the anthesis (Fig. 1F, 4A). There are five sepals fused at the base with imbricate aestivation (Fig. 4B, C). The abaxial surface of sepals is covered by dense simple trichomes on the lower part (Fig. 4B), while adaxial surfaces are mostly glabrous with few sparsely distributed trichomes (Fig. 4D). There are ciliate simple trichomes on the margin (Fig. 4B-D). The corolla consists of five imbricate petals (Fig. 4E, F), but extra petals are occasionally found. On the abaxial surface, there are simple trichomes present on the lower part (Fig. 4C, G) while the adaxial surface is glabrous (Fig. 4H). Few simple trichomes are present on the upper margin but become denser at the lower part (Fig. 4H). Petals are bigger than sepals in mature flowers (Fig. 4A, C). There is no nectary in male flowers. There are about 45 stamens inserted on a slightly concave receptacle (Fig. 4I-K). The outermost stamens are arranged opposite to sepals (Fig. 4J, K). Filaments are extremely short making anthers looks sessile on receptacle (Fig. 4L, M). Pollens are inaperturate with croton-pattern surface (Fig. 4N).

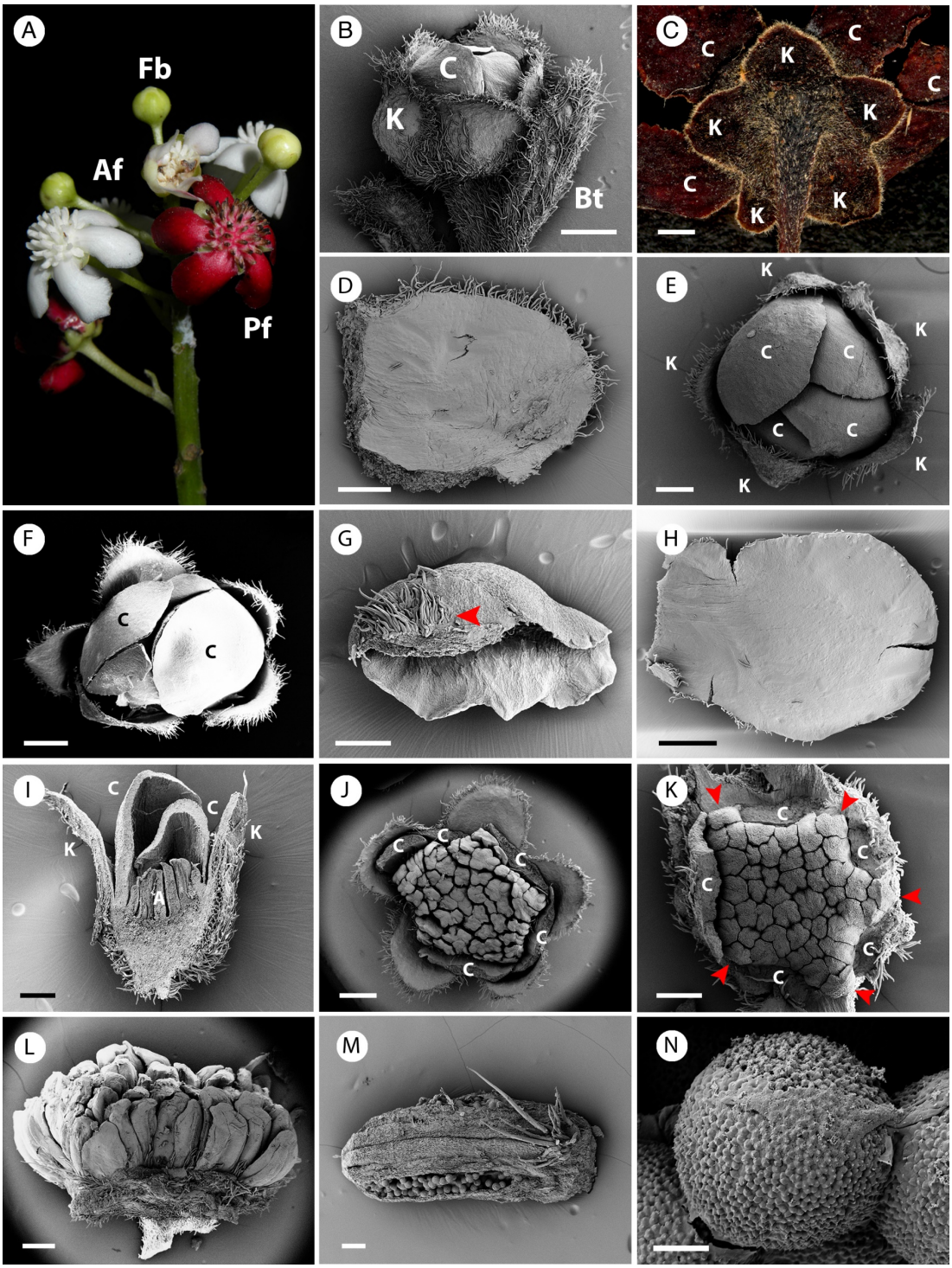

Figure 4. Morphological structure of male flowers in *Sagotia*. (A, B, D, F-J) *Sag racemosa*; (C, E, K, M) *Sag brachysepala*. (A) A male inflorescence with some flowers in bud and some mature flowers. In pre-anthesis and anthesis stage, petals are white then change to red at post anthesis. (Use of image courtesy of Reinaldo Aguilar) (B) A young male flower subtended by two bracteoles. All structures are covered with simple trichomes.

Figure 4 continued. (C) Lower view of a flower at anthesis. There are five sepals visible which are covered with simple trichomes on the abaxial side. The lower part of petals is covered with simple trichomes as well. (D) Adaxial side of a sepal. The margin is fimbriate. (E) A closed flower with cochlear aestivation corolla. (F) Flower in bud showing corolla with quincuncial aestivation. (G) Lower part of the abaxial side of a petal. There is a tuft of simple trichomes visible (arrowhead). (H) Adaxial side of a petal. The margin is fimbriate but trichomes are much less dense than in sepals. (I) Longitudinal section of half flower shows slightly concave receptacle which may be the result of pressing. (J) A flower near anthesis with petals removed. Stamens are arranged in a star shape. (K) A flower bud with perianth removed. The stamens are erect. The outermost stamens are alternate with petals (opposite sepals) (arrowhead). (L) Lateral view of dissected flower showing anthers sessile on the pilose receptacle. (M) Adaxial view of sessile stamen. There is a tuft of simple trichomes at the stamen base. (N) Inaperturate pollen with croton-pattern. Fb, flower bud; Af, anthetic flower, Pf, post-anthetic flower, Bt, bracteole; K, sepal; C, petal; A, stamen. Scale bars: (B, F, H, J, L) = 500 μm; (C) = 1,000 μm; (D, E) = 300 μm; (G, I, K) = 200 μm; (M) = 100 μm.

Male floral vasculature and anatomy

A male flower of *Sagotia* was included in the μCT procedure (Fig. 5A). One of two bracteoles with one vascular bundle was attached to the pedicel (Fig. 5B). In the pedicel and also the base of the flower, vascular bundles are ring-shaped (Fig. 5B, C). Five outermost bundles extend to supply sepals and are branched into several veins (Fig. 5D). Five sepals are fused at the base (Fig. 5D-G). IN the upper part of the receptacle, five bundles alternating with sepal bundles extend to supply petals (Fig. 5E, F). The rest of vascular bundles are arranged in a star shape, which divides to supply stamens (Fig. 5F, G). Five outermost stamens are arranged alternate with petals (Fig. 5F-J). Vascular bundles in the outer whorl branches to supply two stamens (Fig. 5H-J). Stamen arrangements are chaotic except for the outermost whorl (Fig. 5I-J). In the upper part of the petal, a vascular trace branches into several veins (Fig. 5H-J). There are 53 stamens in this flower with tetrasporangiate dithecous anther (Fig. 5J). There are several pairs of stamens that are fused together at the base (Fig. 5J). Stamens are inserted on a flat receptacle (Fig. 5K). There is no nectary in male flowers of *Sagotia* (Fig. 5L).

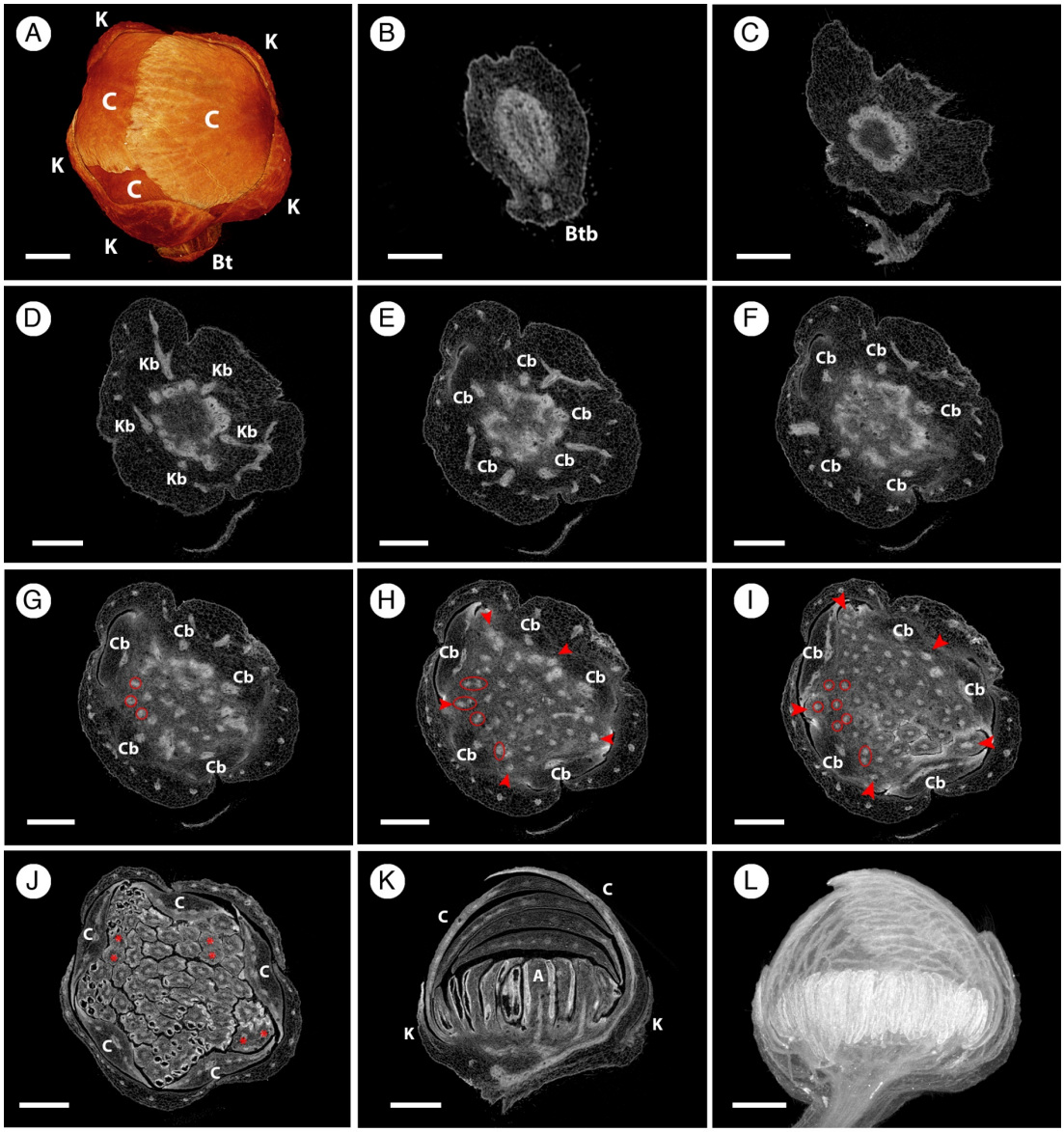

Figure 5. Micro-computed tomography images of a male flower of *Sagotia racemosa*. (A) The top view of a flower bud. (B) Transverse section of the pedicel shows ring-like vasculature with a bundle departing to a bracteole. (C) At the base of the flower vascular bundles are arranged in a ring and the outline of the calyx becomes visible. (D) Sepal traces extend from the central ring and branch into several veins. (E) Petal traces start to separate from the central ring. (F) Five distinct petal traces are visible. The central ring forms a star shape with each angle arranged alternating with petal traces. (G) The central ring disintegrates into stamen vascular traces. There is no distinct whorl of stamens, except for outermost stamens. Circles indicate the start of branching bundles. (H) Some single stamen traces divide radially to supply two separate stamens (circles). (I) Upper part of the receptacle showing the outline of individual stamens. Scale bars: (A-L) = 500µm.

Figure 5 continued. The single outermost stamens alternating with petals are clearly visible (arrowhead). Cleavages of vascular bundles are indicated with circles. (J) Transverse section in the middle of anthers. Stamens are arranged in a star shape with the outermost stamens opposite to sepals. Some stamens are confluent at the base (asterisk). (K) Longitudinal section of the flower shows flat receptacle, (L). There is no trace of a nectary in the flower. Bt, Bracteole; K, sepal; C, petal; Btb, bracteole bundle; Kb, sepal bundle; Cb, petal bundle; A, stamen.

Female floral morphology

Only female flowers of *Sag. racemosa* were studied. Female flowers are polysymmetric with one whorl of perianth (Fig. 6A). The outermost whorl of female flowers is the calyx with five free green sepals (Fig. 6A, B, C). The abaxial surface is covered by simple trichomes (Fig. 6B, D) while the adaxial surface is glabrous (Fig. 6C, E, F). There are simple trichomes present at the margin (Fig. 6D, E)). Minute glandular structures are also present along the sepal margin (Fig. 6G) which after anatomical examination were found to be colleters (Fig. 6H). The corolla is absent. In the centre of the flower there is an ovary consisting of three carpels (Fig. 6A, F, I). The outer ovary surface is densely covered by simple trichomes (Fig. 6A, C, I, J). There are three styles with bifid branching on top of the ovary (Fig. 6J, K). Young styles are white but later change to red after anthesis. (Fig. 1G, 6A).

Female floral vasculature and anatomy

The flower specimen we observed is flattened from the herbarium preparation and some of the sepals were removed for SEM observation (Fig. 7A). Transverse section at the base of flower found that the vasculature forms a ring (Fig. 7B). Each sepal is supplied by a bundle (Fig. 7C) which later branches into several veins (Fig. 7D, E). A bright ring was observed around the base of the ovary from high accumulation of tungsten suggesting a high cellular density (Fig. 7F-H). Thin section slides reveal the structure to be a nectary ring (Fig. 7I, J). There is no vascular supply to the nectary ring. It is also found that the ovary is embedded in the receptacle (Fig. 7I). Simple trichomes on the ovary surface are found to be at the same level with epidermis (Fig. 7K). We could not observe vasculature in the ovary due to damage

during herbarium preparation. On the upper part of the ovary, there are three styles fused together at the base (Fig. 7L). Each style branches into two stigmas each, supplied by a single trace (Fig. 7M).

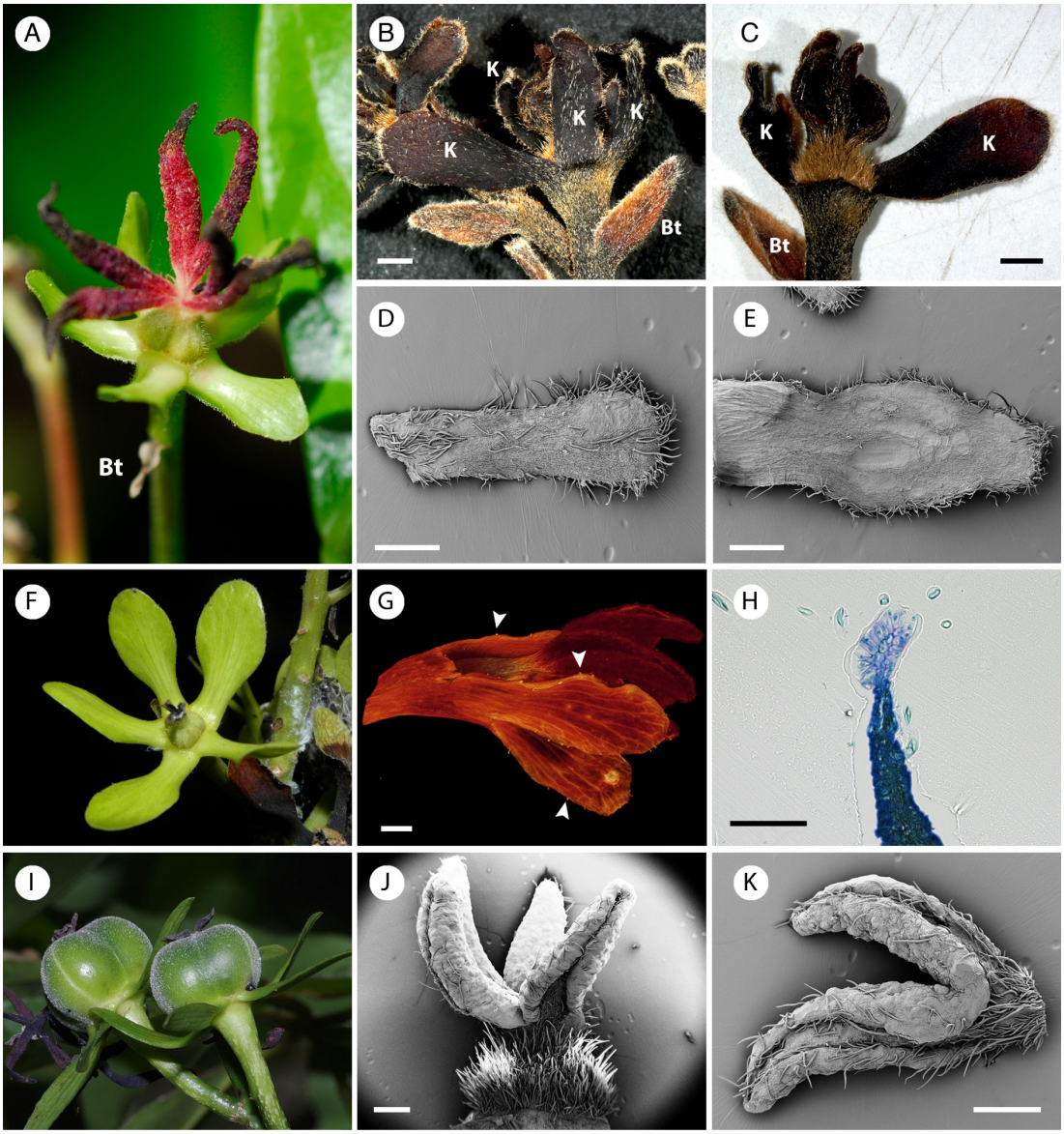

Figure 6. Morphological structures of female flowers in *Sagotia racemosa*. (A) A slightly post anthesis flower with red stigma. A ring of hairs is visible IN the space between sepals and the ovary. (Photographed by Paul E. Berry. Use of image courtesy of Ricarda Riina) (B) A flower extracted from the herbarium. One bracteole is visible densely covered with simple trichomes. (C) The same flower as in B but some sepals are removed. The ovary is densely covered with yellowish simple trichomes. (D) Abaxial side of a sepal. It is sparsely covered with simple trichomes. The margin is lined with simple trichomes. (E) Adaxial side of a sepal with glabrous surface. (F) A young fruit with detached stigma. (Use of image courtesy of Reinaldo Aguilar) (G) Micro-computed tomography image showing several bright granular structures on the sepal margin.

Figure 6 continued. (H) A resin thin section reveals those granular structures are mucilage secreting structures (colleter). (I) Young fruits show enlargement of ovary during the fruit maturation process. (Use of image courtesy of Hervé Galliffet) (J) The ovary wall is covered with simple trichomes. Two stigmas are broken off. (K) A bifid stigma. The style is densely covered with simple trichomes while there are less trichomes on the abaxial side of the stigmatic tips. Bt, bracteole; K, sepal. Scale bars: (B, C) = 1,000 µm; (D, E, G, J, K) = 500 µm; (H) = 100 µm.

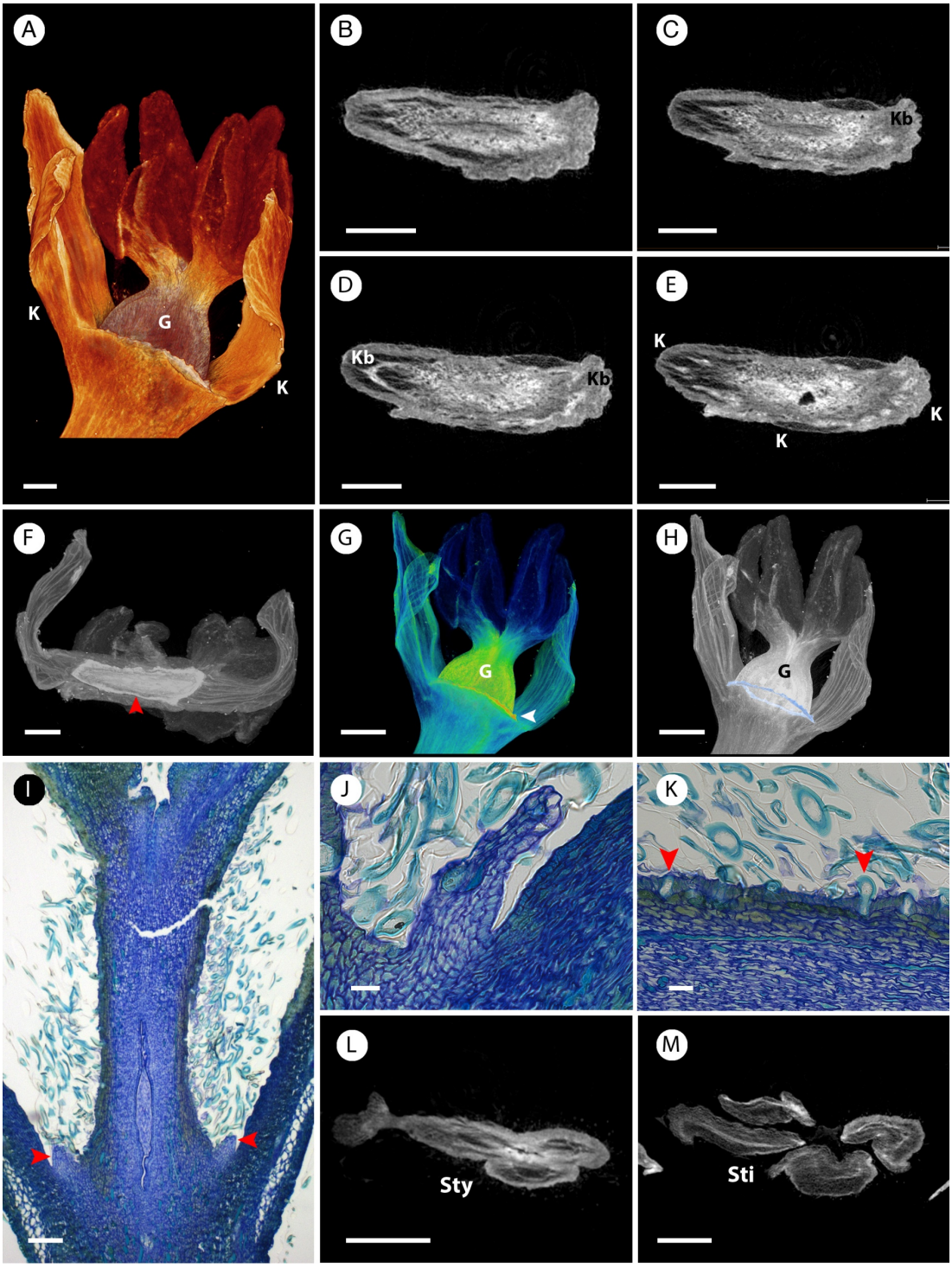

Figure 7 (previous page). Anatomical structures of a female flower of *Sagotia racemosa*. (A-H, L, M) Micro-computed tomography images; (I-K) resin thin sections. (A) Side view of a flower. Some sepals were removed. (B) View of transverse section of the flower at the pedicel. There is a ring of vascular bundles. (C) A sepal trace extends from the central stele. (D) The sepal trace start branching into veins. (E) Several veins are visible in each sepal. (F) A bright ring is visible in the space between sepals and the ovary. (G) The ring is visible as an orange structure. (H) Segmentation object view of the ring structure shows it surround the ovary base. (I) A longitudinal section image shows the ovary slightly embedded into the receptacle. The ring structure is present on the area between sepal and the ovary. (J) The ring structure is very thin and comprises secreting cells. It is also covered with trichomes. (K) The wall of the ovary is covered with simple trichomes. The bases of trichomes are embedded in the epidermis. (L) The area above the ovary shows three styles branching from a common column. (M) Each style then branching into bifid stigma. K, sepal; G, pistil; Kb, sepal's bundle; Sty, style; Sti, stigma. Scale bars:(A-F, L, M) = 500µm; (G, H) = 1,000 µm; (I) =100µm; (J, K) = 20µm.

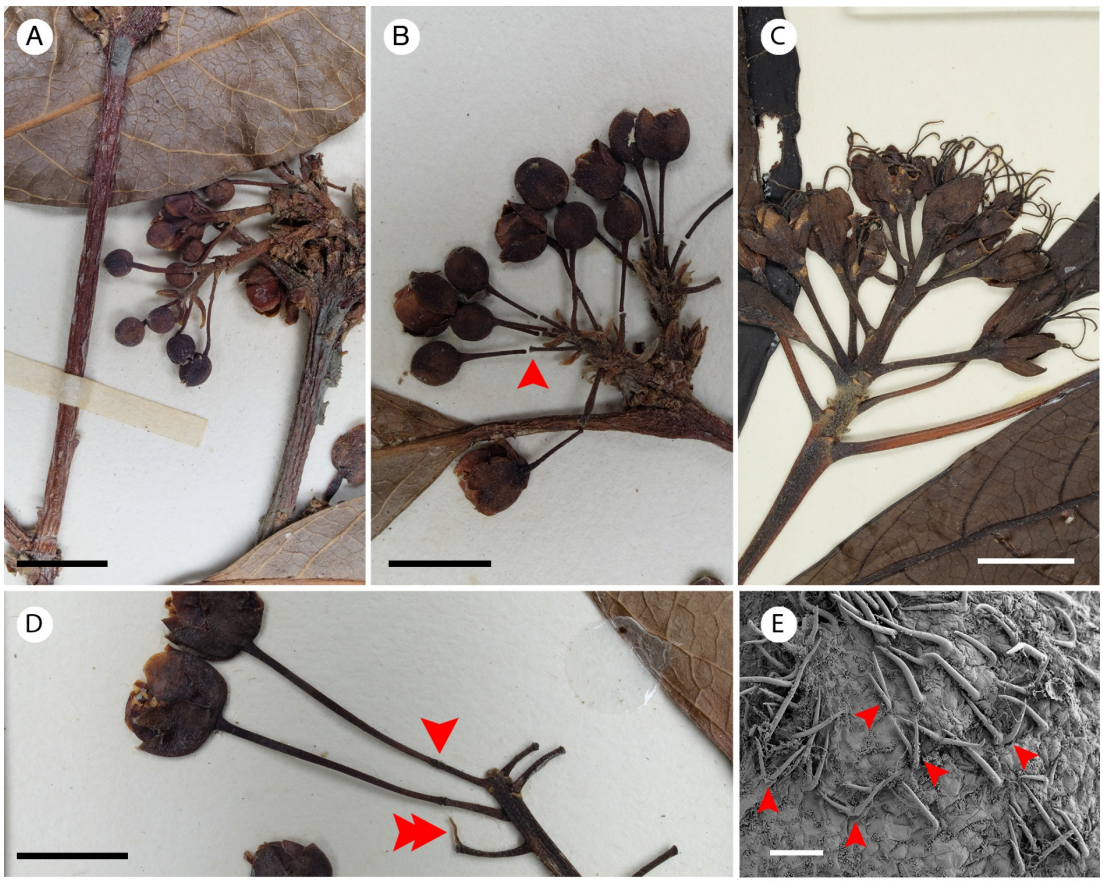

Figure 8. Inflorescence structures in *Sandwithia guyanensis*. (A) A cluster of male inflorescences borne on the old inflorescences from the previous year. The abscission zone on the pedicels are visible. (B) In the herbarium, flowers are likely to break at the abscission zone. (C) Male flower with visible joint on the pedicels (arrowhead). There is a bracteole present on the broken pedicel (double arrowhead). (D) There is no visible joint on the pedicels of female flowers. A bract is visible subtended some pedicels. (E) Simple trichomes are present in sepals. Many of them are arrange in pairs (arrowhead). Scale bars: (A, B, D) = 5 mm; (C) = 1 cm; (E) = 90 µm.

2.4.2 *Sandwithia*

Inflorescence morphology

Both male and female flowers from the monotypic *Sandwithia guyanensis* were included in this study (Table 1). The genus has unisexual inflorescences (Fig. 8A-C). Male inflorescences are of the botryoid type that have grown on the old remnants of the previous year inflorescences (Fig. 1H, I; 8A). The petiole of each male flower has recaulescent growth with an uplifting of two bracteoles while a bract is located at the base similar to *Sagotia* (Fig. 8A, B, D). Female flowers are borne on botryoids on normal pedicels subtended by a bract (observed as a scar, Fig. 8C). We could not observe any trace of bracteoles. The general indumentum consists of simple trichomes commonly found in pairs (Fig. 8E).

Male floral morphology

In male flowers, three to four sepals are fused together in bud which are later split open (Fig. 9A-C). The abaxial surface is sparsely covered with simple trichomes which become denser at the apex (Fig. 9B, D-F), while the adaxial surface is glabrous (Fig. 9E). The corolla comprises three or four petals (Fig. 9C, G). Petals are generally glabrous on both surfaces with a ciliate margin of simple trichomes (Fig. 9G-I). Inside the corolla whorl there are three nectary lobes, each covered with some simple trichomes (Fig. 9J, K). There are about 30 stamens in male flowers (Fig. 9L). Their filaments are long and twisted in bud (Fig. 9M). Simple trichomes are present on the receptacle and the filaments (Fig. 9M). Pollen is inaperturate with ektexine following the *Croton* pattern (Fig. 9N).

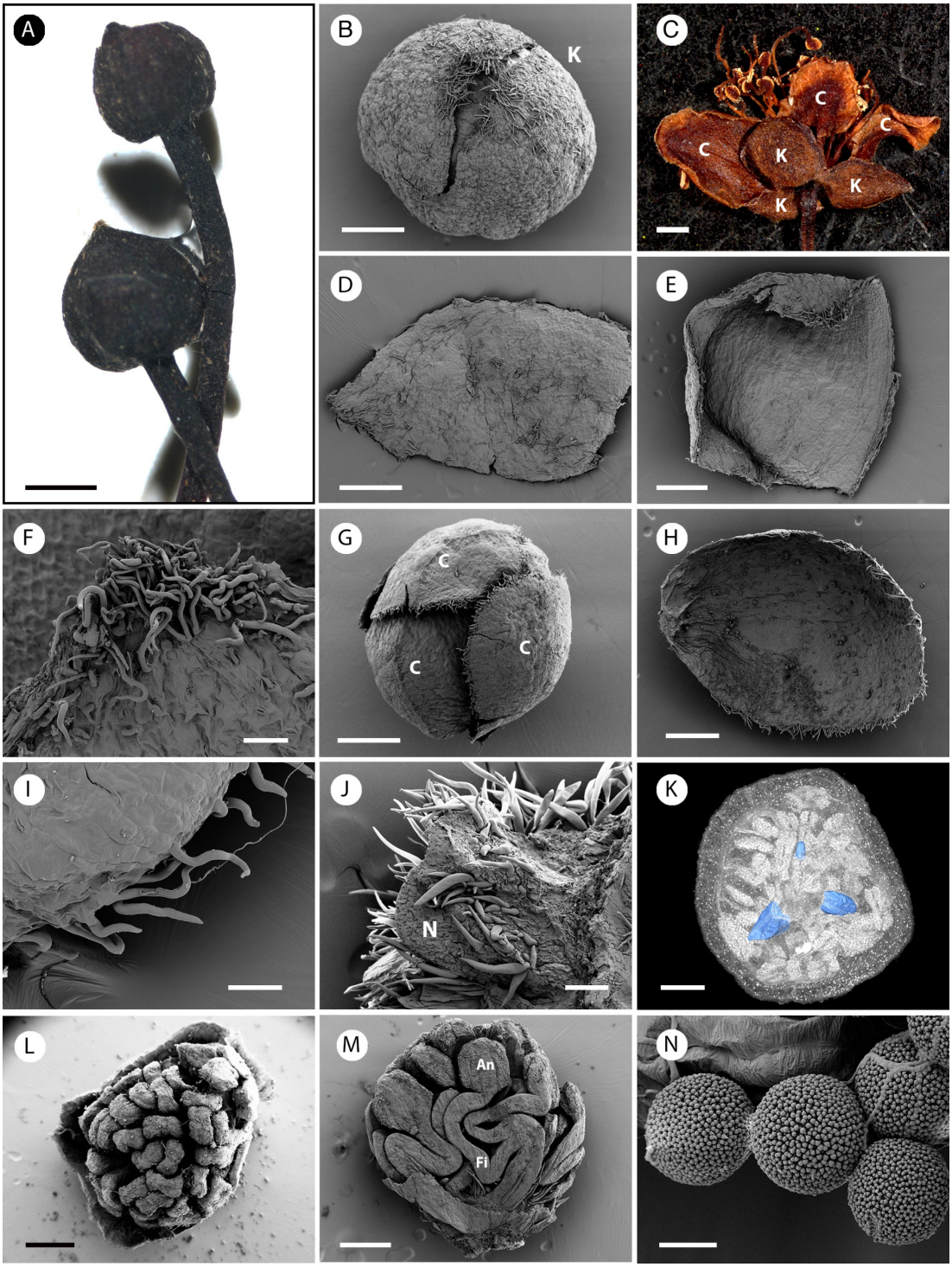

Figure 9. Morphological structures of male flowers of *Sandwithia guyanensis*. (A) Male flowers extracted from herbarium. Sepals are fused together into a calyptra-like hood. (B) The sepal hood breaks apart during the blooming process. (C) A fully open flower with three sepal lobes visible. (D) Abaxial surface of a sepal. There are some simple trichomes present on the surface. (E) Adaxial surface of a sepal with glabrous surface. There are many trichomes present at the tip on the abaxial surface. (F) The apex of the sepal is densely covered with simple trichomes. (G) A flower bud with sepals removed.

Figure 9 continued. The aestivation of the corolla is quincuncial. The abaxial surface is glabrous but the margin is lined with simple trichomes. (H) Adaxial surface of a petal with glabrous surface and fimbriate margin. (I) The margin of a petal with lining of simple trichomes. (J) A nectary with some simple trichomes present. (K) Three nectary glands are revealed by segmentation of a micro-compute tomography image. They are arranged alternate with petals. (L) Top view of stamens with petals removed. (M) Longisection of the stamen and receptacle. Stamens are twisted inside the bud on the pilose receptacle. (N) Inaperturate pollen grains inside the anther with croton-pattern surface. K, sepal; C, peta; N, nectary; Fi, filament; An, anther. Scale bars: (A, C) = 1,000 μm; (B, D, E, G, H, K-M) = 500 μm; (F) = 90 μm; (I) = 40 μm; (J) = 100 μm; (N) = 20 μm.

Male floral vasculature and anatomy

Several male flower buds of *Sandwithia* were included in the μCT and thin section examination. Sepals are fused together at the apex (Fig. 10A). Transverse sections of the pedicel found that vasculature is ring-shape (Fig. 10B). At the base of the flower, three bundles extend to supply three sepals (Fig. 10C). Three inner bundles alternating with sepal bundles extend to supply three petals (Fig. 10C-G). Three nectary glands of unequal size (two big, one small) are arranged opposite to sepals without vasculature supply (Fig. 10C-G). The rest of vascular bundles are in a triangular shape with each angle opposite a petal (Fig. 10F, G). However, the bundles opposite sepals are the first to branch out (Fig. 10H). There are two stamens present opposite each of two big nectaries (Fig. 10F-J), while there is one stamen opposite the small nectary (Fig. 10G-J). The remaining bundles extend to supply the rest of the stamens (Fig. 10I-J). There are more than 20 stamens in a male flower (Fig. 10L-M). Filaments are long, folded and twisted inside the flower bud (Fig. 10J-L). A pattern of stamen whorls could not be observed but there are three stamens at the central of the flower (Fig. 10M). Sections in the upper part of the flower bud found that anthers are tetrasporangaiate dithecal (Fig. 10L-N). Stamens are borne on the convex receptacle (Fig. 10O).

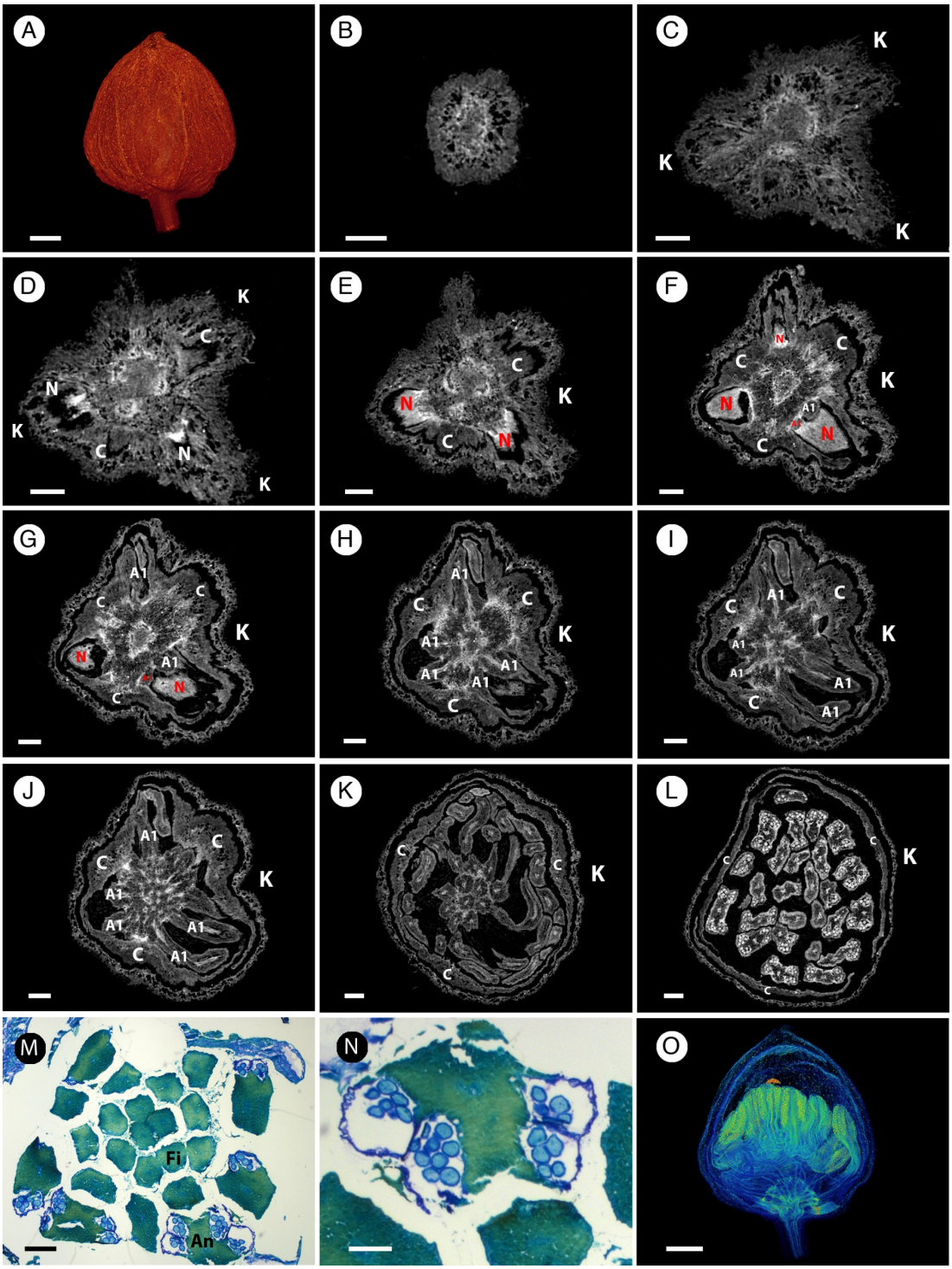

Figure 10. Anatomical structures in male flowers of *Sandwithia guyanensis*. (A-L, O) Micro-computed tomography images; (M, N) resin thin sections. (A) A full flower with fused sepals. (B) The pedicel of a flower; vascular bundles are confluent in a ring. (C) Three sepals are present in the flower. (D) Petals arranged alternating with sepals, while nectaries are opposite to sepals. (E) two bases of petals are visible. Vasculature forms a triangular shape. (F) Three bundles extend to supply three petals. One of them branches into three veins. A pair of stamens appears opposite a nectary.

Figure 10 continued. (G) Three petals are visible. The floral stele is in a triangular shape with each angle pointing toward a petal. (H) Five outermost stamens are visible. Two pairs of stamens are situated opposite two big nectaries. There is a single stamen opposite the smaller nectary. (I) Bundles in floral stele extend to supply the rest of the stamens. (J) There is no whorled pattern present in the bundle. (K) Stamens have long filaments that are twisted in bud. Three imbricate petals are visible. (L) The upper part of a stamen at the anther level. The arrangement of stamens is chaotic due to twisting nature of the filaments. (M) The central stamens are arranged in a triplet. The filaments accumulate pigment; therefore, they appear dark in the sectioning. (N) The anthers are tetrasporangiate dithecous. (O) Stamens fold and twist inside a flower and inserted on the convex receptacle. K, sepal; C, petal; N, nectary; A1, first stamen whorl; Fi, filament; An, anther. Scale bars: (A-M) = 200 µm; (N) =100 µm; (O) = 500 µm.

Female floral morphology

In female flowers, there are five sepals fused together varying from one third to two third of their length (Fig. 11A). The abaxial surface is sparsely covered by simple trichomes with ciliate margin (Fig. 11B). The adaxial surface is glabrous (Fig. 11C). Small petals are present in the flower (Fig. 11D-F). Five nectary glands are also present and arranged alternate with petals (Fig. 11E-G). The *Sandwithia* gynoecium consists of a tricarpellate ovary (Fig. 11 D-G). The outer surface is densely covered by simple trichomes (Fig. 11D, F, H). There are three long styles on a common stalk on top of an ovary with bifid stigmas (Fig. 11A, D, E, I).

Female floral vasculature and anatomy

The samples we examined are polysymmetric. The sepals are fused together for about one third of their length (Fig. 12A). There are four sepals, but one sepal was removed for SEM observation. In the pedicel, vasculature forms a ring (Fig. 12B). At the base of the flower, four outer bundles extend to supply sepals (Fig. 12C). Next, several bundles extend to petals alternating with sepals and nectaries (Fig. 12D, E). There are five nectary lobes surrounding the base of the ovary (Fig. 12E-G). There is no vascular supply the nectary (Fig. 12F). Six petals with variable size are visible (Fig. 12G, H). The base of the ovary is triangular shaped (Fig. 12H). The ovary consists of three carpels with one ovule in each carpel (Fig. 12I, J). The ovule is anatropous with two integuments (Fig.12I). There is a part of the nucellus that elongates beyond

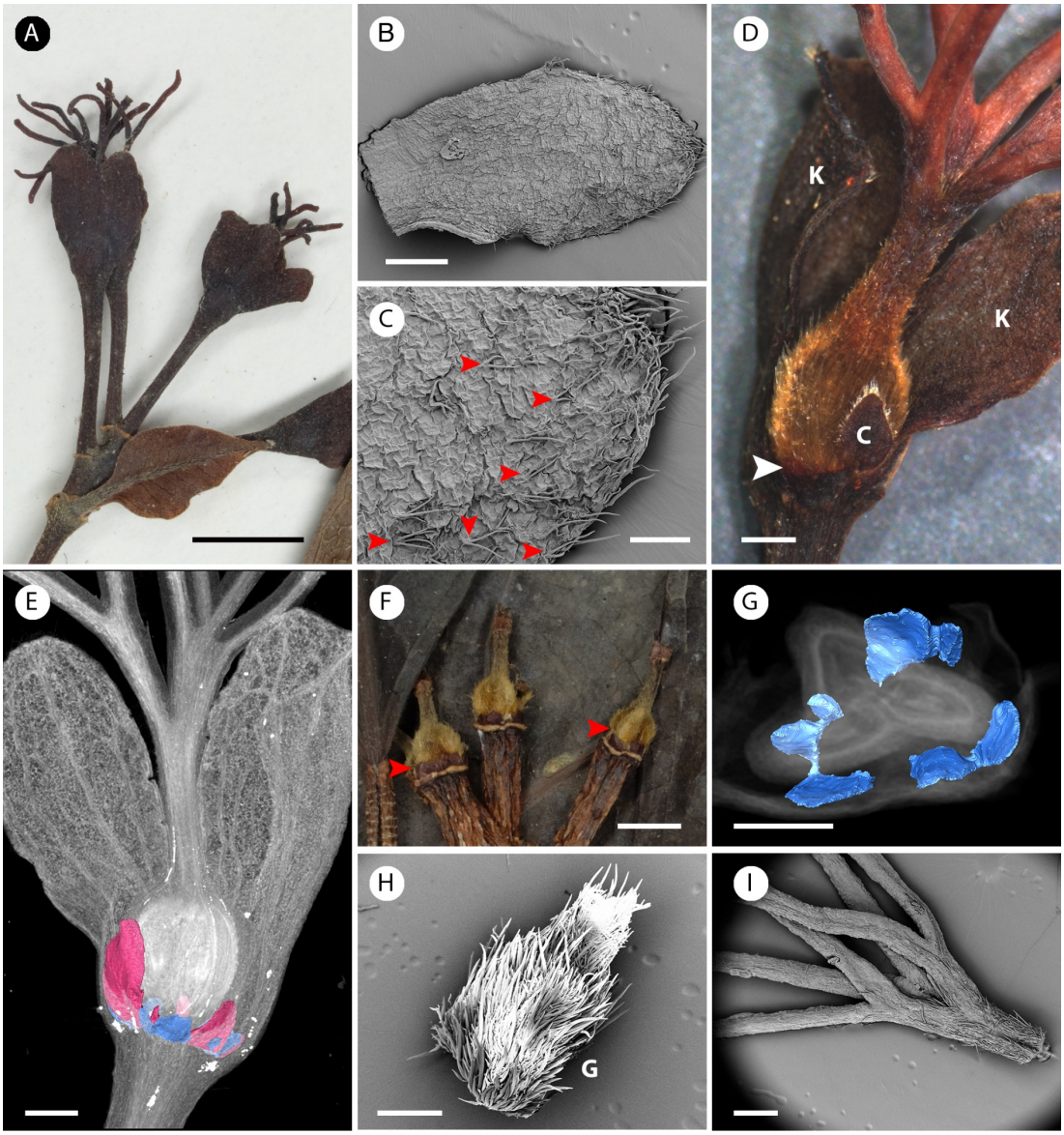

Figure 11. Morphological structures of female flowers of *Sandwithia guyanensis*. (A) Female flowers with sepals fused for more than 2/3 of their length. (D) A female flower with some sepals removed. A nectary lobe (arrowhead) and a small petal are visible. (B) Abaxial side of a sepal. The upper part is covered with some simple trichomes. (C) Enlarged view of C, most of simple trichomes are arranged in pairs. (E) Micro-computed tomography of a flower. Petals and nectaries were highlighted in different colours. Petals with pink and nectaries in blue. (F) Young fruits in the herbarium after sepal drop off. Nectary lobes and reduced petals are visible. (G) Nectariferous structures in the flower surrounding the ovary. (H) The ovary is covered densely with simple trichomes. (I) The stigmas are bifid (total of 6 tips). The lower part is covered with simple trichomes. K, sepal; C, petal; G, pistil. Scale bars: (A) = 5 mm; (B, D, E, G-I) = 500 µm; (C) = 200 µm; (F) = 2.5 mm.

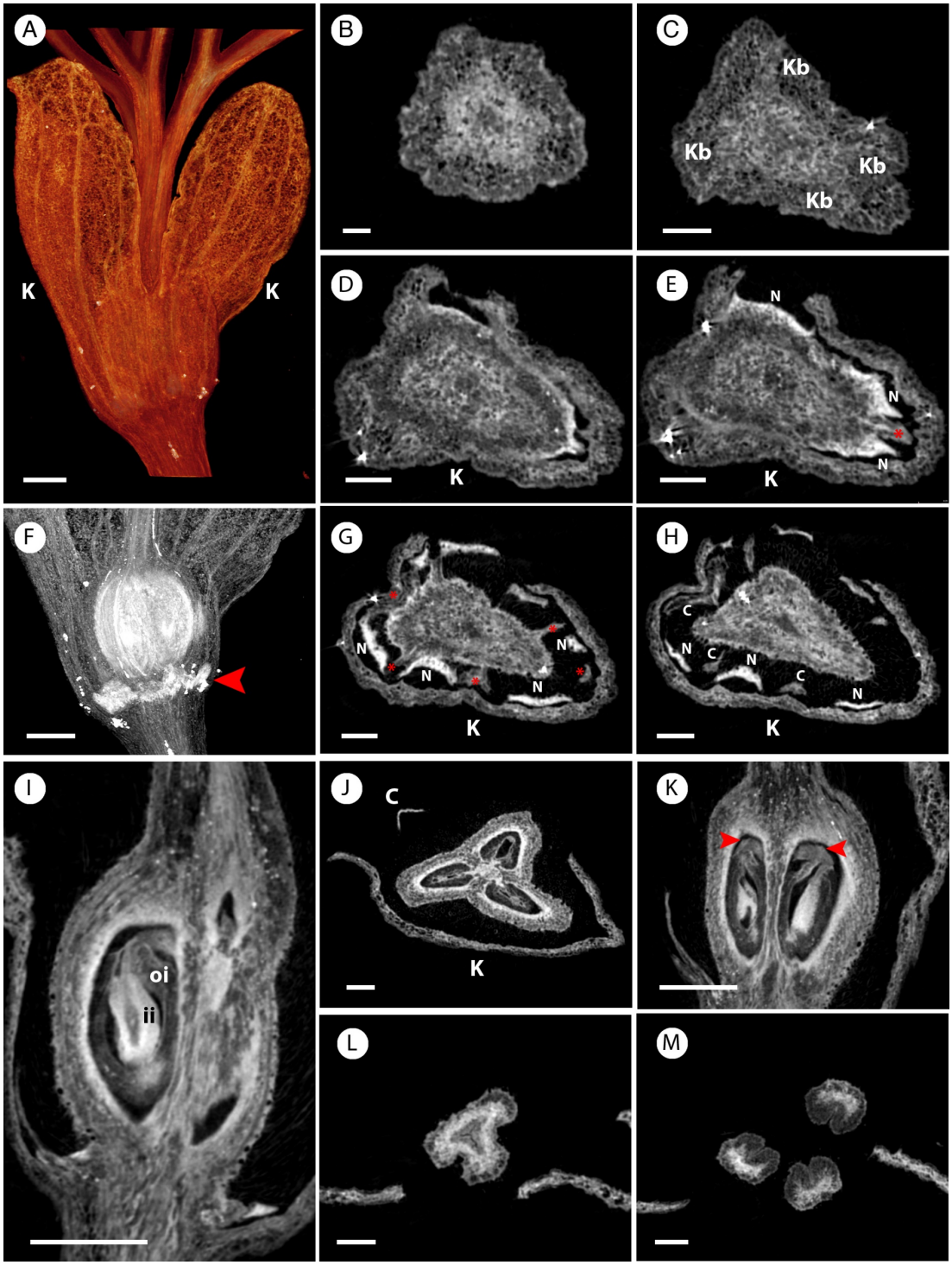

Figure 12. Micro-computed tomography images of a female flower of *Sandwithia guyanensis*. (A) side view of a flower. The sepals are fused for 1/3 of their length. (B) The pedicel of a flower with ring-like vasculature. (C) Sepal bundles start to branch out from the floral stele. (D) Sepals are separated from the other floral organs. Note nectaries starting to appear. (E) A reduced petal is supplied by a vascular bundle. The petal alternates with two zones of nectariferous tissue. (F) Nectary tissue surrounds the ovary in a flower. (G) Six nectary lobes are visible.

Figure 12 continued. (H) Five unequal-shaped petals are visible alternating with the nectary. (I) Longitudinal section of a flower. An ovule is visible with an outer integument, inner integument and nucellus with beak. (H) The placentation is axile. Fusion of sepals could be observed. (K) A longitudinal section of a female flower. Above each ovule, there is an obturator present. (L) The base of a style with three lobes. (M) Higher up the style branches into bifid stigmas. K, sepal; Kb, sepal's bundle, N, nectary, C, petal, oi, outer integument; ii, inner integument. Scale bars: (A, F, I, K) = 500 μm; (B-E, H, J, L, M) = 200 μm; (G) = 20 μm.

The micropyle (nucellar beak) (Fig. 12I). An ovule is covered by an obturator (Fig. 12I, K). The placentation is axile (Fig. 12J). On top of the ovary, three styles are fused at the base and fork higher up into bifid stigmas each supplied with a bundle (Fig. 12L, M).

2.4.3 *Acidocroton*

Inflorescence morphology

Samples included for examination are from two sections of *Acidocroton* (Section *Acidocroton – Ac. oligostemon* (male and female); *Section Ophellnatha – Ac. gentryi* (both flower sexes) and *Ac. spinosus* (male flower). In *Ac. gentryi* and *Ac. spinosus,* inflorescences are borne terminally on the stem (Fig. 13A, B) (Fig. 1 in Fernández-Alonso and Jaramillo-Mejía, 1995). Their inflorescences are bisexual of the raceme type with a basal female flower and several male flowers on the distal part (Fig. 13B-D). We could not examine bract and bracteoles of female flowers. Pedicellate male flowers are subtended by a bract and two bracteoles (Fig. 13C-D). Each of the bract and bracteoles has a trilobed shape with colleters present on the two lateral lobes (Fig. 13E, F). Flowers of *Ac. oligostemon* are borne on spiny stems (Fig. 13G). Inflorescences are bisexual clusters borne in a condensed shoot among some leaves in the axil of two spines and could occur terminally on the stem (Fig. 13H-J). Flowers are arranged following the helical pattern, which suggests that the cluster is actually a condensed raceme (Fig. 13J).

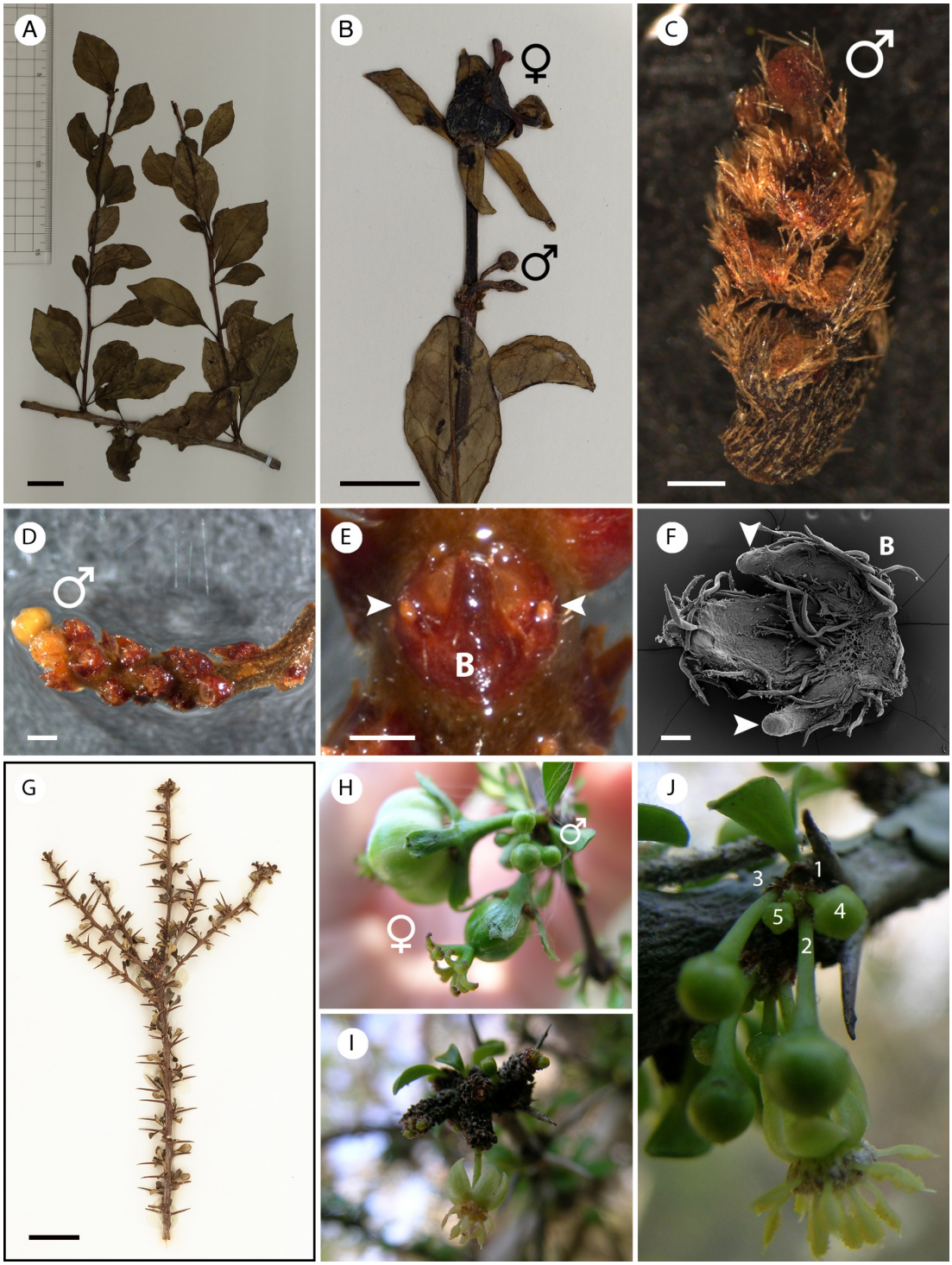

Figure 13. Morphological structure of inflorescences in the genus *Acidocroton*. (A-C) *Ac. spinosus* (Section *Ophellantha*); (D-F) *Ac. gentryi* (Section *Ophellantha*); (G-J) *Ac. oligostemon* (Section *Acidocroton*). (A) Stems of *Acidocroton* section *Ophellantha* bear leaves on the elongate branch. (B) Inflorescence of *Ac. spinosus* is borne apically. (C) Inflorescence of *Ac. spinosus* has an indeteminate raceme pattern. (D) Inflorescences of *Ac. gentryi* is an indeterminate raceme. (E) A flower is subtended by a bract and two bracteoles. (F) A bract has a hastate shape with colleters present on the lateral lamina.

Figure 13 continued. (G) Stem of *Acidocroton* section *Acidocroton* covered with pairs of spines. (Use of image courtesy of the C. V. Starr Virtual Herbarium of The New York Botanical Garden, USA (http://sweetgum.nybg.org/science/vh/specimen-details/?irn=1651025)) (H) Bisexual inflorescence. (I) Inflorescence appears to be condensed but could become elongated by producing flowers for a long time. (J) The arrangement of flowers on the inflorescence follows a helical pattern typical for a raceme. (Image H-J. Photographed by Benjamin van Ee. Use of image courtesy of Ricarda Riina) B, bract. Scale bars: (A, G) = 2 cm.; (B) = 1 cm; (C, E) = 500 µm; (D) = 1,000 µm; (F) =100 µm.

Male floral morphology (Section *Ophellantha, Ac. spinosus* & *Ac. gentryi*)

Male flowers from *Ac. spinosus* and *Ac. gentryi* are similar with a high stamen number (Fig. 14A, B). Sepals in both species are arranged in quincuncial aestivation (Fig. 14C). Both abaxial and adaxial surfaces are glabrous (Fig. 134D, E). There are simple trichomes present on the margin (Fig. 14C-F). Petals are present only in male flowers with cochlear aestivation (Fig. 14F-G). They are ovate shaped with glabrous abaxial and adaxial surfaces (Fig. 14F-H). Few simple trichomes are present basally on the adaxial side of petals (Fig. 14H). There are simple trichomes along the edge (Fig. 14G, H). Petals are bigger than sepals at the mature stage (Fig. 14A, B, F, G). A ring-like nectary comprising several lobes surround the stamens, each covered with simple pilose hairs (Fig. 14I-K). Stamen number is variable for each taxon. There are about 46 stamens in *Ac. gentryi*, while there are about 20 stamens in *Ac. spinosus* (Fig. 14L). Hanan-Alipi and Steinmann (2013) reported that stamens of species in section *Ophellantha* could be highly variable, ranging from 50-100 in *Ac. gentryi* and 16-50 in *Ac. spinosus*. One connective appendage is present in an anther of *Ac. spinosus* (Fig. 14M), while *Ac. gentryi* has two connective appendages per anther (Fig. 14N). The pollen is inaperturate with croton-pattern (Fig. 14O).

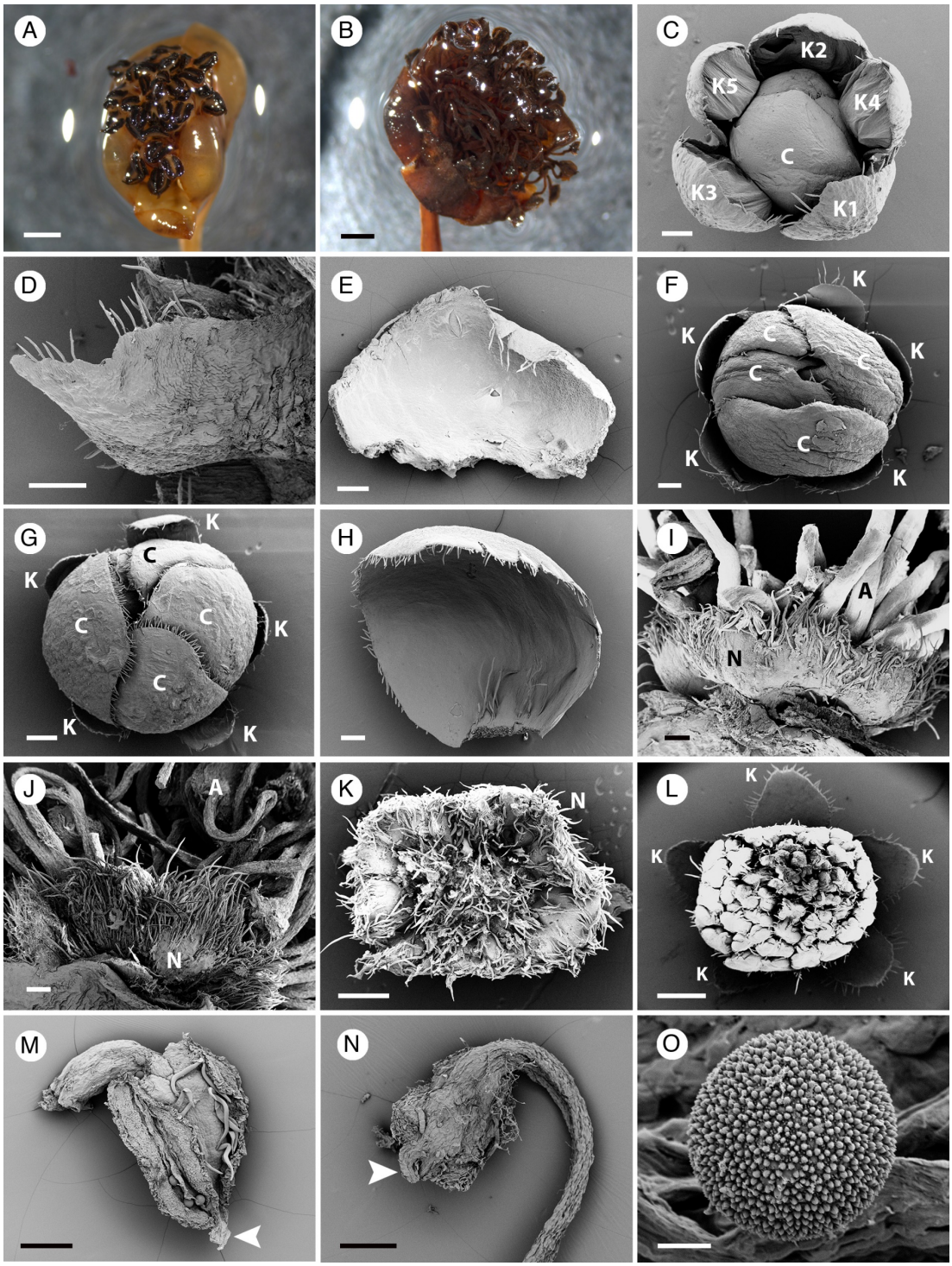

Figure 14. Morphological structures of male flowers of *Acidocroton* Section *Ophellantha*. (A, E, F, I, M, O) *Ac. spinosus*; (B-D, G, H, J, L, N) *Ac. gentryi***.** (A) A male flower of with many stamens. (B) A male flower with high stamen number. (C) A young male flower with sepals arranged in quincuncial aestivation. (D) A sepal with glabrous surface and fimbriate margin. (E) Adaxial side of a sepal. The surface is glabrous with some simple trichomes on the margin. (F) Male flower near anthesis with petals larger than sepals. The aestivation of the corolla is cochlear. (G) flower bud near anthesis with petals arranged in a cochlear aestivation. The margin of the petal is fimbriate.

Figure 14 continued. (H) Adaxial side of a petal. Most of the surface is glabrous except the lower part by the presence of few simple trichomes. (I) A flower with perianth removed. A nectary ring covered with trichomes surrounds the stamens. (J) Nectary ring formed by the fusion of many nectary lobes. (K) Nectary tissue is surrounding stamens (removed). The receptacle is pilose. (L) A flower with petals removed showing numerous stamens. Stamens erect and facing toward the centre of the flower. (M) An anther with presence of simple trichomes on the connective. Not, the presence of aconnective appendage. (N) A stamen of *Ac. gentryi* with similar morphology to the stamen in *Ac. spinosus*. Few simple trichomes are present on the connective tissue. A connective appendage is also present. (O) Inaperturate pollen with *Croton*-pattern. K, sepal; C, petal; N, nectary, A, stamen. Scale bars: (A, B) = 1,000 μm; (C-F, H-J, M, N) = 200 μm; (G) = 300 μm; (K, L) = 500 μm; (O) = 10 μm.

Male floral vasculature and anatomy (Section *Ophellantha*, *Ac. spinosus*)

A male flower bud from *Ac. spinosus* was examined. Petals are bigger than sepals (Fig. 15A). At the pedicel level, the vasculature forms a ring (Fig. 15B). At the base of the flower bud, 10 vascular bundles are visible (Fig. 15C). Two outer bundles extend to the two outermost sepals followed slightly higher by three bundles to the three remaining sepals (Fig. 15C, D). Five inner bundles alternating with lower sepal bundles are extended and supply the petals (Fig. 15E, F). The rest of the vascular bundles forms a ring (Fig. 15F, G). The first five stamen traces extend in alternipetalous position (Fig. 15H). Next 10 bundles extend to supply two stamens adjacent to the first five stamens (Fig. 15I). More bundles supply the rest of stamens on the convex receptacle (Fig. 15J, K). The lowermost part of the nectaries alternates with the petals (Fig. 15G), but at a slightly upper level, the nectary expands to form a ring around the androecium (Fig. 15H, L) and expands further throughout the receptacle engulfing the filaments bases (Fig. 15I-K, M-O). Nectaries are covered with simple trichomes (Fig. 15M, N). The anthers are tetrasporangiate bithecate (Fig. 15O).

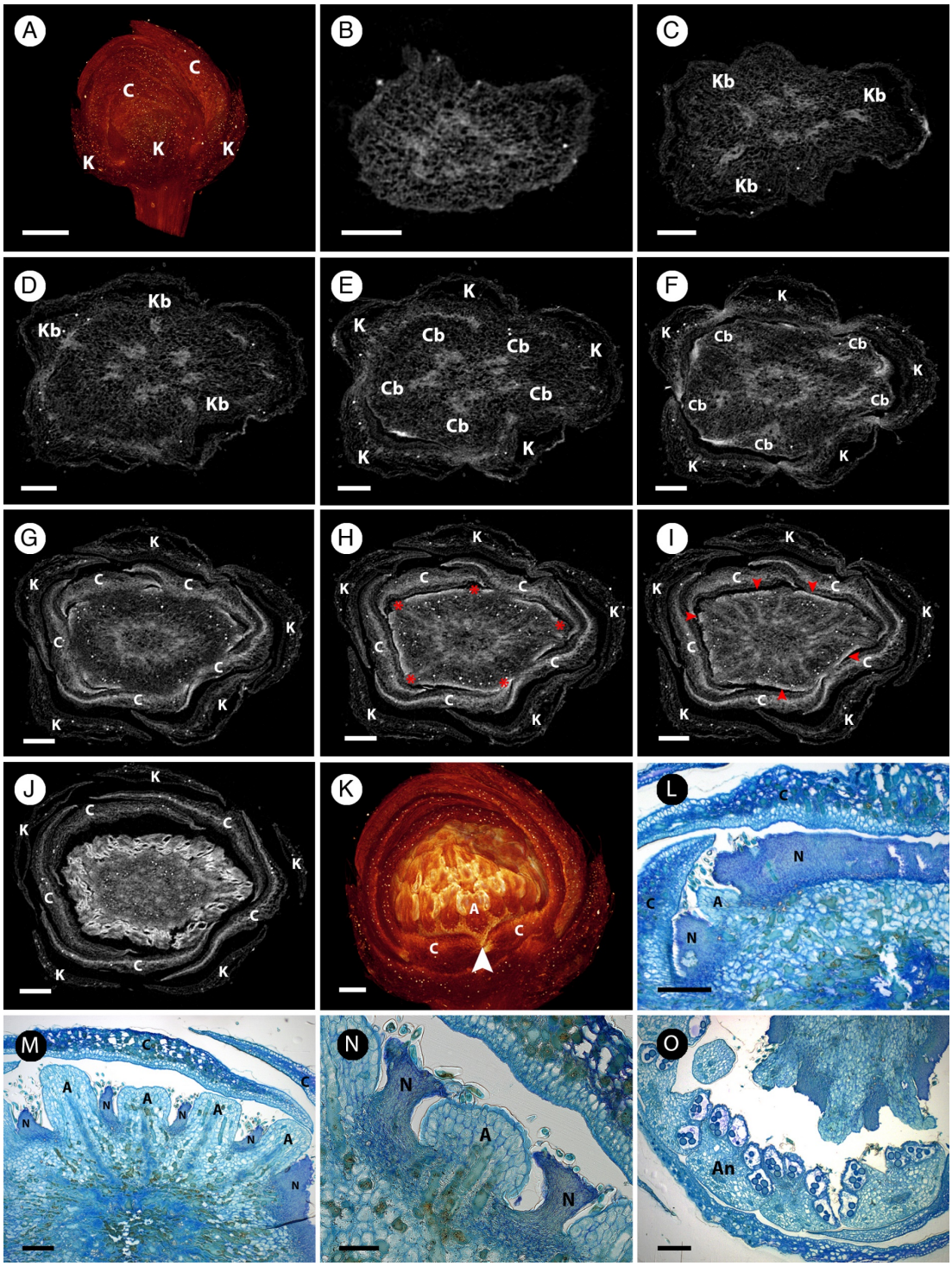

Figure 15. Anatomical structures of male flowers of *Ac. spinosus* (Section *Ophellanatha*). (A-K) Micro-computed tomography images; (L-O) resin thin sections. (A) Side view of a flower bud. Note, petals are bigger than sepals. (B) A transverse section at the base of the flower; vasculature arranged in a ring. (C) Two sepal traces branch out of the central ring. (D) The remaining three sepal traces start to extend out of the floral stele. (E) Five bundles separating from the central ring. (F) Five petal traces branch out leaving a central vascular ring.

Figure 15 continued. (G) Five sepals are visible with quincuncial aestivation, while five petals are arranged in a cochlear pattern. Nectary tissues starts to appear in the alternipetalous area. (H) The first stamen trace to branches out in an alternipetalous position. Nectary tissue is visible on the rim of the receptacle. (I) More stamen traces proliferate from the floral stele. (J) A section on the convex receptacle shows many stamens arranged on the receptacle. Nectary tissue is present at the base of stamens. (K) The outermost stamens are located in alternation with petals. Nectariferous tissue forming a ring surrounding stamens and also expanding toward floral centre. (L) Transverse section through nectary tissue including an outermost stamen. (M) The nectary expands to engulf the base of all filaments. (N) The upper part of each nectary is covered by many simple trichomes. (O) All anthers are tetrasporangiate. K, sepal; C, petal; Kb, sepal bundle; Cb, petal bundle; N, nectary, A, stamen; An, anther. Scale bars: (A) = 500 μm; (B-M, O) = 200 μm; (N) = 100 μm.

Male floral vasculature and anatomy (Section *Ophellantha*, *Ac. gentryi*)

A male flower bud was included in the CT-scan. The flower is pre-anthetic with petals bigger than sepals (Fig. 16A). A transverse section at the level of the pedicel reveals a ring-shaped vasculature (Fig. 16B). At the base of the flower, five bundles extend to supply sepals and branch into three veins (Fig. 16C). Sepals are fused together at the base (Fig. 16D-F). Higher up, five bundles alternating with sepal bundles extend to supply petals and branch into three veins (Fig. 16D-F). The rest of bundles is arranged in a pentagonal shape with each angle pointing towards the sepals (Fig. 16G). The outermost bundles supply alternipetalous stamens (Fig. 16H). At a higher level, more bundles are given off to supply many stamens (Fig. 16I). Stamens are slightly twisted within the flower bud and prevented observation of the stamen arrangement from above (Fig. 16J-M). Stamens are inserted on a convex receptacle (Fig. 16M). The lowermost parts of the nectary appear in alternipetalous position (Fig. 16G). They expand into a ring (Fig. 16H) and higher up divide into many lobes (Fig. 16I, N, O). The nectary ring remains around the androecium and does not expand to engulf the filaments (Fig. 16N, O).

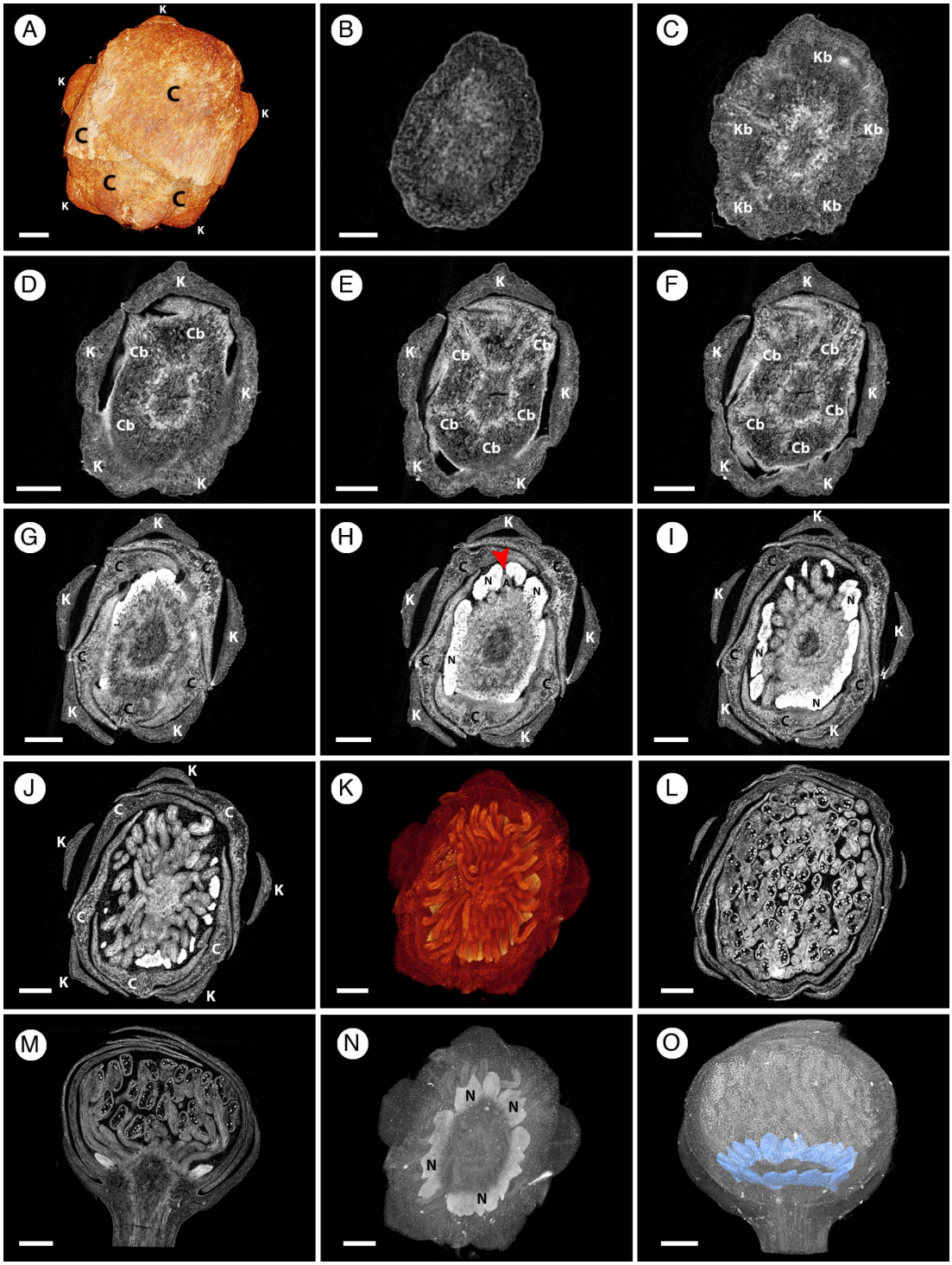

Figure 16. Micro-computed tomography images show anatomical structures of male flowers of *Ac. gentryi* (Section *Ophellanatha*). (A) Top view of a flower bud. The petals are arranged in a cochlear aestivation. (B) A vascular ring is present at the base of a flower. (C) On the upper part, sepal traces extend out of the central ring. (D) Petal traces extend out of the floral stele. (E-F) While vascular traces extend to supply petal, the rest of the floral stele forms a pentagonal shape. (G) The angles of the pentagonal ring alternate with petals. A nectary ring is visible.

Figure 16 continued. (H) The outermost stamens are arranged alternate with petals. Many traces branching to supply the stamens. (I) No clear whorl can be observed due to high stamen number. The upper part of the nectary ring divided into many lobes. (J) Stamens packed chaotically inside a flower bud. (K) Top view of the 3D surface showing many stamens clustered in a flower bud. (L) Transverse section at the level of the anthers show anthers facing toward the central part of the flower (introrse). (M) Due to limited space, filaments are twisted to fit all stamen inside the flower bud. (N) Top view of the nectary ring. K, sepal; C, petal, Kb, sepal bundle; Cb, petal bundle; N, nectary. Scale bars: (A, C-L, N) = 400 μm; (B, M, O) = 500 μm;

Male floral morphology (Section *Acidocroton*, *Ac. oligostemon*)

Male flowers from this species have a green perianth (Fig. 13I, J; 17A). Sepals are arranged in a quincuncial aestivation. Both abaxial and adaxial surfaces are glabrous with ciliate margin (Fig. 17B-D). There are colleters present on the margin of sepals (Fig. 17E, F). Petals are present with cochlear aestivation (Fig. 17G). They are ovate shaped with glabrous abaxial and adaxial surfaces and ciliate margin (Fig. 14G). Petals are bigger than sepals at maturity (Fig. 13I, 17A, G). Nectaries are present at the base of the filament and also form a ring surrounding part of the androecium (Fig. 17 I, J). There are about 16-17 stamens in male flower (Fig. 17A). Each anther has one pollen sac on each side (bisporangiate dithecate) with a connective appendage (Fig. 17A, K, L).

Figure 17 (next page). Morphological structures of male flowers of *Ac. oligostemon* (Section *Acidocroton*). (A: Use of image courtesy of Benjamin van Ee). (A) A flower at anthesis. The outermost stamens alternate with petals (asterisks). There is a connective appendage present on each anther (arrowhead). (B) Abaxial side of a sepal with glabrous surface. There are simple trichomes on the margin. (C) An adaxial side of sepals with glabrous surface. Sepals are fused together at the base. The margin is lined with simple trichomes.

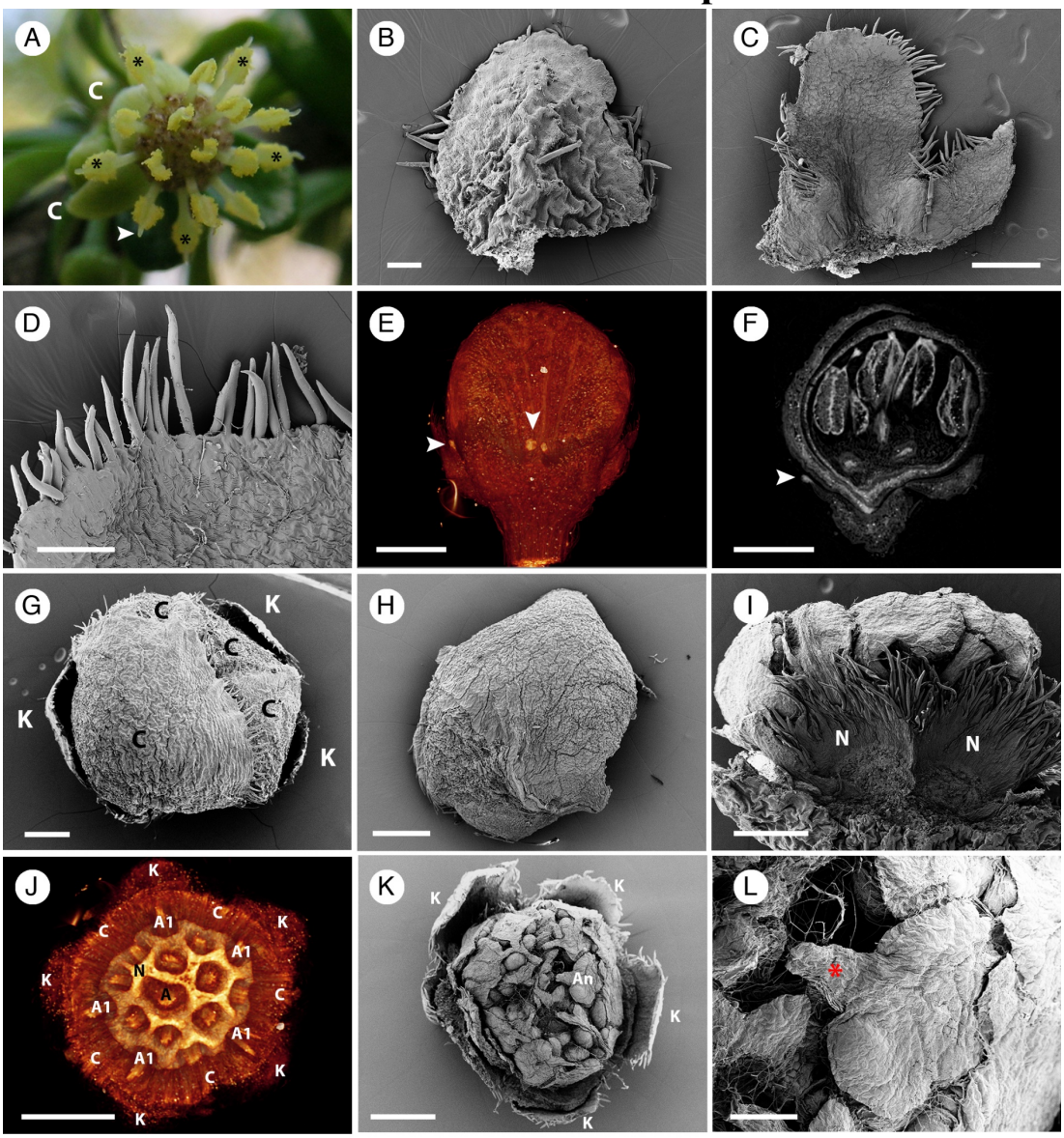

Figure 17 continued. (D) The margin of a sepals with simple trichomes. (E) A side view from µCT shows the presence of glandular structures on sepals (arrowhead). (F) A longitudinal section shows the presence of a gland on a sepal margin (arrowheads). (G) A mature flower bud shows petals with cochlear aestivation. The margin of petasl is lined with simple trichomes. (H) The glabrous abaxial surface of petals. (I) Side view of a flower with petals removed. There is a nectary ring surrounding the androecium. (J) A transverse section image shows the honeycomb-like nectary engulfing the base of filament. Note the outermost stamens are arranged alternate to petals. (K) A mature flower bud with petals removed. Stamens are not inflexed in bud but curved toward the centre. (L) Each anther has a connective appendage with unknown function (asterisk). C, petal; K, sepal; N, nectary, A, stamen, A1, first whorl stamen; An, anther. Scale bars: (B, D, L) = 100 µm; (C, G-I) = 300 µm; (E, F, J, K) = 500 µm.

Male floral vasculature and anatomy (Section *Acidocroton*, *Ac. oligostemon*)

We included a male flower bud in the micro-computed tomography (Fig. 18A). The vasculature is arranged in a ring at the pedicel level (Fig. 18B). At the base of the flower, five bundles extend to supply the sepals (Fig. 18B-D) and later branch into three veins (Fig. 18C, D). Five alternating bundles extend to supply petals (Fig. 18C, D). Sepals are fused together at the base (Fig. 18E, F). The remaining vascular supply is rearranged in a pentagonal ring (Fig. 18F). The lower parts of the nectary appear as five pairs of glands alternating with the petals (Fig. 18G). Higher up, the nectary expands forming a pentagonal ring (Fig. 18H). At the base of the filaments, the nectary expands further between the filament bases forming a honeycomb-like structure (Fig. 18J). The outer whorl of stamens is supplied by five bundles in alternipetalous position (Fig. 18F, G). A second whorl of six stamen bundles is formed in antepetalous position (Fig. 18G). Four of the stamens are solitary, but two form an antepetalous pair (Fig. 18H-K). The remaining six bundles supply the rest of the stamens as the third whorl and a central stamen (Fig. 18I-K). However, two bundles - one from the third whorl and one from the centre – are fused together (fig. 18H-K). In this flower, there is a total of 16 stamens in a 5+6+4+1 pattern (Fig. 18J, K). Sections of the top part of the stamens reveal anthers to be tetrasporangiate dithecate (Fig. 18L).

Figure 18 (next page). Micro-computed tomography images show anatomical structures of male flowers of *Ac. oligostemon* (Section *Acidocroton*). (A) Top view of a flower bud. (B) A vascular ring in the pedicel. (C-D) Five bundles diverge from the floral stele to supply sepals. (E) Alternating with sepal bundles, five other bundles extend to supply petals. Sepals are fused together at the base.

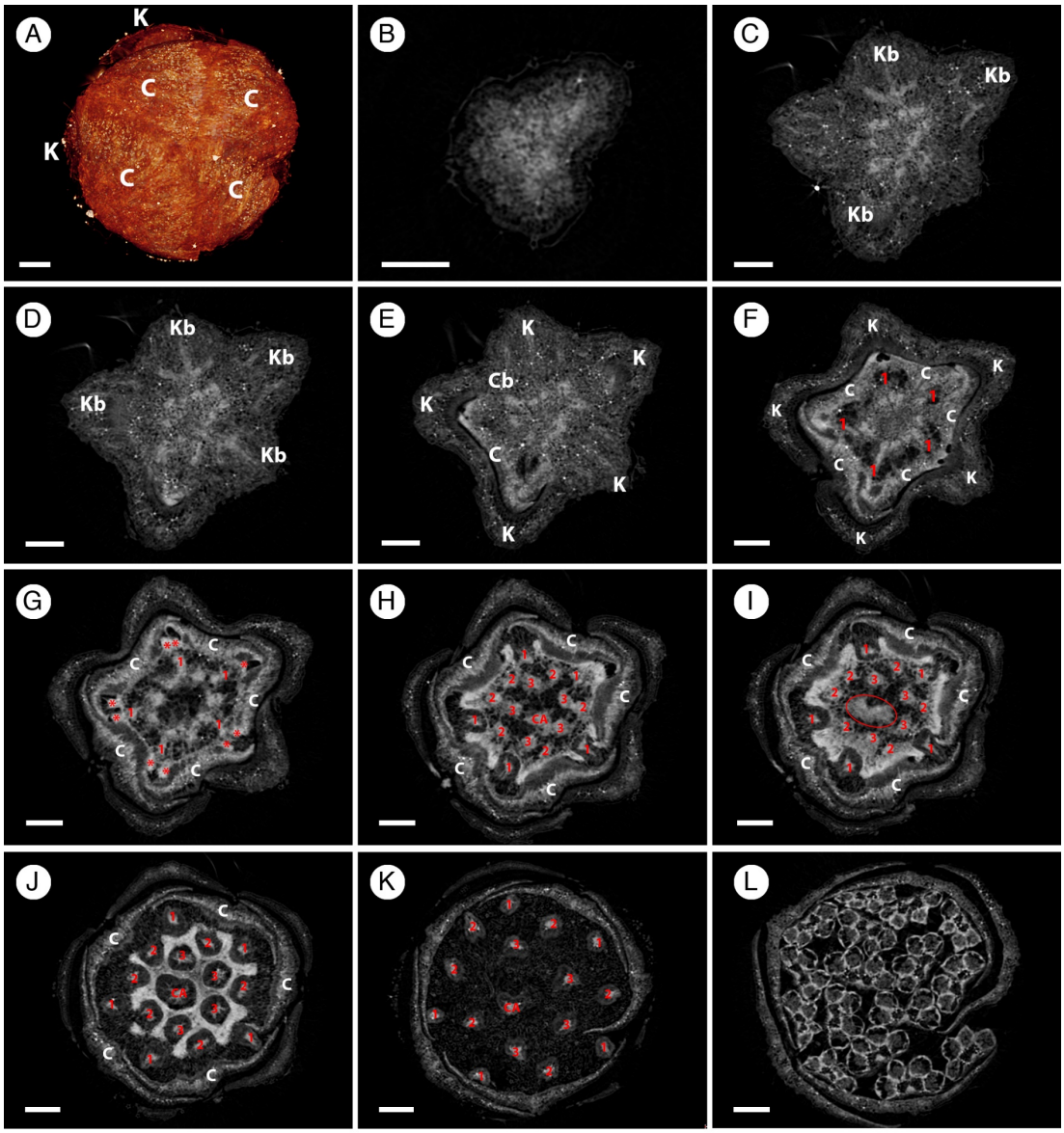

Figure 18 continued. (F) Slightly higher level than the previous image, five bundles extend to supply five outermost stamen in the alternipetalous position. (G) At higher level, a nectary starts to appear in alternipetalous position. (H) At this level, the nectary forms a ring around the androecium. The rest of the floral stele divides to supply the second and third whorls of stamens and also a central stamen. In the second whorl, one bundle splits to supply two stamens. There are 17 stamen's bundle arranged as 5+6+5+1. (I) Slightly higher up, a third whorl stamen's bundle fuses with a central stamen (highlighted by the oval shape). (J) At the base of stamens, nectaries expand to engulf the base of filaments. There is a total of 16 stamens in the flower. (K) A transverse section at the level of middle part of the stamens showing the arrangement of stamen. The outermost stamens are alternating with petals. (L) A section at the upper part of stamens show tetrasporangiate dithecal anthers. K, sepal; C, petal; Kb, sepal bundle; Cb, petal bundle; 1, 2 & 3 indicate stamen whorl; CA, central stamen. Scale bars: (A-L) = 200 µm.

Female floral morphology (Section *Ophellantha*, *Ac. gentryi*)

The calyx consists of six sepals with imbricate aestivation (Fig. 19A). This could be a variation since the presence of generally five sepals was reported in the literature (Fernández-Alonso and Jaramillo-Mejía, 1995). Sepals are mostly glabrous on both surfaces except for some simple trichomes in the middle at the level of the vascular strand on the abaxial side (Fig. 19B, C) and small patches of simple trichomes on the lowermost adaxial side (Fig. 19C). The margins are covered with several long simple trichomes and colleters (Fig. 19A-D). Inside the perianth whorl, there are several nectary lobes, each covered with simple trichomes, surrounding the ovary (Fig. 19F-I). The ovary consists of three fused carpels (Fig. 19H). The outer surface is sparsely covered with simple trichomes (Fig. 19H). Three forked styles are present with a tetrafid end each (total of 12 stigmatic tips) (Fig. 19F).

Female vasculature and anatomy (Section *Ophellantha*, *Ac. gentryi*)

A female flower was included in the micro-computed tomography (Fig. 20A). Two sepals were removed for SEM observation. At the pedicel level, vascular bundles are arranged in a ring shape (Fig. 20B). Five bundles extend and branch into three to five veins supplying the sepals (Fig. 20C-F). Several nectary glands were observed around the base of the ovary (Fig. 20G). Higher up the remaining vascular bundles are rearranged in a triangular shape (fig. 20G) Three outer bundles supply the dorsal veins of the carpel, while three inner bundle supply the ovules (Fig. 20H). The placentation is axile with one ovule in each locule (Fig. 20H). At the top of the pistil, three styles fuse together at the base then fork into bifid stigmas (Fig. 20I, J). Ovules are anatropous, each superposed by an obturator (fig. 20K, L).

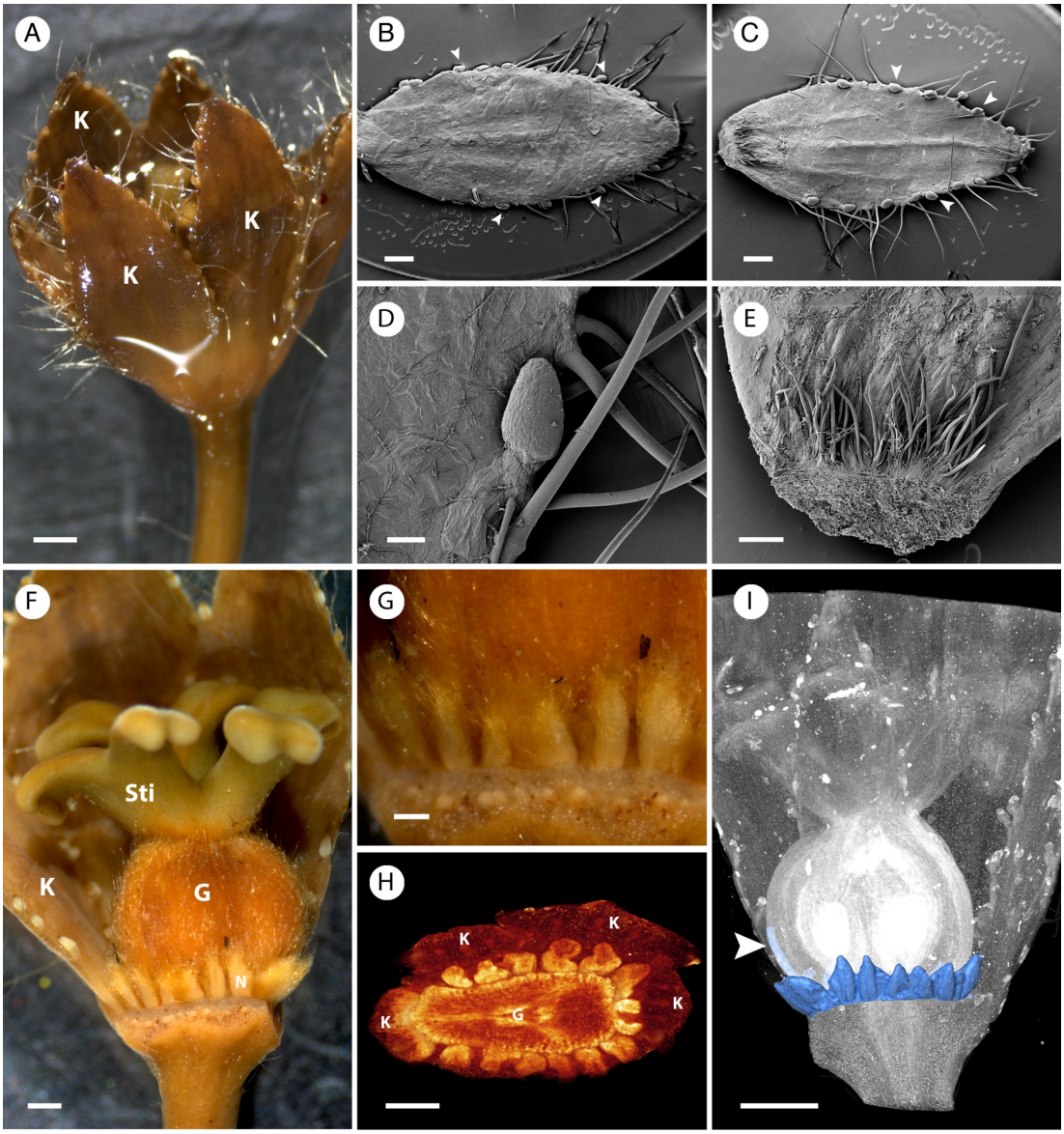

Figure 19. Morphological structures of female flowers of *Ac. gentryi* (Section *Ophellanatha*). (A) An anthetic flower with six sepals. (B) Abaxial side of a sepals with glabrous surface and some simple trichomes along the midrib. There are simple trichomes on the margin with colleters (arrowhead). (C) An adaxial side of a sepal with glabrous surface except for tufts of simple trichomes on the lower most part. There are colleters present along the margin (arrowheads). (D) Close-up of a colleter. (E) Dense simple trichomes on the lower part of the adaxial side of a sepal. (F) A flower with some sepals removed show many nectary glands surrounding an ovary. There are three styles on top of the ovary with tetrafid branching pattern. (G) A close-up image of the nectary structure. (H) A transverse section shows the presence of many nectary glands surrounding an ovary. (I) A reconstruction of the nectary structure in a female flower. There is a long thread-like structure which may be a filamentous structure (arrowhead). K, sepal; G, ovary; Sti, stigma. Scale bars: (A, I) = 1,000 µm; (B, C) = 600 µm; (D) = 100µm; (E, G) = 200 µm; (F, H) = 500 µm;

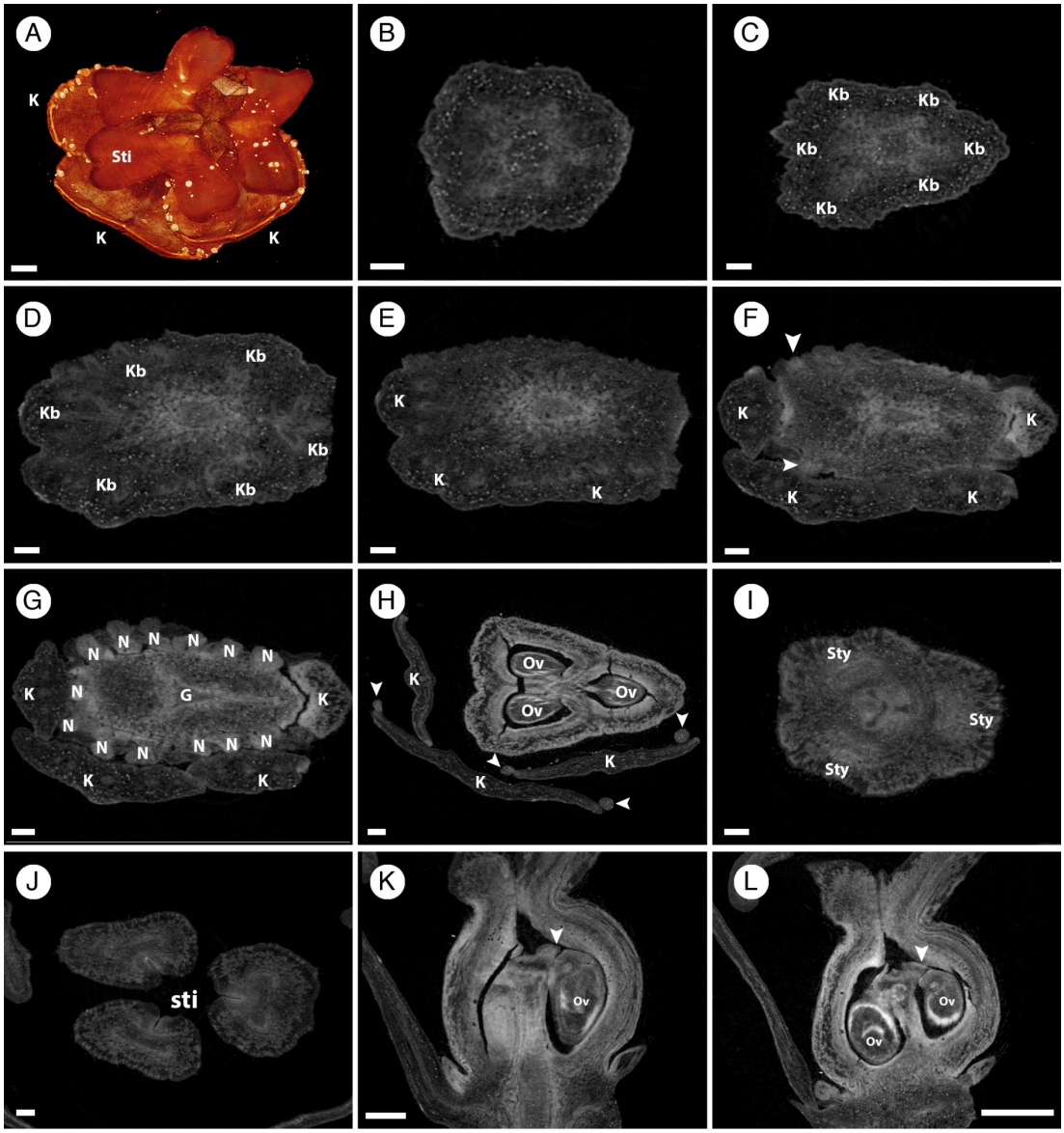

Figure 20. Micro-computed tomography images show anatomical structures of a female flower of *Ac. gentryi* (Section *Ophellanatha*). (A) The top view of a flower show sepals and three bifid styles. (B) A transverse section at the pedicel show a vascular ring. (C) At the base of the flower, six bundles extend from the central ring to supply sepals. (D-E) Each bundle branches into three veins that later branch further into several veins. (F) A non-vascularised nectary starts to appear at this level (arrowhead). Two sepals are removed. (G) Slightly higher up, 13 nectary lobes are visible around the base of the ovary. (H) A section through the middle of the ovary shows three fused carpels with an ovule in each locule with axile placentation. Colleters on the sepal margin are visible (arrowheads). (I-J) On top of the ovary, there are three bifid styles, each supplied by vasculature from the abaxial side of the ovary. (K-L) A longitudinal section shows one ovule in each locule. The ovules are anatropous. There are traces of obturator on top of each ovule (arrowhead). K, sepal; Sti, stigma; Kb, sepal bundle; N, nectary; G, pistil; Ov, ovule; Sty, style. Scale bars: (A, K) = 500 µm; (B-J) = 200 µm; (L) = 1,000 µm.

Female floral morphology (Section *Acidocroton, Ac. oligostemon*)

Female flowers are monoclymedeous with only the calyx whorl present (Fig. 21A-C). Both surfaces are glabrous (Fig. 21B-E). There are colleters present on the sepal margin (Fig. 21B, C) which disappear when flowers develop into fruits (Fig. 21D, E). Inside the calyx whorl, there is a nectary ring surrounding the ovary base (Fig. 21F, G). Ovaries are tricarpellate (Fig. 19H). The outer surface is covered with simple trichomes (Fig. 19H). Three tetrafid styles are present (total 12 stigmatic tips) (Fig. 21I).

Female floral vasculature and anatomy
(Section *Acidocroton, Ac. oligostemon*)

A female flower was included in the micro-computed tomography (Fig. 22A). Vascular bundles form a ring in the pedicel (Fig. 22B). At the base of the flower, five bundles extend to supply sepals (Fig. 22C, D), each bundle branching into three veins (Fig. 22D). There is a bundle extending to the alternisepalous position supplying a filamentous structure (Fig. 22D-F). A nectary ring is observed at the base of the ovary (Fig. 22G, H). The lowermost part of the nectary consists of two glands alternating with the sepals (Fig. 22E, F). The nectary expands higher up forming a ring with five lobes (Fig. 22F-H). However, damage from herbarium preparation prevented us from observing the complete structure (Fig. 22G, H). The ovary is at the centre of the flower inserted on a convex receptacle (Fig. 22J). It comprises three carpels with axile placentation (Fig. 22I). There is one ovule per locule (Fig. 22I, J). At the top of the pistil, each carpel dorsal bundle runs into a separate style which later branches into tetrafid stigmas (Fig. 22K, L).

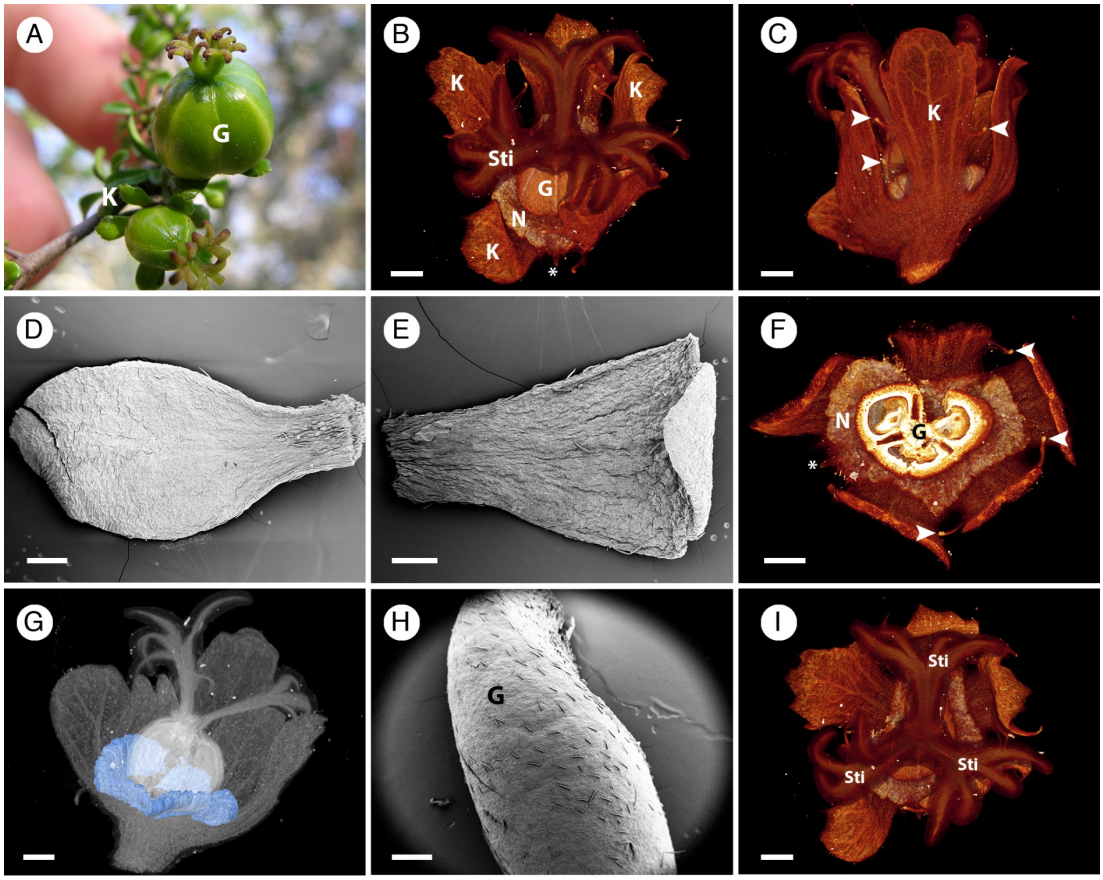

Figure 21. Morphological structures of female flowers of *Ac. oligostemon* (Section *Acidocroton*). (A: Use of image courtesy of Benjamin van Ee). (A) Two young fruits on the stem. All parts are green except the yellow, purplish stigmatic tips. (B) A image of a flower with all floral organs, i.e., sepals, nectary, ovary topped with styles. There is a filamentous-like structure present alternate with sepals (asterisk). (C) A side view of the flower shows the presence of colleters on sepals (arrowheads). (D) An abaxial side of an old sepal from a fruit shows a nearly glabrous surface with few simple trichomes present on the lower part. (E) An adaxial side of an old sepal from a fruit shows a glabrous surface with some simple trichomes on the margin. (F) A transverse section shows a ring of nectary surrounding a tricarpellate syncarpous ovary. Colleters are visible on the sepal margin (arrowheads). A filamentous-like structure is visible alternate with two sepals (asterisk). (G) A reconstruction of the nectary ting surrounding an ovary. (H) The surface of a young fruit is sparsely covered with simple trichomes. (I) There are three styles on top of an ovary with tetrafid pattern (12 stigmatic tips). G, pistil; K, sepal; N, nectary; Sti, stigma. Scale bars: (B, C, E, G, I) = 500 µm; (D, H) = 600 µm;

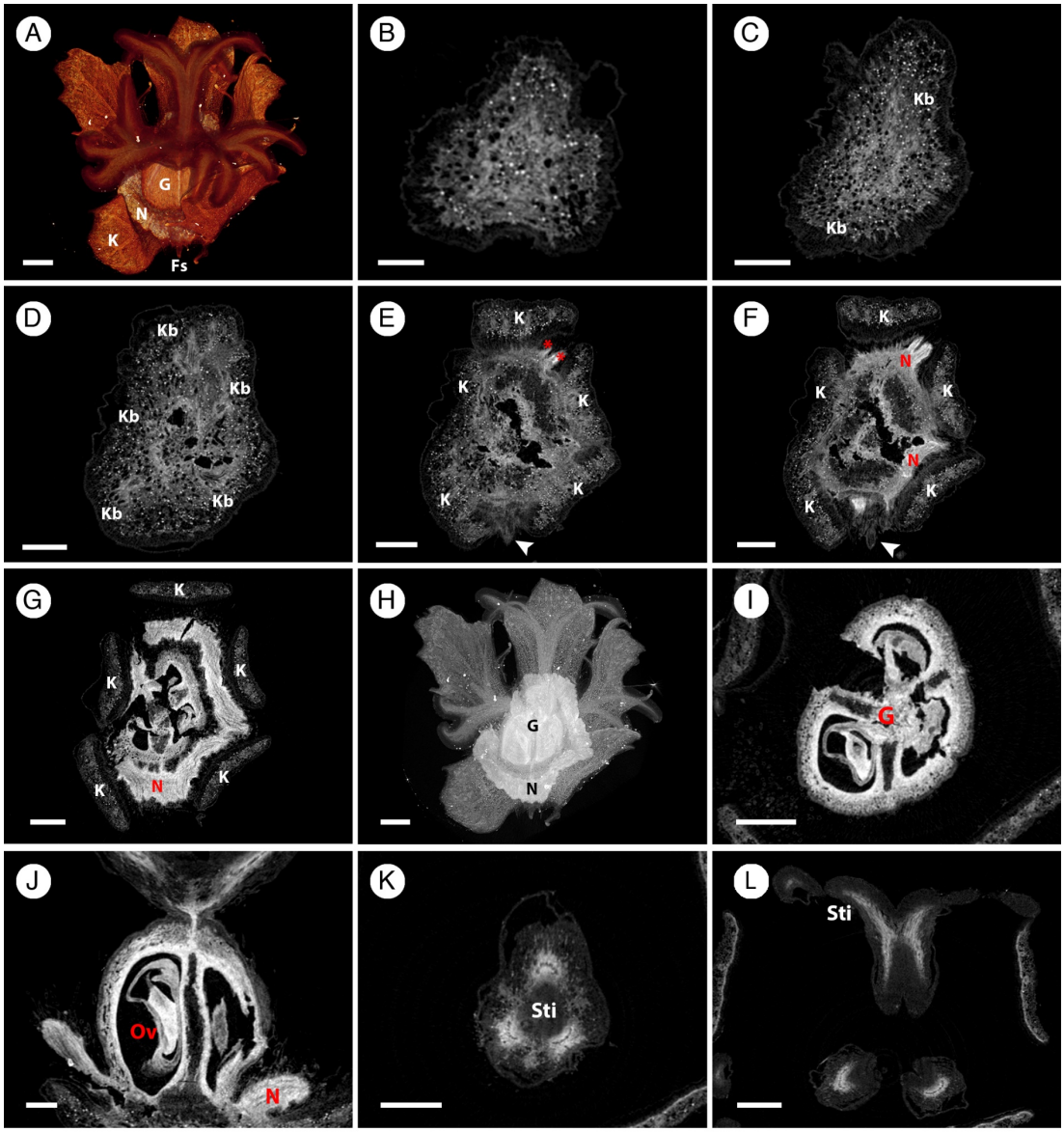

Figure 22. Micro-computed tomography images show anatomical structures of a female flower of *Ac. oligostemon* (Section *Acidocroton*). (A) Top view of a flower shows sepals, a nectary ring, a filamentous structure, an ovary and three tetrafid styles. (B) A section at the base of the flower shows a vascular ring. (C-D) Five bundles extend from the ring to supply sepals. (E) At a slightly higher level from the previous image, there is a bundle extending to supply the filamentous structure (arrowhead). A non-vascularised nectary starts to appear in an alternisepalous position (asterisk). (F). Parts of a nectary ring are visible. There is a filamentous structure at the alternisepalous position. (G) A vascular ring is visible but is damaged on one side. (H) Nectary ring is surrounding the base of the ovary. (I) The ovary consists of three carpels. There is one ovule per locule with axile placentation. One carpel is highly damaged from herbarium preparation. (J) A longitudinal section of the flower shows an ovule in a locule. The surface of the nectary is pilose. (K) There are three styles on top of the ovary. (L) At higher level, each style branches two times becoming tetrafid. K, sepal; N, nectary; G, pistil; Kb, sepal bundle; Ov, ovule; Sti, stigma. Scale bars: (A, H) = 500 µm; (B) = 200 µm; (C-G) = 400 µm.

2.4.4 *Astraea*

Inflorescence morphology

Samples from three species of *Astraea*, *As. comosa, As. lobate* and *As. surinamensis* were examined. Inflorescences are determinate thyrses with male cymules present on the distal part, while there are variations of proximal subunits as solitary female flowers or bisexual cymules (Fig. 23A-C). Florets in each cymule are arranged in a zig-zag pattern (Fig. 23D). Pedicels of male flowers from all three species show recaulescent growth (Fig. 23E). Both stellate and simple trichomes are present in this genus (Fig. 23F).

Male floral morphology

Male flowers from all three species are similar. Their perianths are bipartite. In the calyx, there are five sepals arranged in a quincuncial aestivation (Fig. 24A). Sepals of *As. lobate* and *As. surinamensis* are sparsely covered with simple trichomes on the abaxial surface (Fig. 24A, C), while in *As. Comosa* stellate trichomes and their reduced forms are present (Fig. 24B, D). The adaxial side of sepals is glabrous in all three species (Fig. 24E, F). Male petals of all three *Astraea* are obovate shaped arrange with imbricate aestivation (Fig. 24G). There are no trichomes on both petal surfaces (Fig. 24G-I). In all three species, margins on the middle and upper part are glabrous but the lower part of the petal margin has trichomes consisting of 'string of bead'-like cells (moniliform trichomes) (Fig. 24H-J). Inside the perianth whorls, five bilobed nectaries were observed surrounding the androecium (Fig. 24L). The receptacle is convex and glabrous (Fig. 24K). There are about 12 to 15 stamens per flower in all three species (Fig. 24K, M, N). Filaments are inflexed in bud and also twisted (Fig. 24N). The pollen is inaperturate spherical shaped with the croton pattern (Fig. 24O). There is no trace of a rudimentary ovary.

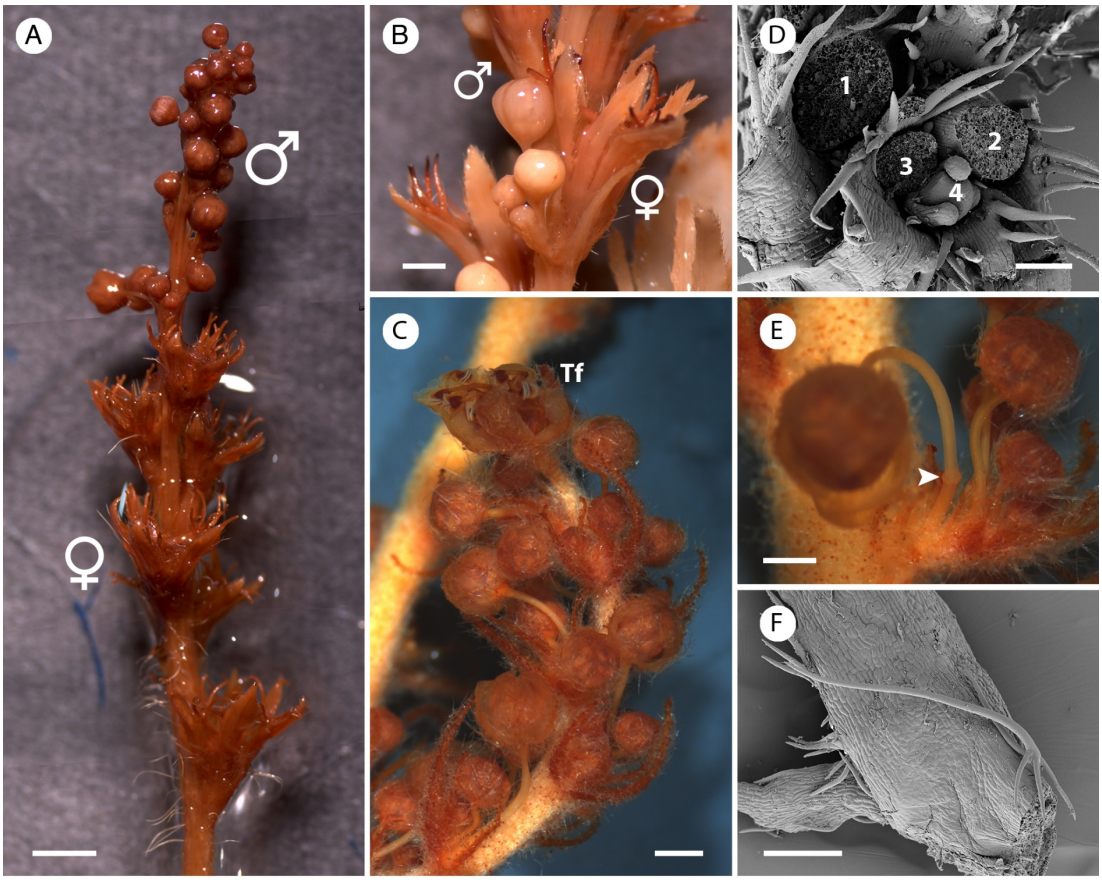

Figure 23. Morphological structures of inflorescences in the genus *Astraea*. (A, F) *As. surinamensis*; (B, D) *As. lobata;* (C, E) *As. comosa*. Tf, terminal flower; Numbers 1, 2 & 3 indicate sequence of flowers in a cyme. (A) An inflorescence with solitary female flowers on the proximal part and male cymules on the distal part. (B) A bisexual cymule with a female flower and several male flowers arranged in a monochasium. (C) A terminal male flower of an inflorescence. (D) The arrangement of flowers in a cyme with cincinus pattern. (E) A side view of a cymule shows the abscission zone on the pedicel. (F) A stellate appressed trichomes with a projection (porrect) on a bract. Tf, terminal flower. Scale bars: (A) = 2,000 µm; (B, C) = 1,000 µm; (D) = 100 µm; (E) = 600 µm; (F) = 200 µm.

Figure 24 (next page). Morphological structures of male flowers of *Astraea*. (A, I, J, M) *As. surinamensis*; (B, D, F, L) *As. comosa*; (C, E, G, H, K, N, O) *As. lobata*. (A) A flower bud with glabrous sepal surface. (B) Sepals of *As. comosa* are covered with stellate trichomes. (C) Abaxial side of a sepal from species with glabrous surface. (D) An adaxial side of a sepal from *As. comosa* is covered with stellate trichomes. (E-F) Adaxial surface of sepals from all three examined species are glabrous. (G) A flower with sepals removed shows petals with a glabrous surface. (H) An abaxial side of a petal shows a glabrous surface. Moniliform trichomes are present on the lower part of the adaxial side (arrowhead). (I) An adaxial side of a petalwith glabrous surface. Moniliform trichomes are present on the lower margin (arrowhead). (J) A close-up image of moniliform trichomes.

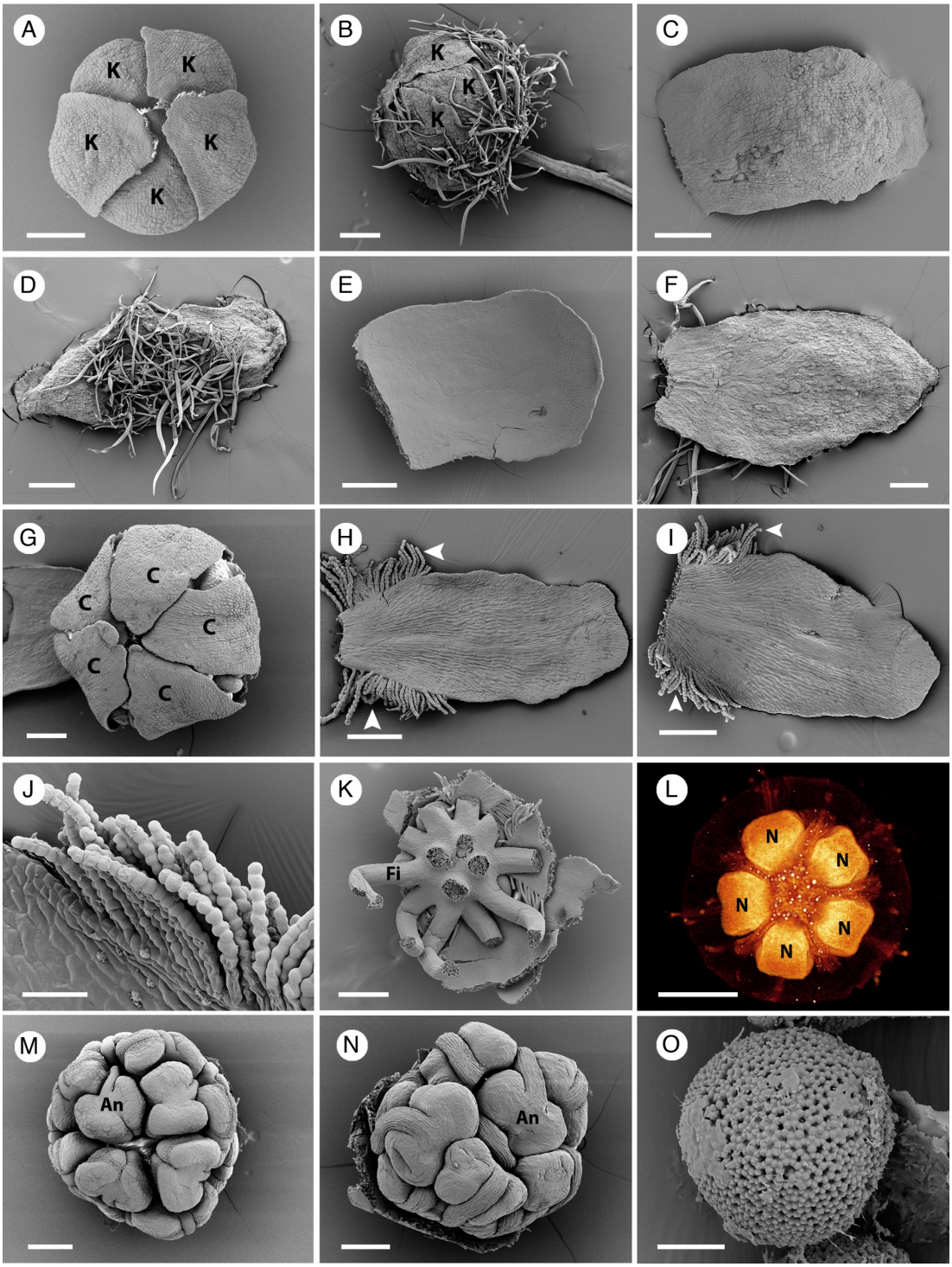

Figure 24 continued. (K) A flower with sepals, petals and upper part of stamen removed shows a glabrous receptacle. There are 14 stamens present in this flower arranged in two whorls of five surrounding four stamens in the middle. The outermost stamens are arranged opposite to petals. (L) A sectioning image shows five separated nectary glands present in a male flower. (M-N) Flowers with perianth removed show inflexed stamens in bud with slightly twisted filaments. (O) An inaperturate pollen with *Croton*-pattern surface. K, sepal; C, petal; Fi, filament; N, nectary; An, anther. (A, C, E-I, K, M) = 200 µm; (B, D) = 300 µm; (J) = 50 µm; (L) = 500 µm; (N) = 100 µm; (O) = 10 µm.

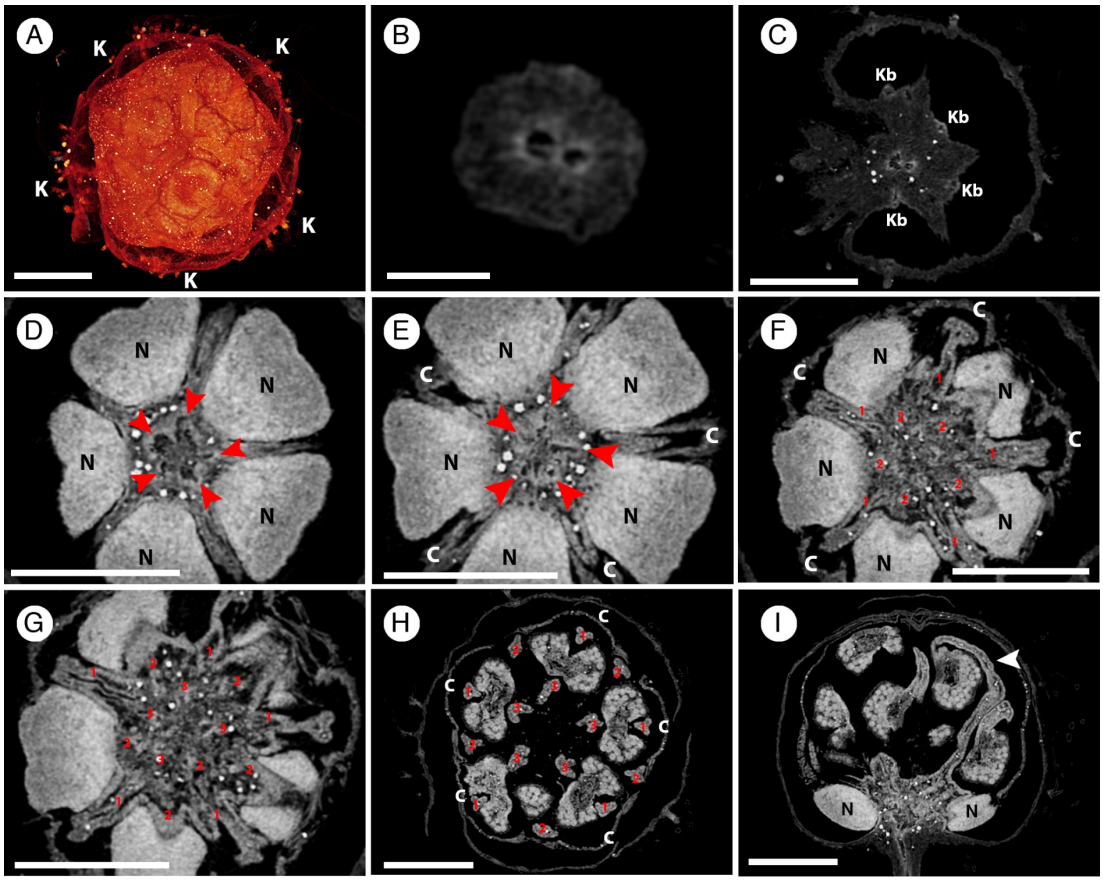

Figure 25. Micro-computed tomography images show anatomical structures of a male flower of *As. comosa*. (A) A top view of a flower reconstructed from image stacks. The outer whorl is the calyx. (B) A vascular ring in the pedicel. (C) Five vascular bundles extend to supply sepals. (D) At a slightly higher level, five alternisepalous bundles diverge to supply petals (arrowheads). (E) Next, five bundles extend to supply the outermost whorl of stamens, which are opposite to petals. (arrowheads). (F) Alternating with the previous whorl, five additional bundles extend to supply the second whorl of stamens. (G) The third whorl of stamens is supplied by another five bundles alternating with the previous whorl. (H) There is a total 15 stamens in the flower arranged in three whorls of five. (I) A longitudinal section shows inflexed stamens in bud (arrowhead). There are nectary glands below the androecium. Kb, sepal bundle; N, nectary; C, petal. Scale bars: (A, C-I) = 500 µm; (B) = 100 µm.

Male floral vasculature and anatomy

A male flower was included in the micro-computed tomography (Fig. 25A). At the level of the pedicel, the vasculature is ring shaped (Fig. 25B). At the base of the flower, five outer bundles extend to the sepals (Fig. 25C, D). Next, five inner bundles alternating with previous bundles extend to supply the petals (Fig. 25E). Sepals fuse

together at the base (Fig. 25). Five bilobed nectary glands are present opposite to sepals (Fig. 25). The outermost bundles of the stamens supply the outermost stamen whorl in antepetalous position (Fig. 25F). Higher up bundles extend alternately with the previous whorl to supply the second alternipetalous stamen whorl (Fig. 25G, H). The third and last whorl of five stamens are supplied by the rest of bundles alternating with the previous whorl (Fig. 25I, J). Stamens are inflexed in the flower bud (Fig. 25K). Their filaments are also twisted to fit in the space of the flower bud (Fig. 25L).

Female floral morphology

Female flowers are pentamerous with five sepals fused at the base and arranged in quincuncial aestivation (Fig. 26A). In *As. lobata* and *As. surinamensis*, there are few simple trichomes along the mid vein on the abaxial surface (Fig. 26B). Simple trichomes are also found lining the sepal margin in these two species (Fig. 26B). In *As. comosa*, the abaxial side is covered by dense stellate trichomes (Fig. 26C). The adaxial surface of *As. lobata* and *As. surinamensis* is glabrous (Fig. 26D). There are colleters presente on the lower margin of sepals in both species (Fig. 26D). In *As. comosa*, the adaxial surface is also glabrous but dense stellate trichomes on the margin prevented us to observe the presence of colleters with SEM view (Fig. 26E). Micro-computed tomography image reveals a row of colleters present on the lower part of the sepals in *As. comosa* as in other two species (Fig. 26F, G). Inside the calyx, there is a ring of distinct nectary lobes (Fig. 26 H-K). Lobe number is basically five (Fig. 26K) but additional lobes occur frequently (Fig. 26H, I). Filamentous structures of various length are present between nectary lobes (Fig. 26H-J), except in *As. comosa* without filamentous structures (Fig. 26K). At the centre of the flower, there is a tricarpellate ovary with axile placentation (fig. 26K). The outer surface is sparsely covered with simple trichomes in *As. lobata* and *As. surinamensis* (Fig. 26L) or dense stellate trichomes as in *As. comosa* (Fig. 26M). Styles are highly branched with tetra- to hexa-fid stigmas (Fig. 26L, N, O). Similar to sepal and ovary surfaces, styles of *As. lobata* and *As. surinamensis* are covered with simple trichomes (Fig. 26N), while styles of *As. comosa* are covered with stellate trichomes (Fig. 26O).

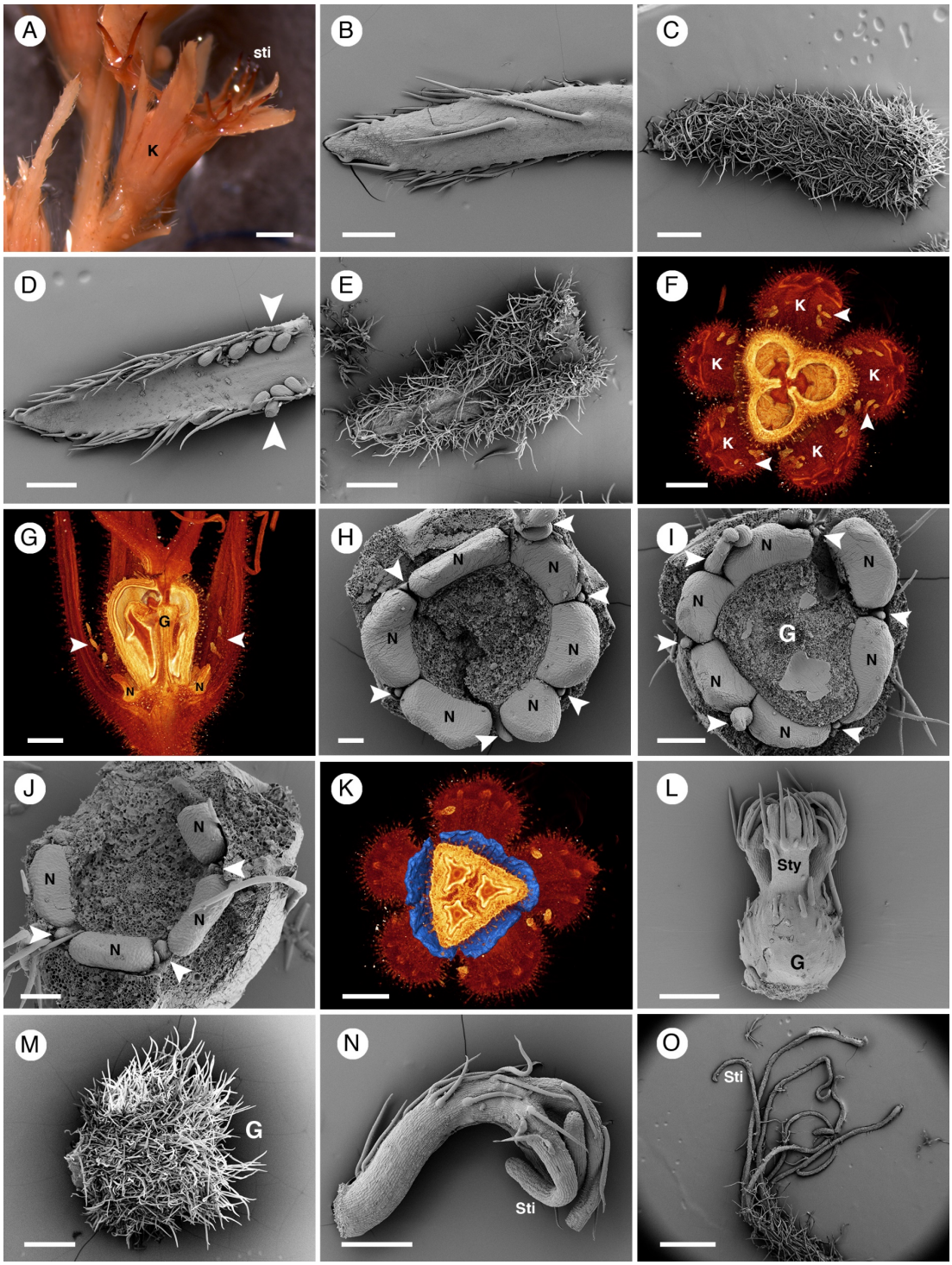

Figure 26. Morphological structures of female flowers of *Astraea*. (A, B, D, H, I, N) *As. lobata*; (C, E, F, G, K, M, O) *As. comosa*; (J, L) *As. surinamensis*. (A) A side view of a female flower with sepals and style/stigma visible. (B) Abaxial side of a sepal found in *As. lobata* and *As. surinamensis* with few simple trichomes along the midrib; there are many simple trichomes lining the margin. (C) Abaxial side of a sepal from *As. comosa* which is covered with dense stellate trichomes.

Figure 26 continued. (D) Adaxial side of a sepal with glabrous surface in *As. lobata* and *As. surinamensis*. There are simple trichomes present on the upper part of the sepal margin, while there are colleters present on the lower part of the margin (arrowheads). (E) Adxial side of a sepal from *As. comosa* showing dense stellate trichomes covering the magin. (F) A sectioning of a flower shows the presence of colleters at the lower margin of sepals (arrowheads) in *As. comosa*. (G) A longtitudinal section of a female flower shows the presence of colleters along the lower margin of sepals (arrowheads). (H-J) Female flowers with sepals and ovaries removed show the unusual presence of six separate nectary glands alternating with filamentous structures (arrowheads). (K) Five nectary lobes are arranged opposite to sepals. (L) A young ovary with surface covered with simple trichomes. Styles are multifid. (M) The ovary surface in *As. comosa* is covered with dense stellate trichomes. (N) A style with multifid branching pattern. (O)A style *As. comosa* with multifidfid branching pattern. K, sepal; Sti, stigma; N, nectary, G, pistil; Sty, style. Scale bars: (A, O) = 1,000 μm; (B, D, L, N) = 300 μm; (C, E-G, K, M) = 500 μm; (H)= 100 μm; (I)=200 μm; (J) = 90 μm.

Female flower vasculature and anatomy

A female flower of *As. comosa* was included in the micro-computed tomography (Fig. 27A). Vascular bundles are arranged in a ring in the pedicel and base of flower (Fig. 27B). Five bundles extend to supply the sepals (Fig. 27C-E). Each trace branches into three veins (Fig. 27E). Below the ovary base, nectary tissue appears in antesepalous position without vasculature, while the rest of the vascular bundles form a ring (Fig. 27F). At the base of the pistil, nectaries fuse together, while the rest of the vasculature is triangular shaped (Fig. 27G). On the lower part of the pistil, the nectary ring starts to separate into five sections (Fig. 27H, I); three outer dorsal bundles also separate from the central bundles (Fig. 27H). The pistil comprises three carpels with axile placentation (Fig. 27 I-L). There is one ovule in each locule (Fig. 27I, J, L). Each ovule is superposed by an obturator (Fig. 27K, L). *As. comosa* has three styles united at the base on top of the pistil (Fig. 27M). Each style is supplied by a vascular trace (Fig. 27N).

Figure 27. Micro-computed tomography images show anatomical structures of a female flower of *As. comosa*. (A) A side view of a flower constructed from image stacks. (B) A section at the pedicel level shows vascular bundles forming a ring. (C-E) Five bundles extend from the floral stele to supply sepals. At the base of sepals, they branch to become sepal veins. (F) Five non-vascularised nectary glands are located opposite to sepals. (G) Five nectary lobes fuse together forming a ring around the base of the ovary. There is a small space between the nectary lobes which could be small filamentous structures.

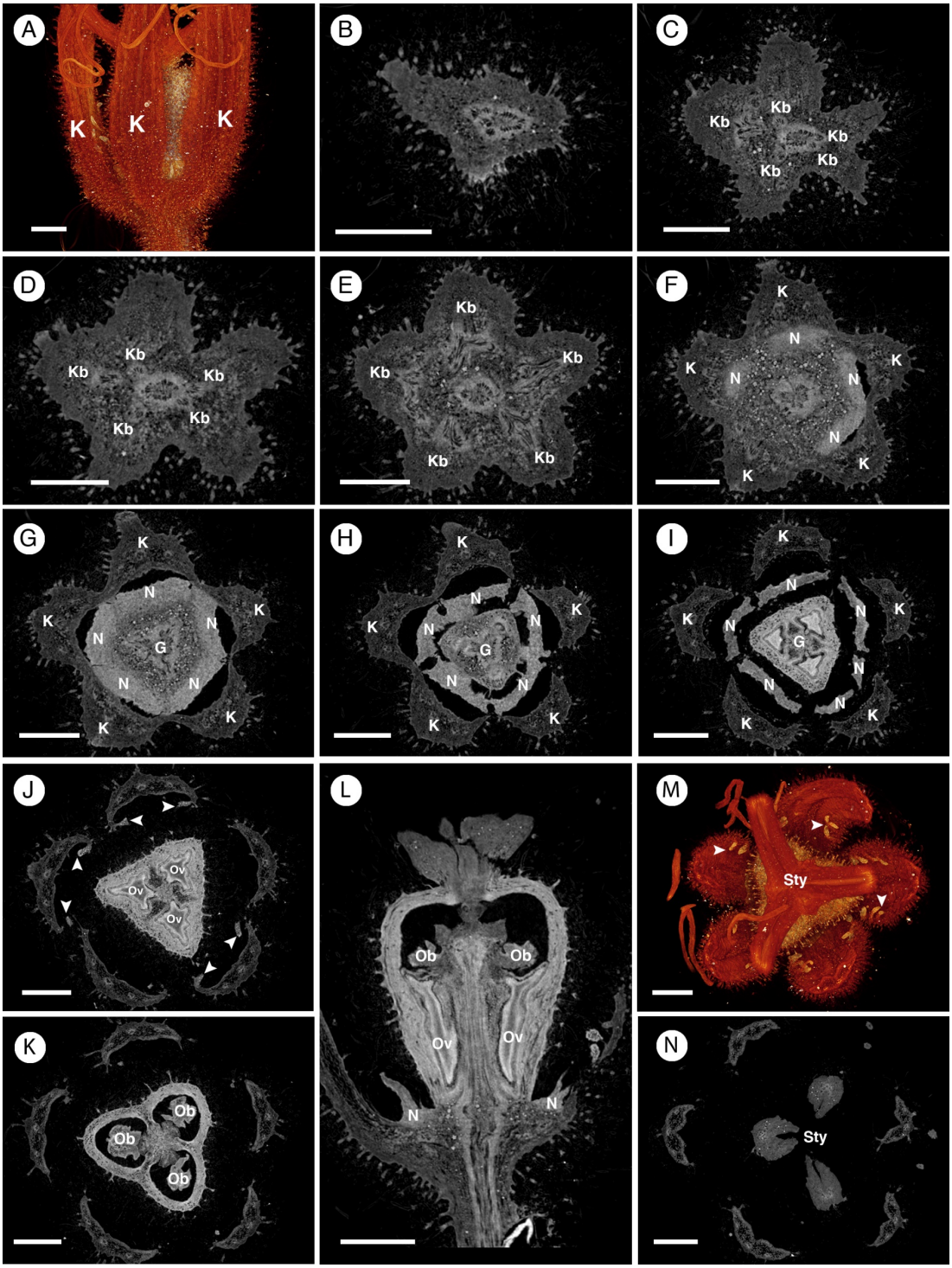

Figure 27 continued. (H) At the lower part of the nectary, five nectary glands start to separate. (I-J) The ovary has three locules with one ovule each on an axile placentation. Colleters are present on the sepal margins (arrowheads). (K-L) There is an obturator superposed on each ovule. (M) There are three styles on top of the ovary. Colleters on sepal margins are visible (arrowheads). (N) At a higher level, higher level, styles branch into several parts. K, sepal; Kb, sepal bundle; N, nectary; G, pistil; Ov, ovule; Ob, obturator, Sty, style. Scale bars: (A-N) = 500 µm.

2.4.5 *Brasiliocroton*

Inflorescence morphology

Inflorescences from both species of *Brasiliocroton*, i.e., *B. mamoninha* and *B. muricatus*, were examined from several herbarium sheets and the online herbarium. *B. mamoninha* has bisexual terminal panicles with ramified side branches (Fig. 28A-B). Side branches on the proximal part are longer and gradually shorter toward to apex of the inflorescence main axis (Fig. 28A). Each side branch comprises a female flower in a distal position; in the proximal part there are few branchlets of male flowers (Fig. 28C). The apex of the inflorescence is generally delicate and falls off during herbarium preparation. However, we found some specimens with the inflorescence apex ending with a terminal female flower (G.L. Farias 20, K; J.R. Pirani 3411, NY) (Fig. 28A, B, D, E). *B. muricatus* usually has unisexual inflorescences (Fig. 28F, G) but bisexual inflorescences are also present (Fig. 28H, I). The inflorescences in this species are borne axillary on the stem as botryoids (determinate raceme) (Fig. 28F, G) or determinate thyrses (Fig. 28I). Female cymules occur in some inflorescences (Fig. 28J). All parts of the inflorescence are covered with dendritic trichomes in *B. mamoninha* (Fig. 28K) or with rosulate trichomes in *B. muricatus* (Fig. 28L). Each flower is subtended by a bract and two bracteoles.

Figure 28 (next page). Morphological structures of inflorescences of *Brasiliocroton*. (A-E, K) *B. mamoninha*; (F-J, L) *B. muricatus*. (A) A cluster of a paniculate inflorescence. The tip of each side branch is terminated with a female flower. Note, there is terminal female flower at the tip of an inflorescence (arrowhead). (B) A close-up image of (A) shows a distal female flower on a side branch. (C) A close-up image of (A) shows a terminal female flower of an inflorescence. (Use of image A-C courtesy of the Royal Botanic Gardens Kew, UK (http://specimens.kew.org/herbarium/K001210233). (D-E) Images show a female terminal flower on the inflorescence. (Use of image D, E courtesy of the C. V. Starr Virtual Herbarium of The New York Botanical Garden, USA (https://sweetgum.nybg.org/science/vh/specimen-details/?irn=1016936) and (http://sweetgum.nybg.org/science/vh/specimen-details/?irn=1101691)). (F) An inflorescence bearing young fruits. (G) A male inflorescence. (H) Inflorescence of *B. muricatus* is borne axillary. In this sample, they are bisexual inflorescences, each topped by a terminal female flower.

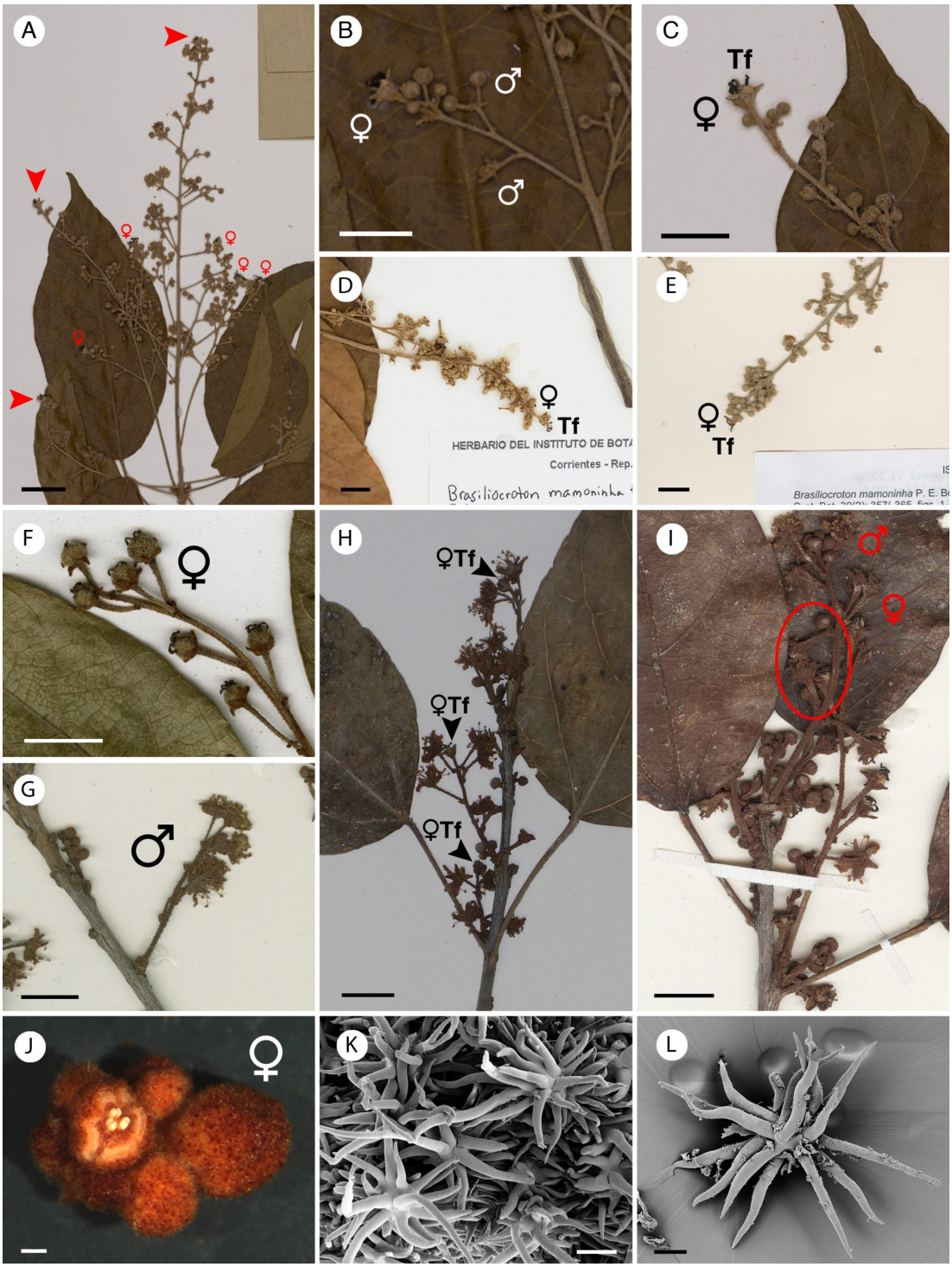

Figure 28 continued. (I) This sample has several bisexual inflorescences. The oval shape highlights a bisexual cymule without elongation. (Use of image courtesy of the UC Davis Center for Plant Diversity, USA) (J) Female cymule in female inflorescence. (K) Many dendritic trichomes with several layers branching. (L) A rosulate type trichome. Tf, terminal flower. Scale bars: (A) = 2 cm; (B-I) = 1 cm; (J) = 500 µm; (K) = 30 µm; (L) = 10 µm.

Male floral morphology (*B. mamoninha*)

Flowers are pentamerous with two whorls of perianth parts. The outermost whorl is a calyx with five sepals arranged in valvate aestivation (Fig. 29A). The outer surface of the sepals is covered with dendritic trichomes with at least two layers of rays (Fig. 29B, C). The inner perianth whorl is the corolla of five petals with free aestivation (Fig. 29D). The abaxial side is covered with simple trichomes (Fig. 29E) while the adaxial part is glabrous but is covered by long simple trichomes along the margin (Fig. 29F). Inside the corolla, there are five nectary glands surrounding the androecium (Fig. 29G, H). The centre of the flower is occupied by stamens inserted on a pilose receptacle. There are about 20 Stamens which are curved but not inflexed in the bud (Fig. 29H). Pollen is inapeturate with croton-pattern surface (Fig. 29I). There is no trace of a gynoecium.

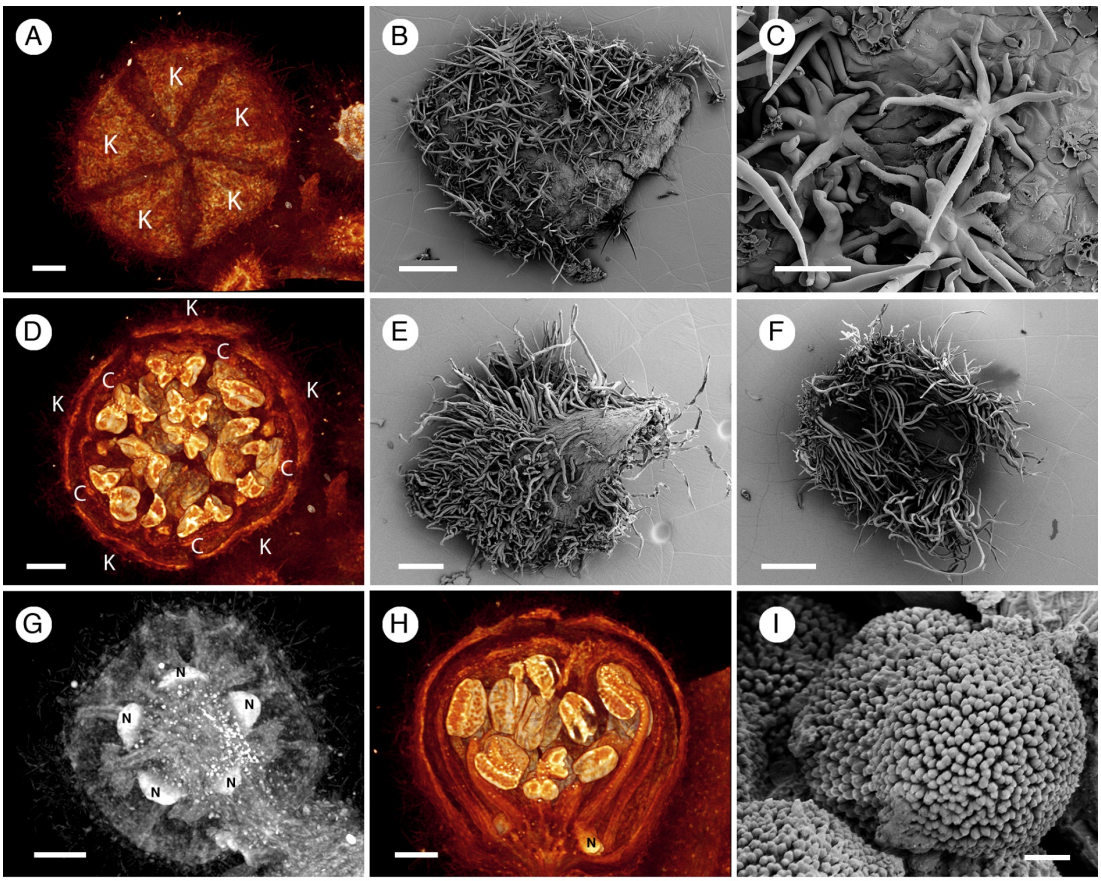

Figure 29. Morphological structures of male flowers of *B. mamoninha*. (A) An image obtained from micro-computed tomography shows a flower bud with five sepals. (B) An abaxial side of a sepal covered with stellate-derived trichomes.

Figure 29 continued. (C) Short dendritic trichomes with two layers of rays. (D) A transverse section shows the presence of non-inflexed stamens in bud. (E) Abaxial side of a petal covered with simple trichomes on the upper part. (F) An adaxial side of a petal shows simple trichomes densely packed along the margin. (G) A sectioning image shows the presence of five nectary glands. (H) A longitudinal section image shows non-inflexed stamens in bud, but they are curved toward the centre. (I) Inaperturate pollen with *Croton*-pattern. K, sepal; C, petal; N, nectary. Scale bars: (A, D, E, G, H) = 200 µm; (B, F) = 300 µm;(C) = 60 µm; (I) = 5µm.

Male floral vasculature and anatomy (*B. mamoninha*)

A male flower bud was included in the micro-computed tomography (Fig. 30A). Vascular bundles are arranged in a ring shape in the pedicel (Fig. 30B). At the base of the flower, five bundles extend to supply sepals (Fig. 30C, D). Then, five alternating bundles extend to supply petals (Fig. 30D-F). A nectary starts to appear at the same level as the base of petals but is without vascular supply (Fig. 30F, G). The rest of vascular bundles are arranged in a pentagonal shape with each angle pointing toward the petals (Fig. 30G). There are one or two outermost stamens in each angle and each stamen is supplied by a bundle (Fig. 30H). The rest of bundles are arranged alternating with each other (Fig. 30I). There are 20 stamens in this flower arranged as A 6+5+6+3 (Fig. 30I, J). Anthers are tetrasporangiate dithecous (Fig. 30K). From above, anthers are not at the same level as a result of bending of filaments (Fig. 30 L).

Figure 30 (next page). Micro-computed tomography images show anatomical structures of a male flower of *B. mamoninha*. (A) A top view of a flower with five sepals arranged in valvate aestivation. (B) A ring of vascular bundles in the pedicel. (C) Five bundles diverge to supply sepals. (D) Slightly higher up, another five bundles alternating with previous bundles supplying two adjacent sepals. (E) Five bundles supplying petals diverge from the base of previous alternisepalous bundles.

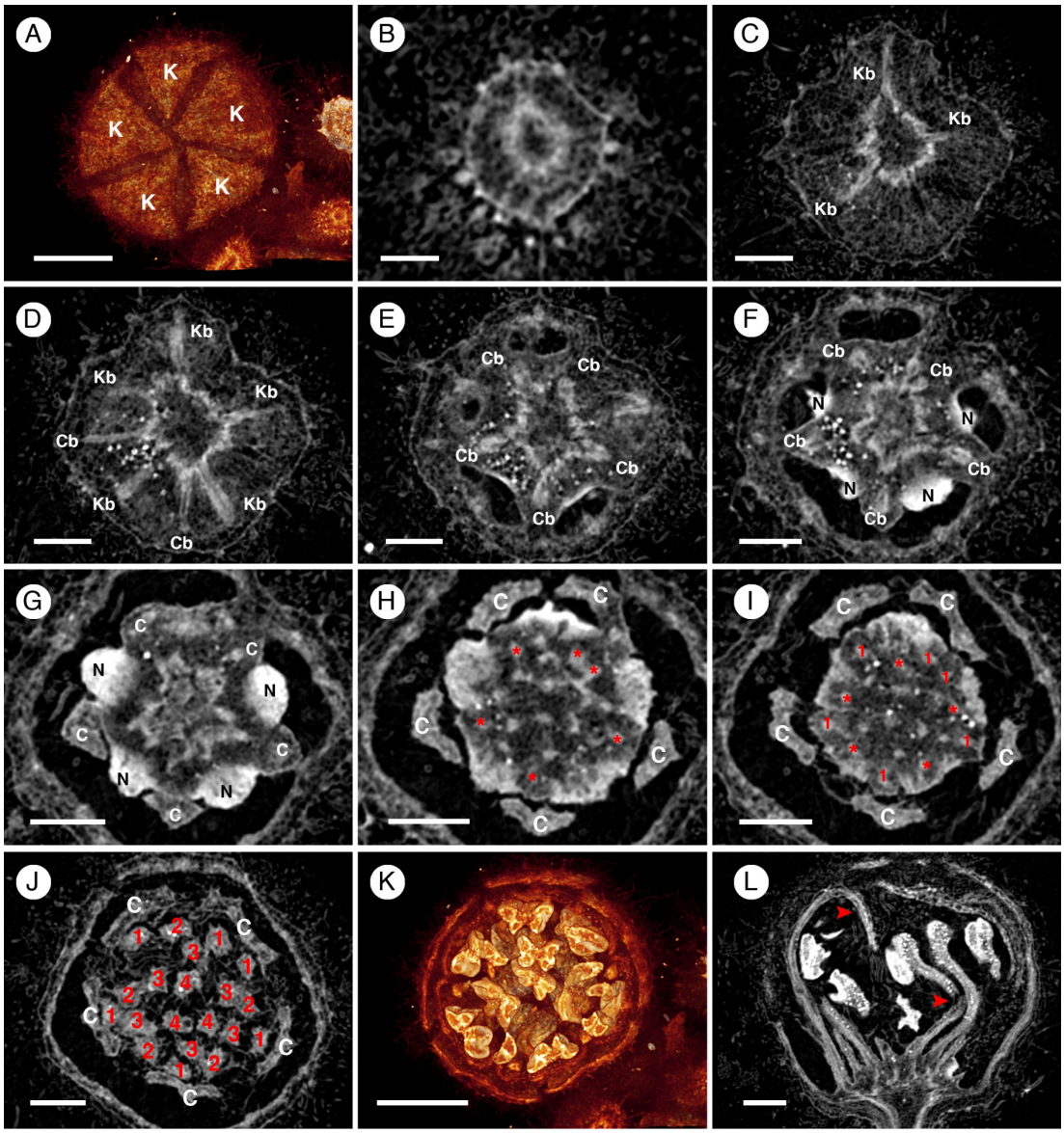

Figure 30 continued. (F) The nectary lobes are visible at the base of the petals. (G) Four nectary lobes are visible alternating with petals. The outermost bundles in the stele point toward the petals. (H) Slightly higher level, six outermost bundles extend to supply stamens (asterisks). There is one position with two stamens. (I) The next whorl of stamens alternates with the first whorl (asterisks) and the third whorl alternates with the second whorl (small circle). (J) In total there are 20 stamens. (K) A transverse section shows the stamens in the flower bud. Stamens are not inflexed in bud. (L) A longitudinal section of the flower bud shows non-inflexed stamens. However, they are curved toward the centre (arrowhead). Kb, sepal bundle; Cb, petal bundle; N, nectary; number indicate stamen whorl. Scale bars: (A, K) = 500; (B)=100 µm; (C-J, L) = 200 µm.

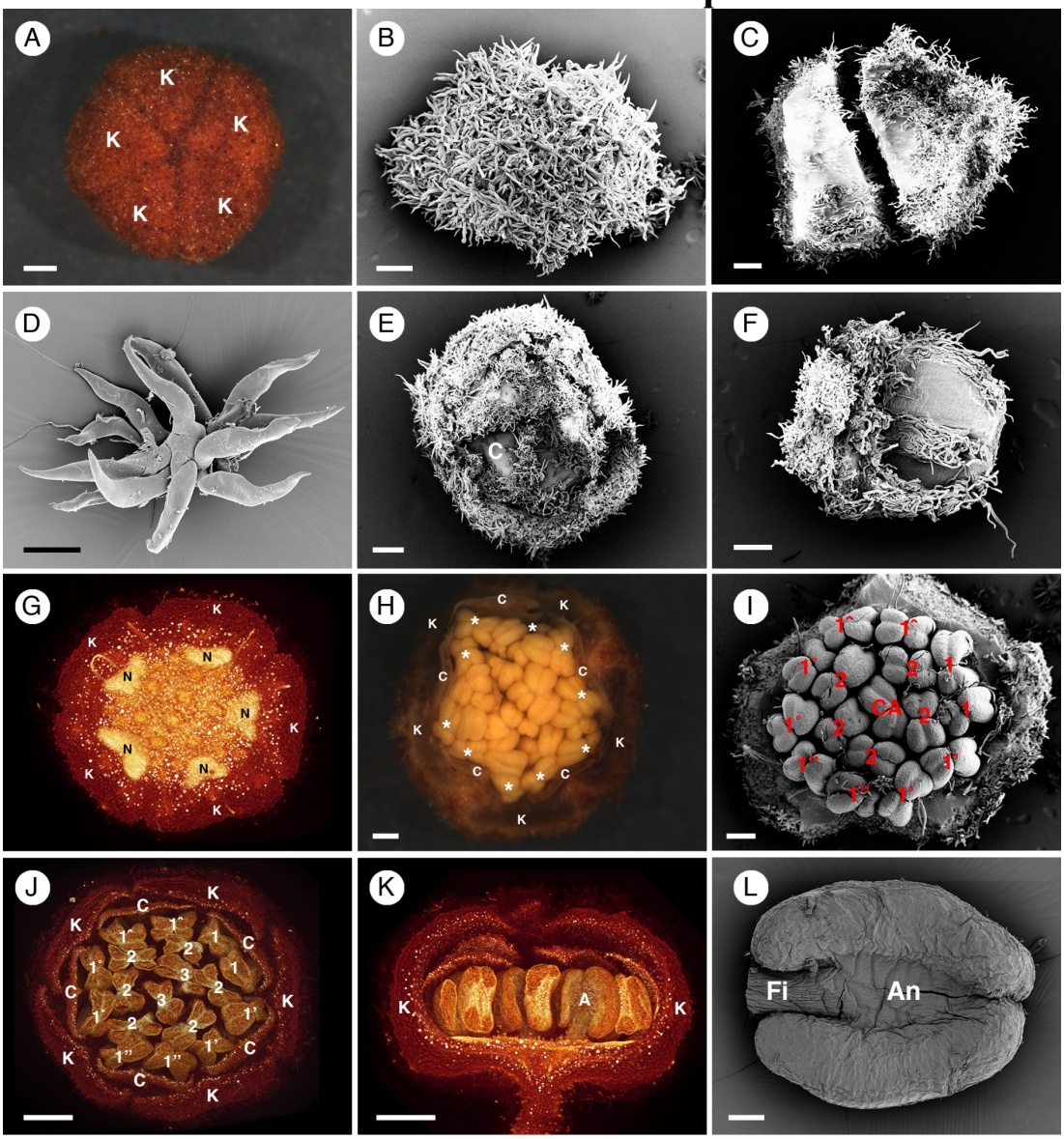

Figure 31. Morphological structures of male flowers of *B. muricatus*. (A) A flower bud with five sepals. (B) Abaxial side of a sepal densely covered with dense trichomes. (C) An adaxial side of a sepal with dense trichome along the margin. (D) A rosulate trichomes. (E) A flower with sepals removed shows adaxial side of petals with dense trichomes on the margin. (F) An adaxial side of a petal shows dense simple trichomes on the margin and midrib. (G) An image from sectioning shows five nectary glands opposite to sepals. (H) A flower bud with upper part of perianth removed shows arrangement of stamens in bud. There are five pairs of stamen arranged opposite to petals (asterisk). (I) A flower bud with 16 stamens (A 10+5+1) (J) An image from transverse sectioning shows the presence of 17 stamen in a flower bud (A 10+5+2). (K) A longitudinal section image shows erect stamens in bud. (L) A stamen with short filament making it erect in bud. K, sepal; C, petal; N, nectary; CA, central stamen; superscript sign indicates a pair of stamens. Scale bars: (A) = 500 µm; (B, C, F, H, I) = 200 µm; (D) = 50 µm; (E) = 300 µm.

Male floral morphology (*B. muricatus*)

Five sepals of male flower in *B. muricatus* are arranged in quincuncial aestivation (Fig. 31A). The abaxial surface of sepals is covered with stellate/rosulate trichomes (Fig. 31B, D). The adaxial side is glabrous on the lower part while the upper part is covered with stellate/rosulate trichomes (Fig. 31C, D). The abaxial surface is covered by dense trichomes on two edges (Fig. 31E). On the adaxial surface, the surface is glabrous, but areas on the two edges and along the midrib are densely covered with trichomes (Fig. 31F). Five bilobed nectary glands were observed surrounding the androecium. There are about 16-20 stamens present (Fig. 31H-J) inserted on flat receptacle (Fig. 31K). The outermost stamens are arranged in pair opposite the petals (Fig. 31H, I). In bud, filaments are short, so the anthers cannot be inflexed in bud (Fig. 31K, L). An ovary is absent.

Male floral morphology and vasculature (*B. muricatus*)

A male flower was included in the micro-computed tomography procedure (Fig. 32A). All parts of the flower are covered with stellate trichomes (Fig. 32B). The vasculature is ring shape in the pedicel (Fig. 32C). There are five bundles extend to supply the sepals (Fig. 32D-F); five alternating bundles extend to supply the petals (Fig. 32E, F). Each petal bundle further extends and provides lateral traces to adjacent sepals (Fig. 32E-G). There are five nectary glands located opposite to sepals (Fig. 32H). The rest of bundles is segregated into several whorls of stamens (Fig. 32H, I). There are 18 stamens in this flower with 10 outermost stamens forming five pairs opposite the petals surrounding another whorl of six stamens and two central stamens (A 10+6+2) (Fig. 32I-K). Petals have an outgrowth along the midrib of the adaxial side (Fig. 32L). The anthers are tetrasporangiate dithecous (Fig. 32L).

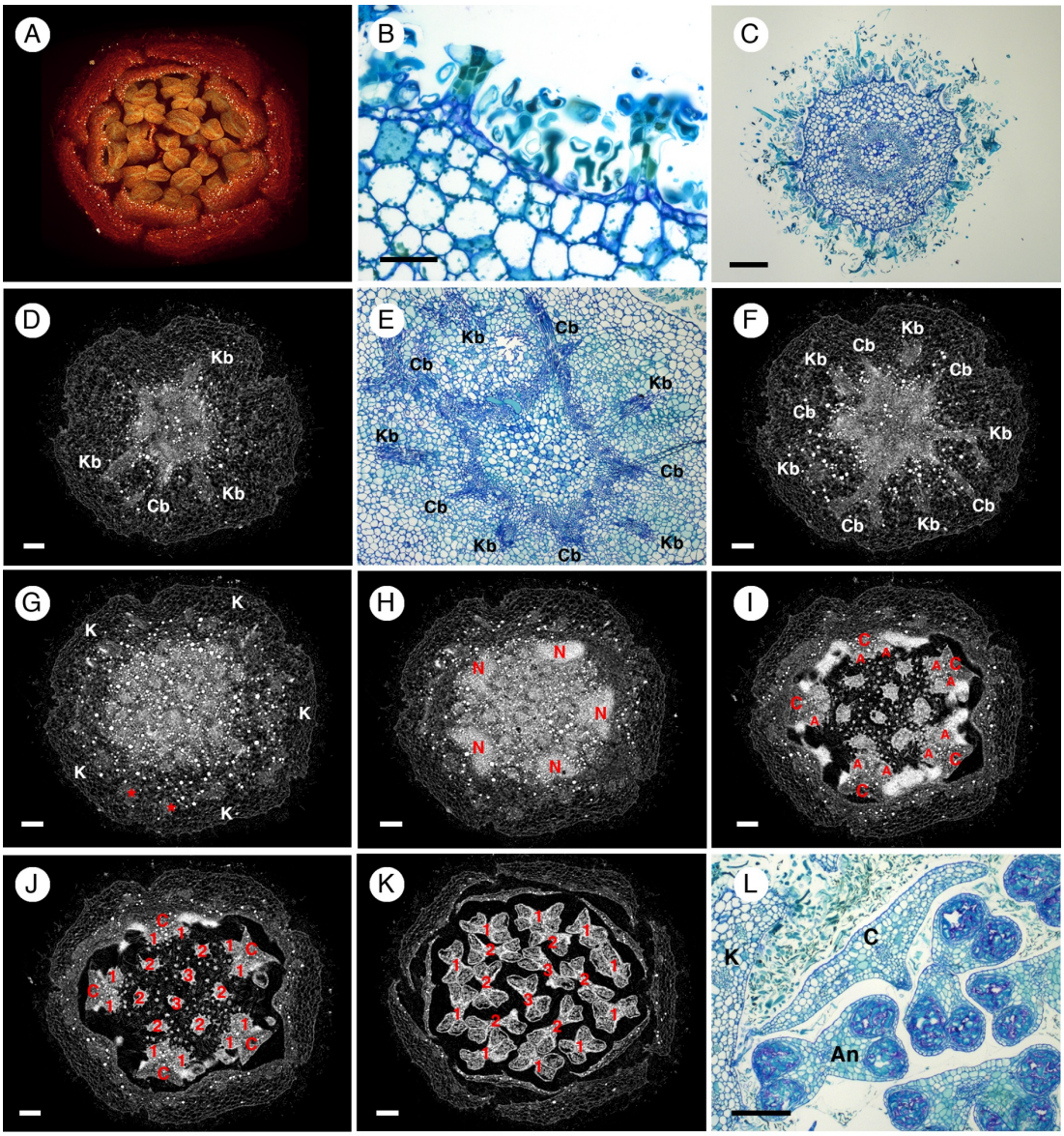

Figure 32. Anatomical structures of a male flower of *B. muricatus*. (A) A transverse section of a flower bud with visible calyx, corolla and androecium. (B) All parts of the flower are covered with stellate trichomes. (C) In the pedicel, vascular bundles form a ring. (D-F) Five outermost bundles extend to supply sepals while the next whorl of bundles extends to supply the petals. The petal bundles also branch to supply the lateral part of two adjacent sepals. (G) Two asterisks highlight the lateral vein of two sepals that are supplied from the same bundle that supply a petal. (H) At a higher level, five nectary lobes start to be visible. Nectary glands alternate with petals. (I) At the level above the receptacle, petal bases connect with the bases of two stamens. (J-K) There are 18 stamens in the flower. The outermost whorl comprises five pairs of stamens arranged opposite to petals. Alternate to the outermost whorl, the inner whorl comprises six stamens surrounding two central stamens. (L) Anthers are tetrasporangiate dithecal. Each petal has an outgrowth which may be formed from pressure of two adjacent stamens. Kb, sepal bundle; Cb, petal's bundle; N, nectary; C, petal; An anther; number indicate stamen whorl. Scale bars: (B) = 60 µm; (C-D, F-L) =200 µm.

Young fruit morphology and vasculature of B. mamoninha

A young fruit extracted from a herbarium sheet was included in this study (Fig. 33A). Five sepals are present below a verrucose ovary (Fig. 33A). In the pedicel, vasculature is ring shaped (Fig. 33B). At the base of the fruit, there are bundles supplying sepals (Fig. 33C-F) alternating with five bundles supplying senescent organs which may be reduced petals (Fig. 33E, F). There is remnant of five nectary lobes superposed to sepals (Fig. 33G, H). One of the previous nectary lobes has glandular structures present on the surface (Fig. 33H). At the centre of the fruit, there is a tricarpellate pistil with axile placentation (Fig.33I). There is one anatropous ovule per locule superposed by an obturator (Fig. 33I, J). Three bifid styles are present on top of the pistil (Fig. 33A, K). There are dehiscence lines visible, so the fruit is a loculicidal capsule (Fig. 33I, K).

Female flower morphology of B. muricatus

There are five sepals with dense stellate/rosulate trichomes on the abaxial surface (Fig. 34A, B). The adaxial surface of sepals is also covered with stellate/rosulate trichomes (Fig. 34C). Inside the calyx, there are five miniature petals present (Fig. 34D-H). On two sides of a petal, two primordia appear (Fig. 34E-H) with sometimes a third primordium in between two petals (Fig. 34E). Alternate with petals, there are five nectary glands present (Fig. 34D). Inside the nectary whorl, there is a tricarpellate ovary with axile placentation. The styles are bifid with a total of six stigmatic tips (Fig. 34H).

Female flower vasculature and anatomy (*B. muricatus*)

A young female flower was included in the micro-computed tomography (Fig. 35A). The sectioning reveals that in the pedicel, the vasculature forms a ring (Fig. 35B). At the base of the flower, five bundles extend to supply sepals (Fig. 35C). Alternating to the sepal bundles, five bundles extend and fork to supply adjacent sepals (Fig. 35C, D). Moreover, the same bundles also branch to supply small petals (Fig. 35D, E). There are two or more structures adjacent to each petal and they were supplied

by additional bundles (Fig. 35E-G). A nectary appears on the hypanthial area above the level of the receptacle (Fig. 35E, H, I). The pistil comprises three carpels with axile placentation and one ovule in each locule (Fig. 35J). There are three styles each supplied by a bundle; styles fork in the upper part to supply bifids stigma (Fig. 35L).

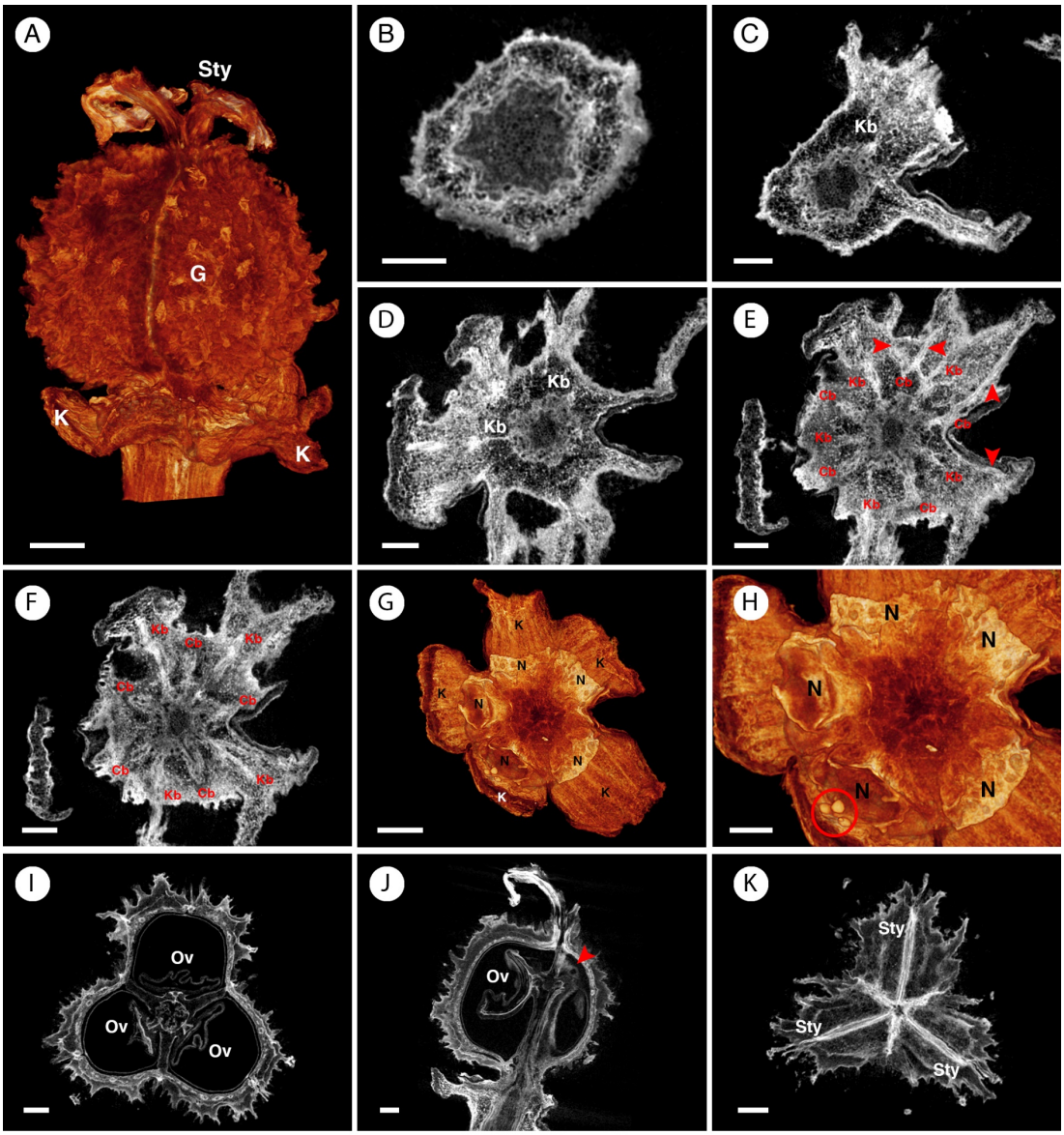

Figure 33. Anatomical and morphological structures of a fruit of *B. mamoninha.* (A) A side view with sepals, ovary and style visible. (B) A transverse section shows a vascular ring in the pedicel. (C-D) Vascular bundles extend from the vascular ring to supply sepals. (E) Alternating with sepal bundles, five bundles diverge and fork to supply lateral veins of two adjacent sepals (arrowheads). (F) slightly higher view from the previous image; the alternisepalous bundles extend to supply organs which could be reduced petals. (G) At the base of the ovary, there are five lobes of nectary glands. (H) One of the nectary glands have several glandular structures.

Figure 33 continued. (I) The ovary is tricarpellate syncarpous with axile placentation. (J) An ovule (or a young seed) is present inside a locule superposed by a remnant of obturator. (K) There are three bifid styles on top of the ovary. K, sepal; G, pistil; Sty, style; Kb, sepal bundle; Cb, petal bundle; N, nectary; Ov, ovule. Scale bars: (A, G) = 1,000 µm; (B, D-F, H-K) = 500 µm; (C) =100 µm.

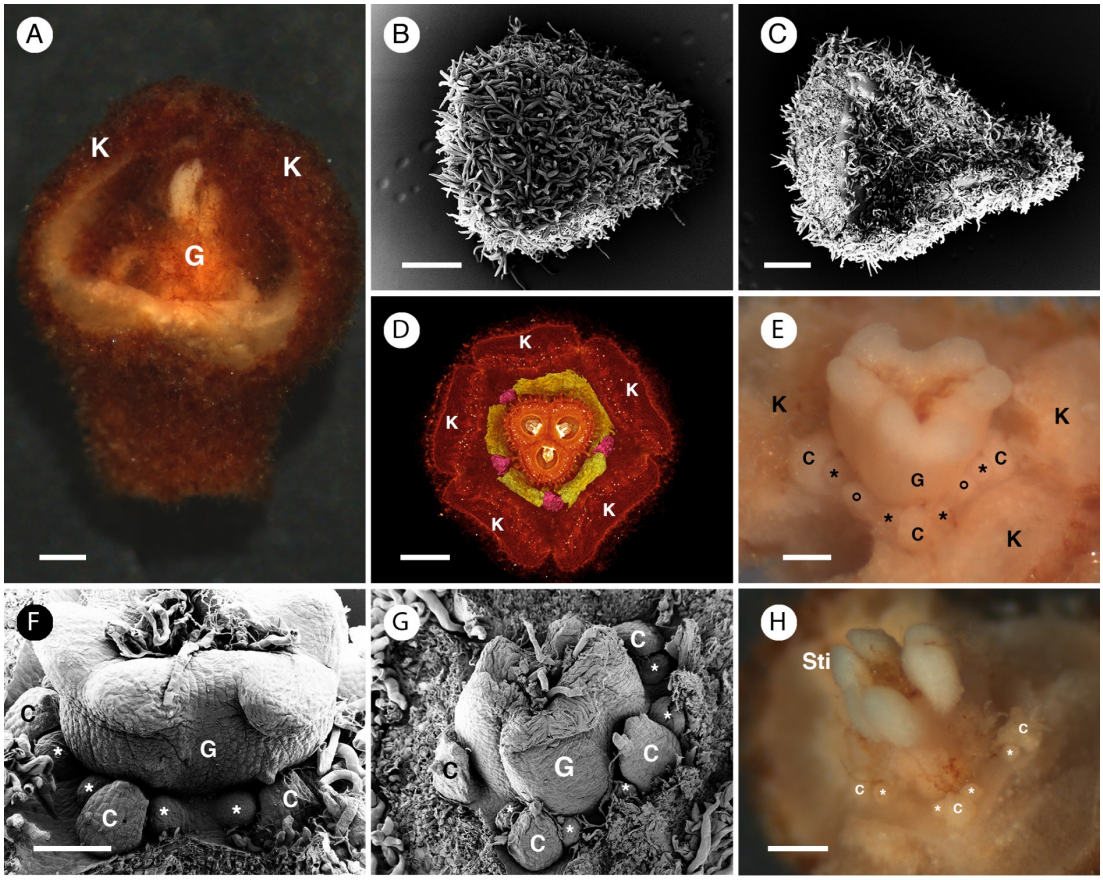

Figure 34. Morphological structures of female flowers of *B. muricatus.* (A) A flower bud with partially removed sepals shows the presence of minute petals (asterisk) and an ovary. (B) Abaxial side of a sepal is covered with trichomes. (C) Adaxial side of a sepal is covered with trichomes. (D) An image from sectioning shows the presence of five sepals surrounding five minute petals (pink colour), five nectary lobes (yellow colour) and a tricarpellate ovary. (E) A dissected young flower bud shows the presence of minute petals adjacent to two primordia (asterisk). There is a primordium in the middle between two petals as well (small circle). (F-G) There are two primordia adjacent to each petal (asterisk). (H) There are three bifid styles on top of the ovary. Note, three petals are visible, each with two adjacent primordia. K, sepal; G, pistil; C, petal. Scale bars: (A-C) = 300 µm; (D) = 500 µm; (E-G) = 100 µm; (H) = 200 µm.

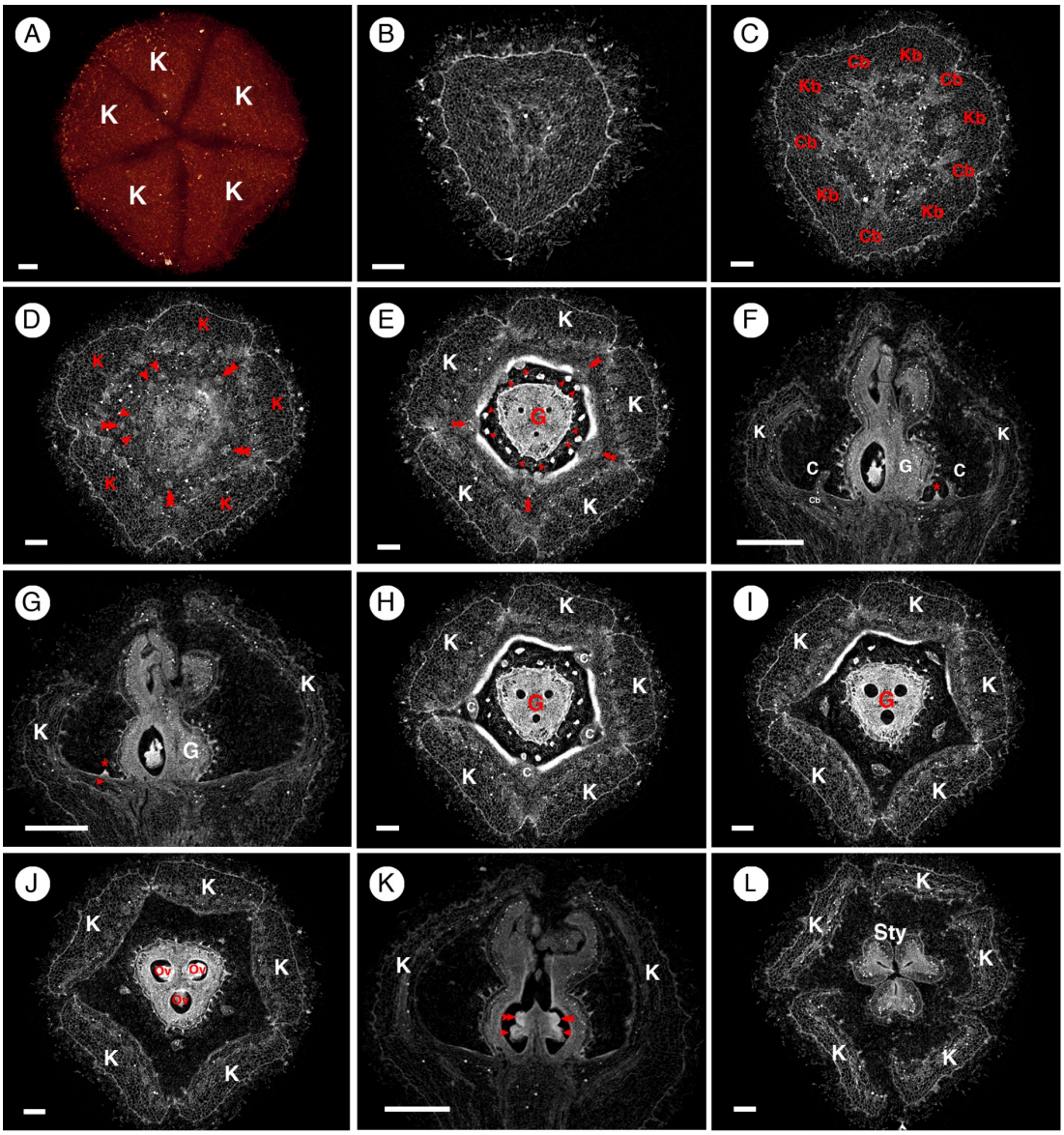

Figure 35. Micro-computed tomography images show anatomical structures of a female flower of *B. muricatus*. (A) A top view of a flower bud with five sepals arranged in valvate aestivation. (B) There is a vascular ring in the pedicel. (C) Five sepal bundles are the first whorl to diverge followed by five alternating petal's bundles. Each of the petal bundles further extend and fork to supply lateral veins in two adjacent sepals. (D-E) In this flower, there are four petals visible (double arrowhead). Adjacent to each petal, there are two primordia present (arrowhead). (F-G) A longitudinal section through the flower shows a petal supplied by a bundle. The unknown structure is also supplied by a bundle (asterisk). (H) Five nectary glands are opposite to sepals. (I-J) There are four petals present in this flower. The ovary is tricarpellate syncarpous with axile placentation. (K) In a locule, there is a structure resembling an ovule (arrowhead) superposed by an obturator (double arrowhead). (L) There are three bifid styles on top of the ovary. K, sepal; kb, sepal's bundle; Cb, petal's bundle; G, pistil; Ov, ovule; Sty, style. Scale bars: (A-E, H-J, L) = 200 µm; (F, G, K) = 500 µm.

2.4.6 *Croton*

Inflorescence morphology

Several species from all four subgenus of *Croton* were included in this study (Table 1). Additional data were obtained from the herbarium and descriptions in the literature. Inflorescences of *Croton* are basically thyrses (indeterminate raceme with cymes) with variable subunits (cymules) (Fig. 36A). Cymules could be bisexual (Fig. 36B, C) or unisexual (Fig. 36D, E). Moreover, from a structural perspective, cymules could be classified as dichasial cymes (Fig. 36B) or monochasial cymes (Fig. 36C-E). In many species of *Croton*, cymules are reduced to solitary flowers which could be described as racemes or spikes (Fig. Fig. 36F0-J). Combination of cymes and solitary flowers could be found in the same inflorescence (Fig. 36A). Sometimes partial thyrse inflorescence is occur by the presence of few cymules in the inflorescence with solitary flowers as the majority. In some groups, reduction of flower number result in inflorescences appearing determinate but without an older terminal flower (Fig. 36H, I). In the section *Julocroton*, capitate inflorescences are actually condensed racemes (Fig. 36J). Apart from reductions appearing in the genus, some unique *Croton* species have elongated subunits. *C. nubigenus* from the monotypic section *Nubigeni* has elongated cyme (Fig. 37A-C). Older flowers are produced on the proximal part while younger flowers emerge on the distal part (Fig. 37B, C). *Croton rusbyi* from the section *Cyclostigma* has panicles (Fig. 37D) with elongated subunits ending with a terminal female flower subtended by male cymules (Fig. 37E). In each subunit, there is generally one terminal female flower (Fig. 37E) but two or more female flowers could be found in some subunits in some specimens (Fig. 37F, G). Along the inflorescence axis, lower branches are longer than upper branches similar to inflorescences of *Brasiliocroton* (Fig. 37D). In the herbarium specimens, the terminal part is generally broken, but in one sample we found a male flower at the apex of the inflorescence apex (Fig. 37H). However, examination of fresh samples would verify our observation. We found one inflorescence of *C. rusbyi* with short branch subunits resembling the general thyrse of the genus (Fig. 37I).

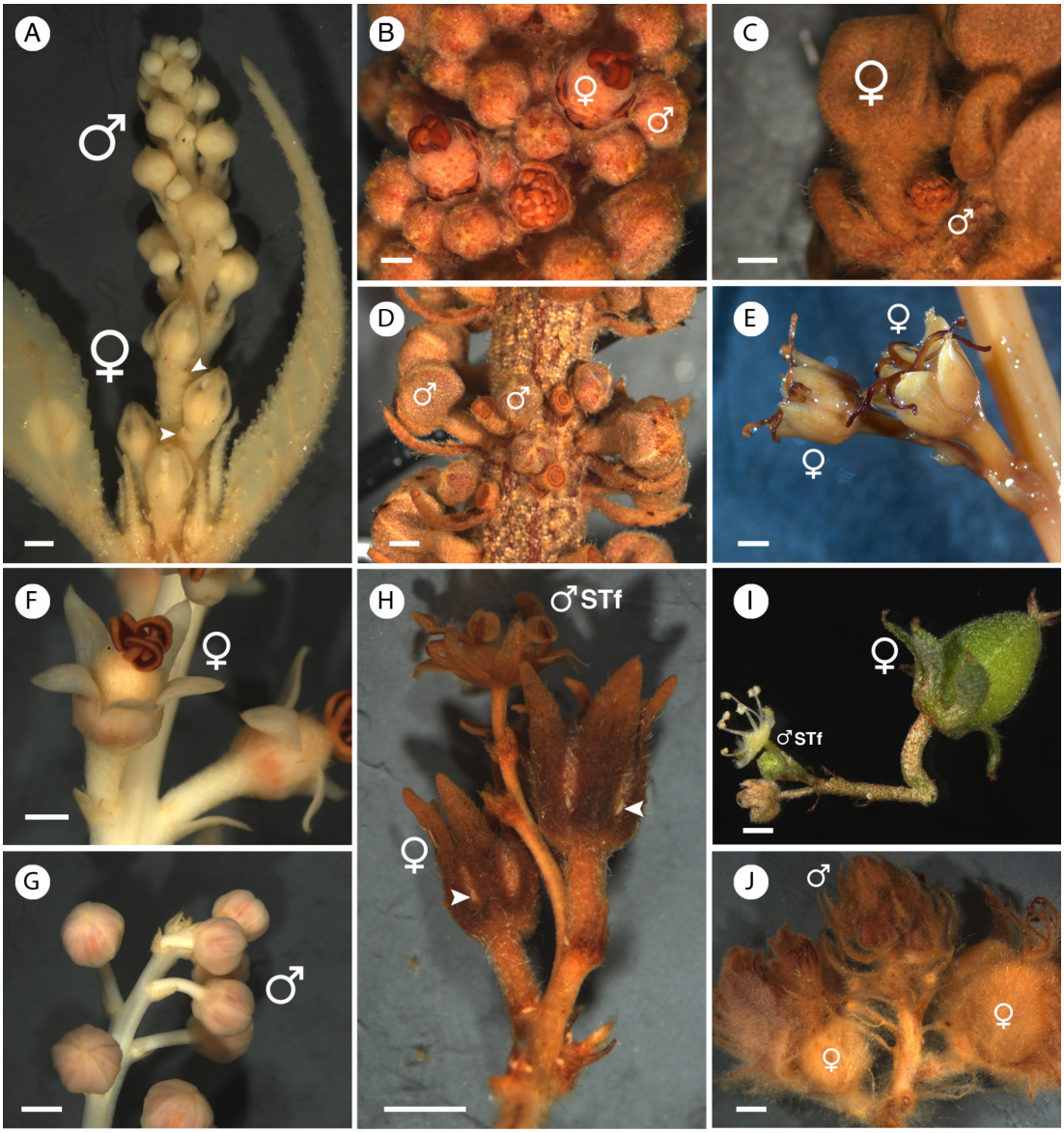

Figure 36. Morphological structure of inflorescence of *Croton*. (A) *C. bonplandianus*; (B) *C. urucurana*; (C-D) *C. floribundus*; (E) *C. fluviatilis*; (F-G) *C. tiglium*; (H) *C. trinitatis*; (I) C. monanthogynus; (J) *C. argenteus*. (A) An inflorescence with female flowers on the proximal part and male flowers on the distal part. Some female flowers have a male flower borne on the side (arrowhead). (B) An inflorescence with bisexual dichasial cymules each with a central female flower surrounded by male flowers. (C) Bisexual monchasial cymules with a female flower grouped with a male flower. (D) Monochasial male cymules. (E) A cymule comprises only female flowers. (F) An inflorescence with solitary female flowers. (G) An inflorescence with solitary male flowers. Note the apical part is indeterminate. (H) A raceme with all flowers solitary. The inflorescence ends with a subterminal male flower subtended by bracts. Note the presence of filamentous structures in female flowers (arrowhead). (I) An old raceme with a fruit on the proximal part. The inflorescence ends with a subterminal male flower. (J) A condense raceme of *Croton* section *Julocroton*. STf, subterminal flower. Scale bars: (A, B) =500 µm; (C-J) = 1,000 µm

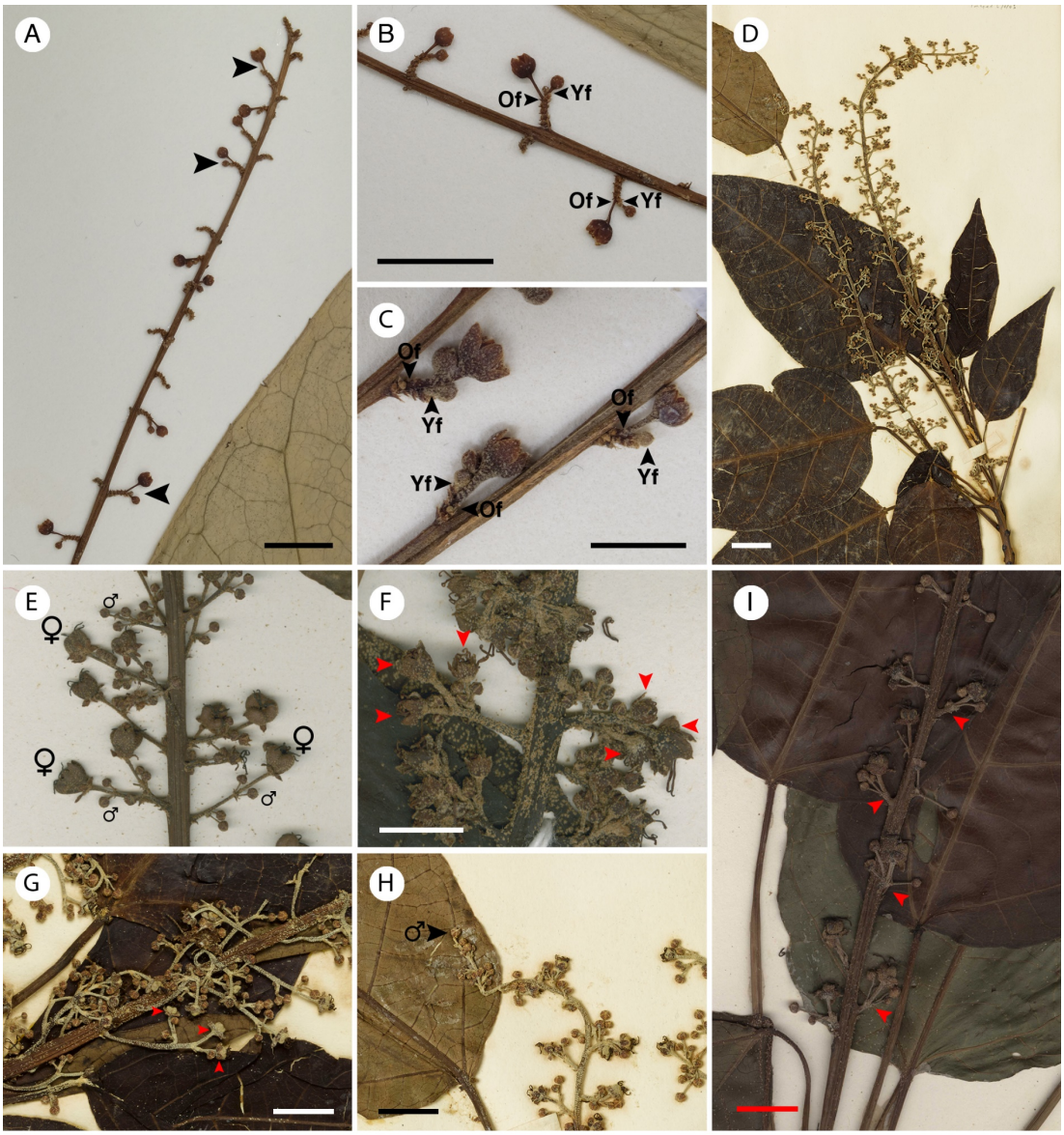

Figure 37. Inflorescences with elongated subunits in *Croton*. (A-C) *C. nubigenus*; (D-I) *C. rusbyi*. (A) An inflorescence with elongation of cymule primordia (arrowhead). (B-C) On the elongated cymules, older flowers are found at the lower part and younger flowers on the upper part. (D) A panicle type inflorescence with long side branches. (E) Detail of long side branches with a terminal female flower subtended by two male cymes. (F-G) In some inflorescences, there are several female flowers (arrowheads) on one side branch. (H) An inflorescence with a terminal male flower. (I) An inflorescence with short side branch similar to typical condense cymules of general *Croton* (arrowhead). Of, older flower; Yf, younger flower. Scale bars: (A-C, F-I) = 1 cm; (D) = 2 cm.

Male floral morphology

Male flowers of *Croton* are usually pentamerous with two perianth whorls. Sepals of *Croton* are generally densely covered with trichomes (Fig. 38A), but some species may have very few trichomes (Fig. 38B). The apex of each sepal is usually lined with simple trichomes that are entangled with trichome from other sepals (Fig. 38B). Trichome types on the surface of sepals are highly variable in the genus, e.g, stellate (Fig, 38C, D), stellate-lepidote (Fig. 38E) and lepidote (Fig. 38F). Petals are generally glabrous on both surfaces with simple trichomes on the margin (Fig. 38G-I). In many species, long simple trichomes are found on the lower part of the adaxial side of petals (Fig. 38H). Some species, e.g., *C. alabamensis* and *C. sampatik*, produce only short trichomes on the margin (Fig. 38I). Inside the corolla whorl, there are five nectary glands alternating with petals and outermost stamens (Fig. 38J). Stamens in *Croton* are generally arranged in several whorl of five (Fig. 38K). However, previous literature reported a high variation in the number of stamens ranging from two to more than 100 (Alain, 1960; van Ee et al., 2008; Riina et al., 2009; Webster, 2014). The outermost stamens are opposite the petals in most species (Fig. 38K). In nearly all species, filaments are inflexed in bud making anthers point to the outside (Fig. 38K). Few species, e.g., *C. alabamensis*, have non-inflexed stamens (Fig. 38L). Generally, stamens are inserted on a convex receptacle (Fig. 38K). However, stamens in *C. alabamensis* are inserted on concave receptacle and the outermost whorl alternates with petals (Fig. 38L).

Figure 38 (next page). Variation of morphological structures of male flowers of *Croton.* (A, K) *C. urucurana*; (B) *C. bonplandianus*; (C, H) *C. monanthogynus*; (D, I) *C. sampatik*; (E) *C. polyandrus*; (F, L) *C. alabamensis*; (G) *C. rusbyi*; (J) *C. tiglium*. (A) A flower bud covered with stellate trichomes. (B) A flower bud with glabrous sepals. (C) Stellate-appressed type trichomes with a central projection (porrect). (D) Stellate type trichomes with short porrect. (E) Stellate-lepidote type trichomes.

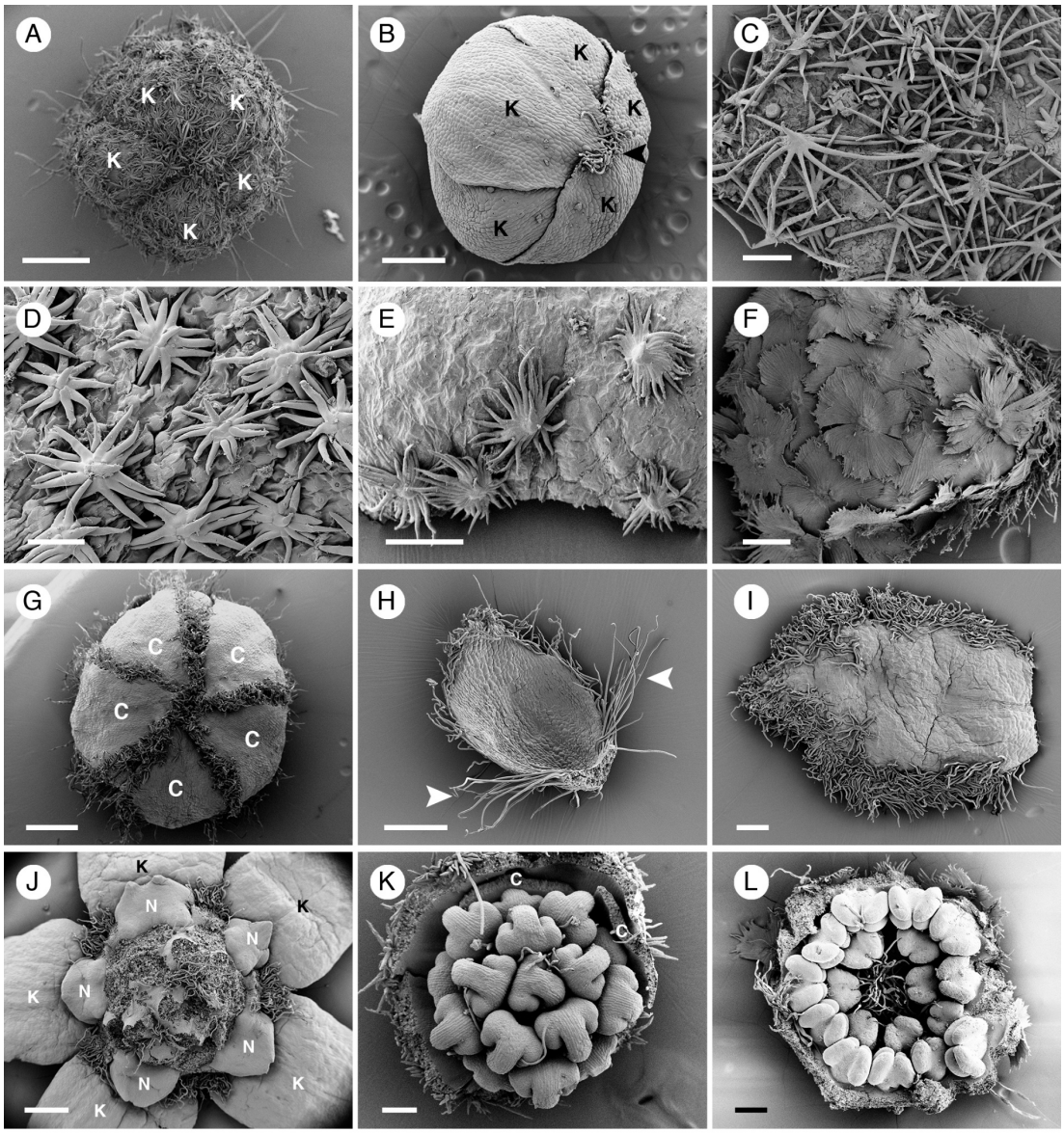

Figure 38 continued. (F) Lepidote type trichomes. (G) A flower bud with sepals removed. Five sepals with fimbriate margin are visible. (H) A petal with clusters of long simple trichomes at the lower part on the adaxial side (arrowhead). (I) Abaxial side of a petal with fimbriate margin lined with dense simple trichomes. (J) A flower with petals and stamens removed shows five nectary lobes opposite to sepals. (K) A flower with 16 stamens arranged in three whorls of five surrounding a central stamen. The outermost whorl is opposite petals. (L) A flower with 18 stamens inserted on the hypanthium with central empty space. K, sepal; C, petal; N, nectary. Scale bars: (A) = 500 µm; (B, F, H, I, L) = 200 µm; (C-E, K) = 100 µm; (G) = 500 µm; (J) = 50 µm;

Male floral vasculature and anatomy

Male flowers from six species of *Croton* were examined (Fig. 39). At the pedicel level, vascular bundles form a ring (Fig. 39A). At the base of the flower, five bundles extend to supply sepals (Fig. 39B, C). Slightly higher, five bundles extend to supply petals in alternation with the sepal bundles (Fig. 39B, C). Sepals are fused near the base (Fig. 39B, C). Five bilobed nectary glands appear above the sepal base (Fig. 39C). In *C. chilensis*, *C. floribundus*, *C. polyandrus* and *C. sampatik*, the outermost stamen bundles supply the antepetalous stamens (Fig. 39D, E). Subsequently bundles depart in alternation with the previous whorl (Fig. 19D, E). Stamen number is variable, ranging from two (*C. maestrensis*), 11 (*C. polyandrus*), 16 (*C. floribundus*), 17 (*C. alabamensis*) and 20 (*C. chilensis*). In *C. maestrensis*, the flower is tetramerous with four sepals, four petals, four nectary glands and two alternipetalous stamens (Fig. 39F). In most species, stamens are inserted on the convex receptacle (Fig. 39G). There are five pairs of nectary glands in the male flower of *C. alabamensis* adjacent to each stamen (Fig. 39H). Each nectary gland is supplied by a vascular strand branching from a sepal bundle (Fig. 39I).

Female floral morphology

Five sepals are fused at the base. Sepals of *Croton* are densely covered on the abaxial surface with trichomes of the stellate or lepidote types (Fig. 40A, B). However, in some species, the surface may be glabrous (Fig. 40C). On the adaxial side, the surface is generally glabrous or with some sparse trichomes (Foig. 40A, C). Dense crushed trichomes are found at the margin of sepals. Dense simple trichomes are generally found at the apex of sepals (Fig. 40C), but they could be absent in some species (Fig. 40A). Alternating with sepals, there are filamentous structures in the same position of the petals in male flowers (Fig. 40D, E). In a few species, fully developed petals are present (Fig. 40B). Inside the perianth whorl, five nectaries alternate with the filamentous structures and are opposite to sepals (Fig. 40D, E). Nectary lobes could be separate or form a ring (Fig. 40D, E). The *Croton* gynoecium consists of a tricarpellate ovary with axile placentation (Fig. 40F). The outer ovary

surface is densely covered by trichomes (Fig. 40F, G). There are three styles on top of the ovary (Fig. 40G). The branching pattern of the styles is extensive, ranging from simple to bifid or multifid (Fig. 40B, G, H).

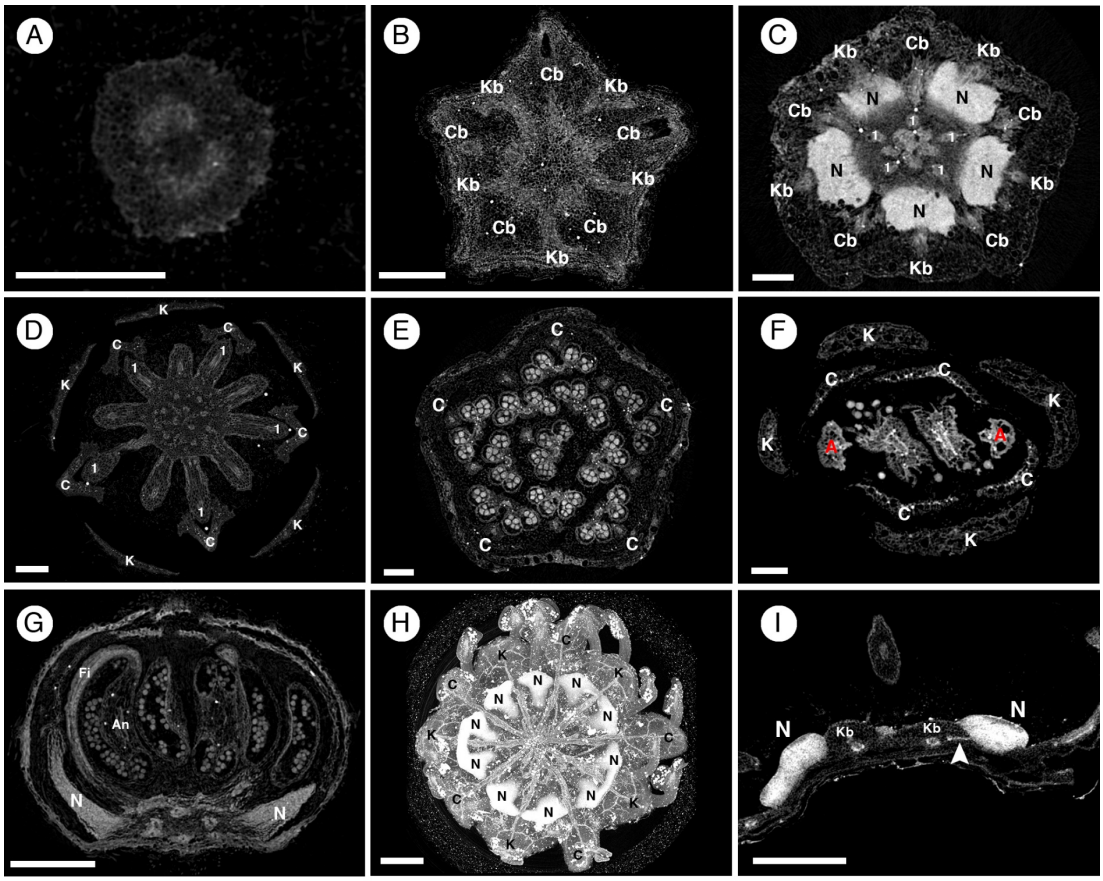

Figure 39. Micro-computed tomography images show anatomical structures of male flowers of *Croton*. (A, D) *C. chilensis*; (B) *C. floribundus*; (C, E) *C. polyandrus*; (F) *C. maestrensis*; (G) *C. sampatik*; (H, I) *C. alabamensis*. (A) A ring vascular bundle in the pedicel. (B) At the base of flower, there are five bundle diverge to supply sepals alternating with five bundle supplying petals. (D) A transverse section of a flower shows the arrangement of stamens with five outermost stamens arranged opposite to petals. (E) A flower with 11 stamens arranged in two whorls surrounding a central stamen. The outermost whorl is opposite to petals. (F) A flower with two stamens arranged alternating with petals. (G) A longitudinal section of a flower shows inflexed stamens in bud. (H) A flower with ten nectary lobes. (I) A longitudinal section of a flower shows a trace diverge from a sepal bundle to supply a nectary gland (arrowhead). Kb, sepal bundle; Cb, petal bundle; N, nectary; K, sepal; C, petal; A, stamen; Fi, filament; An, anther. Scale bars: (A, B, D, G) = 500µm.; (C, E, F) = 200µm; (H, I) = 1,000 µm.

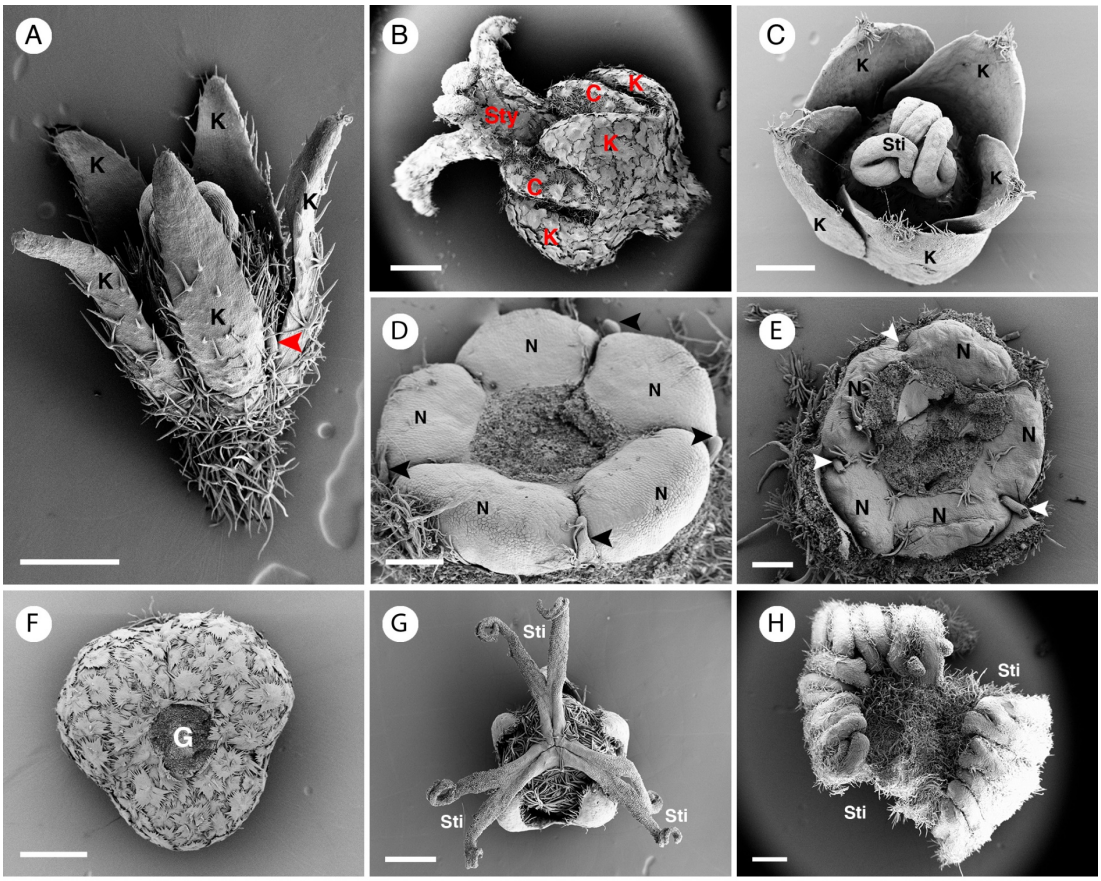

Figure 40. Variation of morphological structures of female flowers of *Croton.* (A) *C. trinitatis*; (B). *C. alabamensis*; (C, F) *C. fluviatilis*; (D) *C. chilensis*; (E) *C. urucurana*; (G) *C. bonplandianus*; (H) *C. floribundus*. (A) Lateral view of a flower with conspicuous sepals covered with some trichomes. There is a minute glandular filamentous structure present alternating with sepals (arrowhead). (B) A female flower with fully developed petals. (C) A top view of a flower with cochlear aestivation of sepals. Three bifid styles are present on the ovary. (D) A flower with sepals and ovary removed shows five nectary glands alternating with filamentous structures. (E) A five-lobed nectary ring with traces of filamentous structures. (F) A three-lobed ovary covered with lepidote trichomes. (G) A flower with three bifid styles (six stigmatic tips). (H) Styles with multiple branching (multifid). K, sepal; C, petal; Sty, style; Sti, stigma; N, nectary; G, pistil. Scale bars: (A, C, F-G) = 500 µm; (B) = 1,000 µm; (D) = 300 µm; (E) = 200 µm.

Female floral vasculature and anatomy

Samples from four species of *Croton* were included in this study (Fig. 41). In the receptacle, a vascular ring is present (Fig. 41A). Higher up the five outermost bundles extend to supply sepals (Fig. 41B, C) and five alternating bundles extend to

supply the filamentous structures (Fig. 41B) or petals (Fig. 41C). Five nectary glands are visible opposite the sepals (Fig. 41C, D). In most species, there are five alternating filamentous structures present outside the nectary whorl (Fig. 41D, E). In some species, the filamentous structures become glandular (Fig. 41E). The ovary of all examined species is comprised of three fused carpels with axile placentation (Fig. 41F). In female flower of *C. alabamensis,* there are petals present instead of filamentous structures (Fig. 41G). In all species, there is one ovule per locule in the ovary (Fig. 41F-H). The ovule is bitegmic, anatropous with the presence of a nucellar beak (Fig. 41H). Each ovule is superposed by an obturator (Fig. 41H). On top of the pistil, three bifid styles are commonly found. In some species, e.g., *C. floribundus*, multifid styles are present (Fig. 41I).

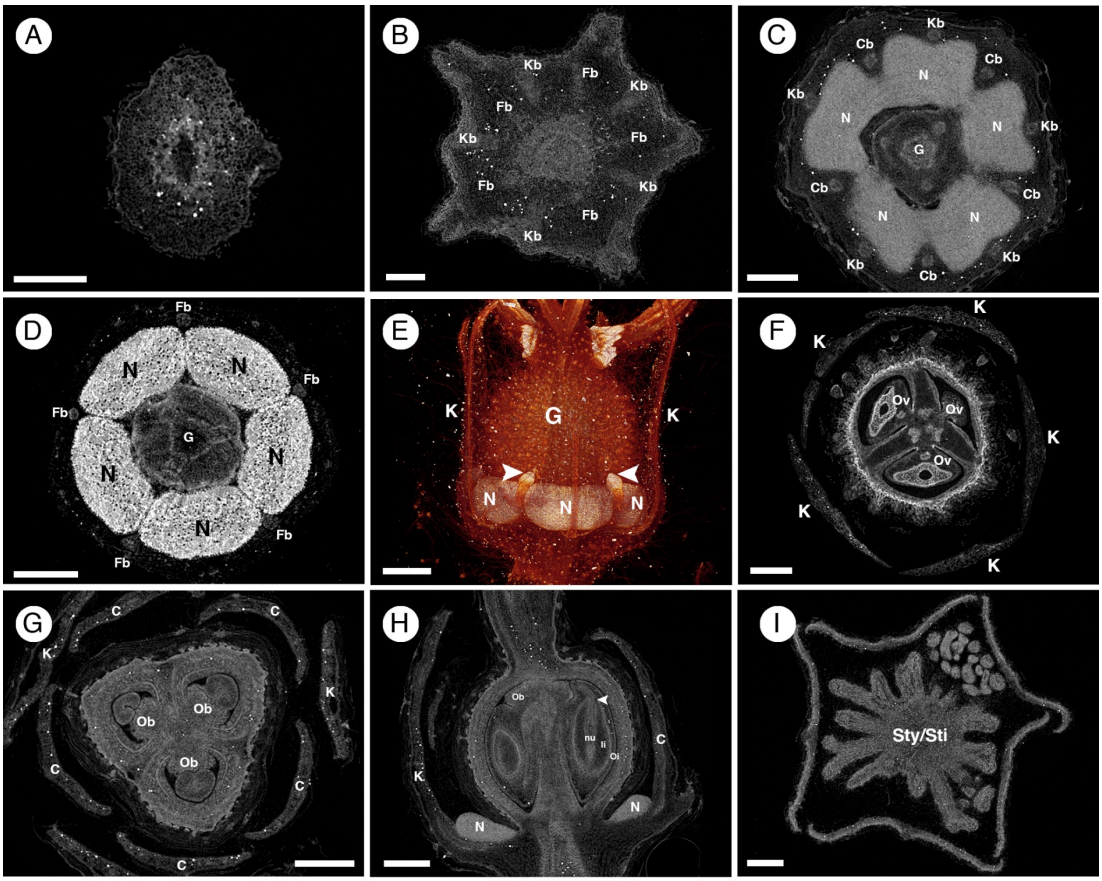

Figure 41. Micro-computed tomography images show anatomical structures of female flowers of *Croton*. (A, F) *C. polyandrus*; (B, I) *C. floribundus*; (C, G, H) *C. alabamensis*; (D, E) *C. chilensis*. (A) Ring vasculature in the pedicel. (B) A transverse section through the base of a flower show five bundle extending to supply sepals, while the other five alternating bundles extend to supply filamentous structure.

Figure 41 continued. (C) A nectary ring comprising five lobes is present in female flower. Alternating with nectary lobes, there are five bundles supplying the fully developed petals. (D) A five-lobed nectary in a female flower. (E) Side view of a flower shows glandular filamentous structures alternating with nectaries (arrowheads). (F) A transverse section shows a tricarpellate ovary with axile placentation. (G) A tricarpellate ovary with axile placentation surrounded by fully developed petals and sepals. (H) A longitudinal section shows bitegmic ovule with thick inner integument. A part of the nucellus protrudes past the micropyle (nucellar beak) (arrowhead). (I) A transverse section through a multifid style. Kb, sepal's bundle; Fb, filamentous structure's bundle; Cb, petal bundle; N, nectary; G, pistil; Ov, ovule; Ob, obturator; Oi, outer integument; Ii, inner integument; nu, nucellus; Sty, style; Sti, stigma. Scale bars: (A-I) = 500 μm.

2.4.7 Phylogenetic tree of *Croton* and related genera

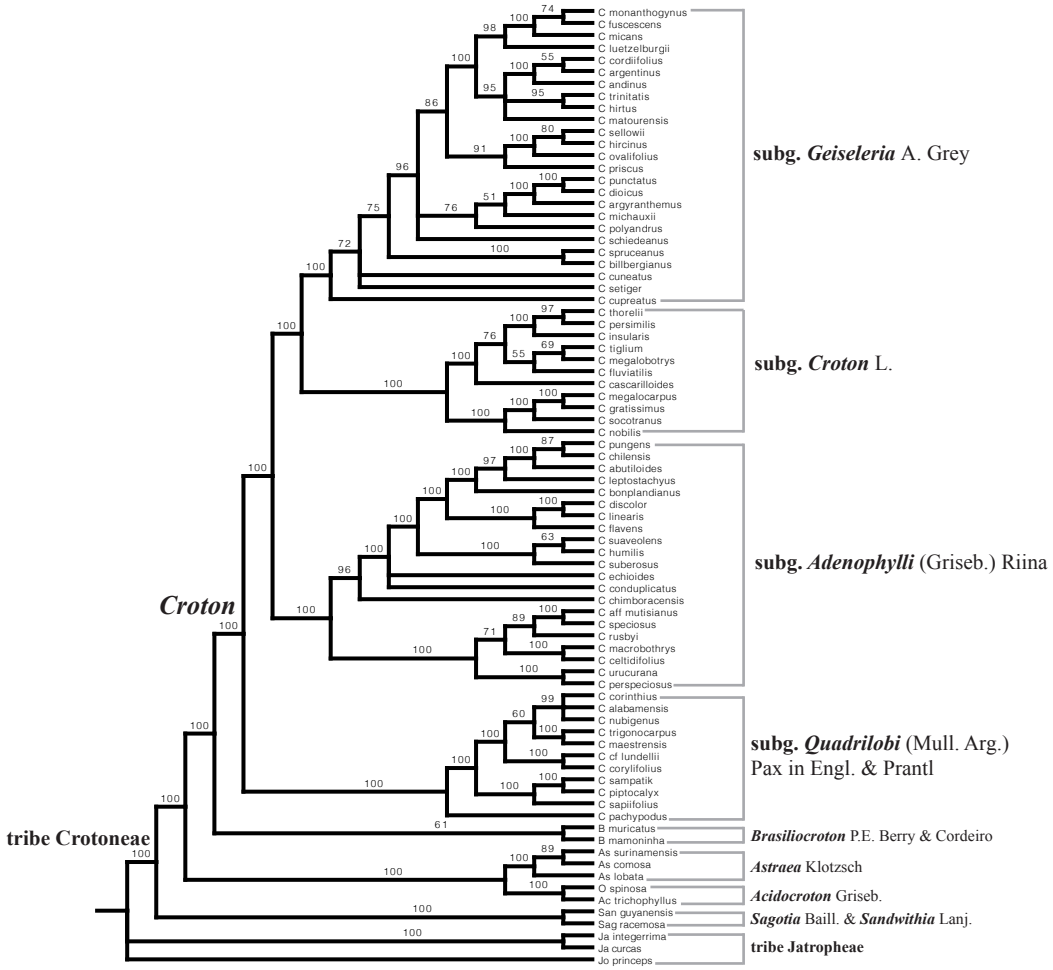

Figure 42. Cladogram from the Bayesian analysis of combined ITS and *trnL-F* of *Croton* and related genera. Bayesian posterior probability values are shown on each branch. Labels indicate tribe, genus and subgenus.

The topology of the phylogenetic tree we obtained is similar to many previous studies (Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2011; van Welzen et al., 2020). The tribe Crotoneae is divided into two clades. The first clade (SS clade) comprises two genera, i.e., *Sagotia* and *Sandwithia* (Fig. 42). The second clade (AABC clade) comprises four genera with *Astraea-Acidocroton* subclade and *Brasiliocroton-Croton* subclade (Fig. 42). Within *Croton*, four monophyletic subgenera are reconstructed. We exclude section *Olivacei* (*C. olivaceus*) from the analysis due to its uncertain identity (R. Riina, personal communication). There are some polytomies present but most of the position are well-resolved.

2.4.8 Character evolution

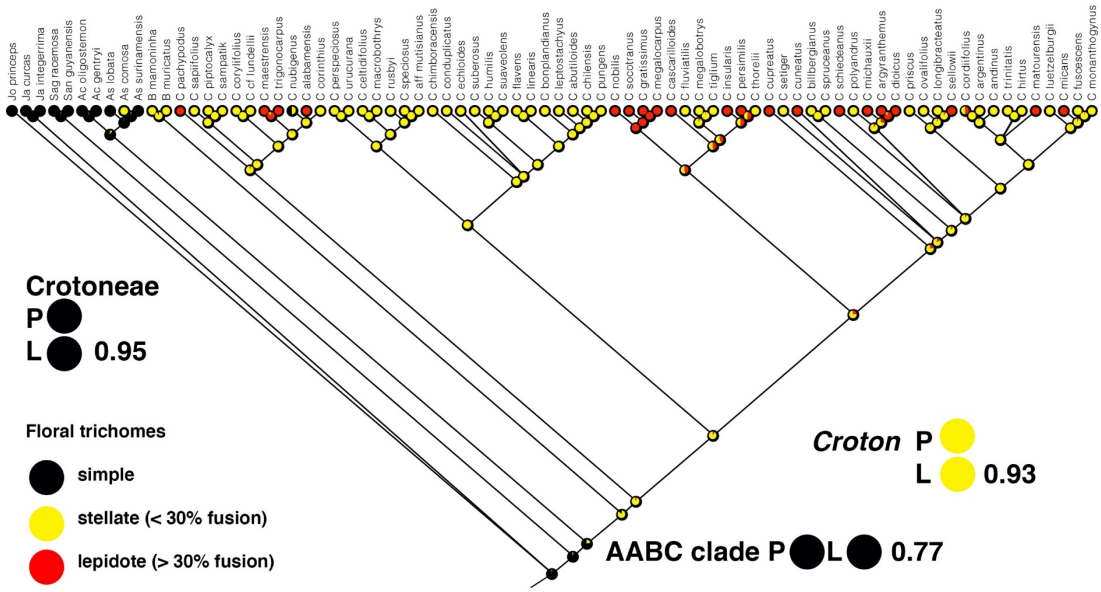

Figure 43. The reconstruction of ancestral states of the character 'floral trichomes' using parsimony and likelihood approaches. Colour codes of each character are shown on the left of the figure. P = Parsimony character state; L = likelihood character state with proportional likelihood score.

Four highly diverse and systematically informative floral characters were chosen to analyse ancestral character states, i.e., floral trichomes, stamen number, stamen arrangement and inflexed stamens in bud. Many types of trichomes could be found in flowers of *Croton* and related genera. However, in this ancestral character state reconstruction we recognised only trichomes on the abaxial surface of sepals or surface of the ovary because reduced stellate trichomes mixed with many simple trichomes are generally present on the abaxial side of sepals and both sides of petals

making it difficult to perform the study. Three characters states were recognised, i.e., simple trichomes, stellate trichomes and lepidote trichomes (Fig. 43). Simple trichomes are defined by a singular trichome without rays. Both stellate and lepidote trichomes have rays with different degree of fusion, less than 30% in stellate trichomes and more than 30% in lepidote trichomes. From the ancestral character state reconstruction, it is found that the ancestor of the tribe Crotoneae is likely to have simple trichome (Fig. 43). Simple trichomes are also inferred to be an ancestral character of the AABC clade (Fig. 43). The stellate trichome is probably an ancestral character present in the ancestor of *Croton* supported by both a parsimony approach and likelihood approach (proportional likelihood 0.93). Moreover, ancestors of each neotropical clade are found to likely have stellate trichomes supported by both approaches. However, in the subgenus *Croton*, a parsimony approach predicts the lepidote trichomes as an ancestral character, while stellate trichomes is selected by the likelihood approach but with low support value (0.52).

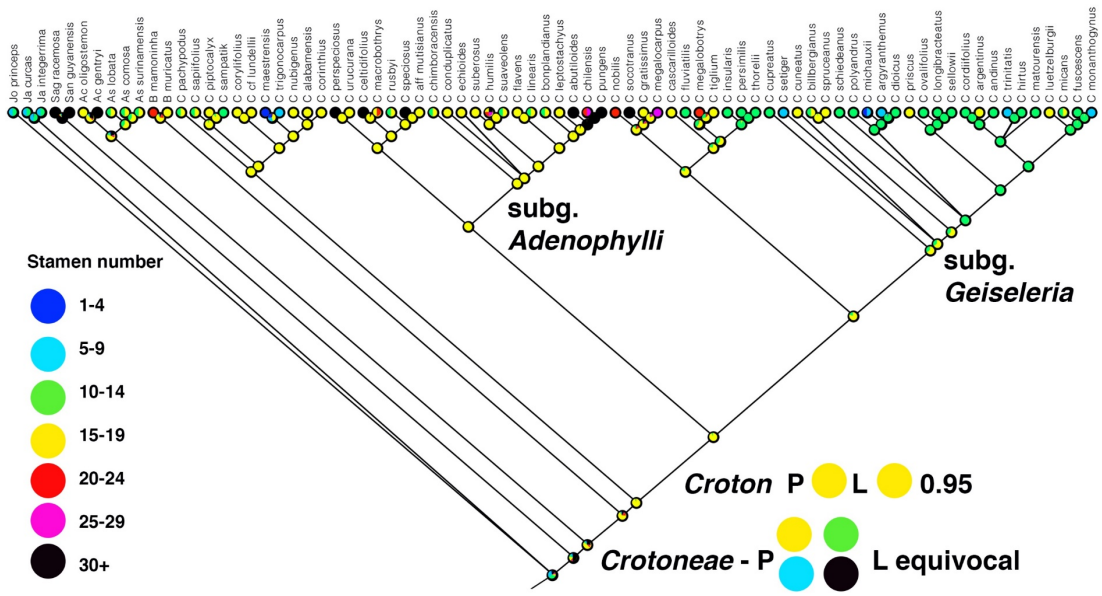

Figure 44. The reconstruction of ancestral states of the character 'stamen number' using parsimony and likelihood approaches. Colour codes of each character are shown on the left of the figure. (P = Parsimony character state; L = likelihood character state with proportional likelihood score.

Our ancestral character state reconstruction found that the ancestral stamen number for the tribe Crotoneae is inconclusive. However, a stamen number of 15 to 19 is inferred to be an ancestral character of *Croton* and also all four subgenera (Fig.

44). There is a trend of stamen increase in the subgenus *Adenophylli* (Fig. 44). In the subgenus *Geiseleria*, there is a trend of stabilising stamen number to 10 to 14 in the upper part of the clade (Fig. 44). Moreover, there are several cases of independent stamen number decrease in this group as well.

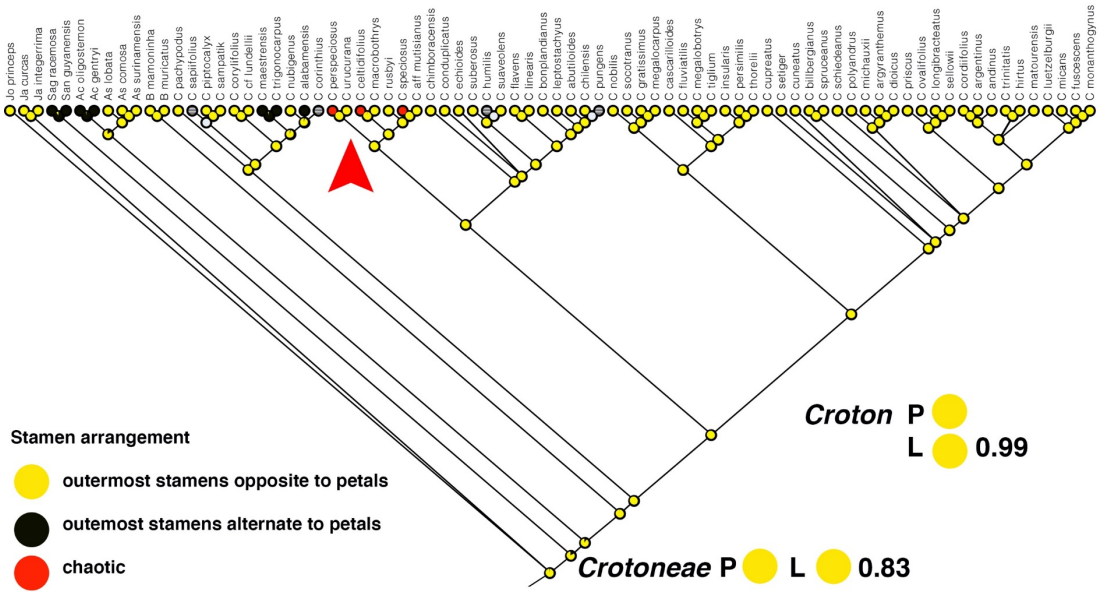

Figure 45. The reconstruction of ancestral states of the character 'stamen arrangement' using parsimony and likelihood approaches. Colour codes of each character are shown on the left of the figure. P = Parsimony character state; L = likelihood character state with proportional likelihood score.

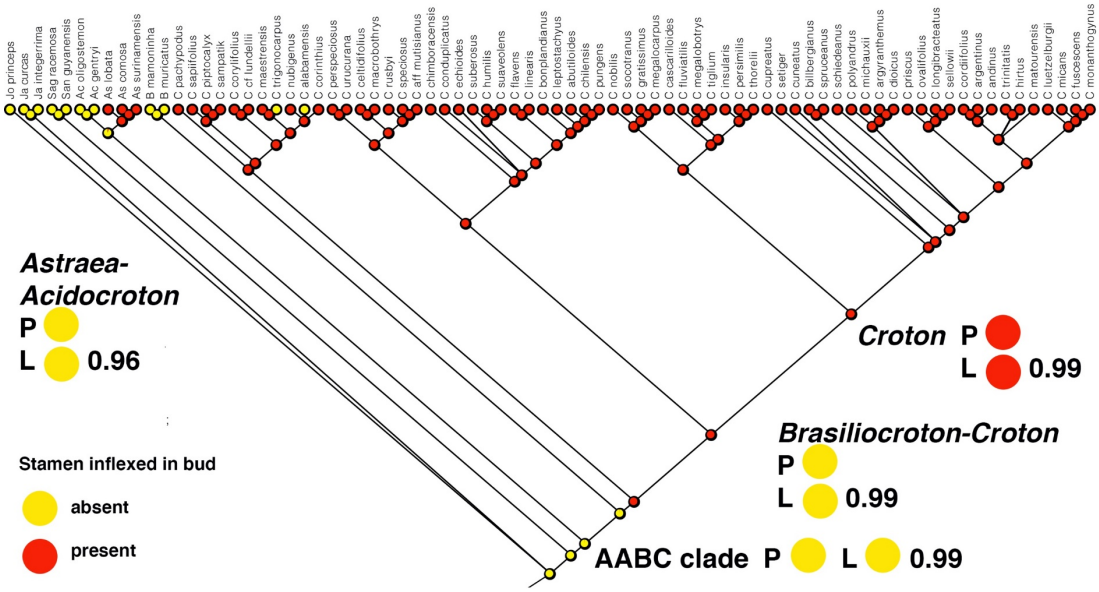

Figure 46. The reconstruction of ancestral states of the character 'stamen inflexed in bud' using parsimony and likelihood approaches. Colour codes of each character are shown on the left of the figure. P = Parsimony character state; L = likelihood character state with proportional likelihood score.

Arrangement of stamens with the outermost whorl opposite to petals is widespread in *Croton* and in the tribe Crotoneae and is likely to be the ancestral state of the tribe supported by both parsimony and likelihood approaches (Fig. 45). However, *C. maestrensis*, *C. trigonocarpus* and *C. alabamensis* from the present study have their outer stamen whorl arranged alternate with petals (Fig. 45). A chaotic stamen arrangement is found in some species from the section *Cyclostigma* (Fig. 45).

Inflexed stamens in bud is a shared character between *Astraea* and *Croton*. In both genera, it is suggested to be an ancestral character (Fig. 46). However, it is found that this character may not an ancestral state of the tribe nor the AABC clade. Two species in *Croton*, i.e., *C. trigonocarpus* and *C. alabamensis* do not have inflexed stamens in bud which is highly unusual among all *Croton* species (Fig. 46).

2.5 Discussion

2.5.1 Floral and inflorescence diversity and relationship within the tribe

In the present molecular based classification, the monophyly of the tribe Crotoneae is well support (Berry et al., 2005b; Wurdack et al., 2005). However, its internal relationships are far from stable. Three hypothetical relationships in the tribe Crotoneae were proposed in the previous literature (Fig. 2). The first topology, *Brasiliocroton-Croton* sister to *Acidocroton-Astraea*, is supported by several molecular phylogenetic studies with a combination of two or more genes from the nucleus, chloroplast and mitochondria (Berry et al., 2005a; b; Wurdack et al., 2005; van Ee et al., 2011; Riina et al., 2014; van Welzen et al., 2020). The second topology, *Acidocroton-Astraea* sister to *Sagotia-Sandwithia*, was obtained from a phylogenetic study with dense sampling of many taxa in the Rosids and involving multiple regions (Sun et al., 2016). However, many samples from the Crotoneae included in the mentioned study lack data from most of the DNA regions included. We think that the second topology may be an artefact result from highly incomplete data. The newly published third topology, *Brasiliocroton-Croton-Acidocroton-Astraea* grade, was obtained from a phylogenetic analysis from a combination of two chloroplast regions (*TrnL-F* and *psbA-trnH*) and a nuclear regions (ITS) using RNAsalsa program

(Stocsits et al., 2009) to align ITS region sequences based on the predicted secondary structure of their RNA sequences (Silva et al., 2020). The mentioned paper also indicated incongruence of the phylogenetic topology among data from different genomes (nuclear and chloroplast) (Silva et al., 2020). Since the second topology may be derived from incomplete data, in the present study we will discuss character evolution within the phylogenetic framework of the first and third topologies.

In the first and third topologies, the tribe Crotoneae could be divided into two main clades (Fig. 2; Berry et al., 2005b; Wurdack et al., 2005; Silva et al., 2020; van Welzen et al., 2020). The first clade consists of two genera, i.e., *Sagotia* and *Sandwithia* (SS clade), and the second clade consists of the rest of genera, i.e., *Acidocroton*, *Astraea*, *Brasiliocroton* and *Croton* (AABC clade). For the SS clade, our study reveals the presence of a few common characters in both *Sagotia* and *Sandwithia*. Inflorescences from both genera are determinate with recaulescent petioles containing uplifting bracteoles that later become an abscission zone (Fig. 3, 8). Recaulescence is caused by partial fusion of bract/bracteoles primordia with the superposed axis (could be inflorescence, rachis or pedicel); the elongation of the axis during growth displaces the bract/bracteoles by moving them up along the axis (Webberling, 1992; Vrijdaghs et al., 2010). Petals from the two genera are also similar with a mostly glabrous surface (Fig. 4E-H; 9G, H) and bigger size than sepals at anthesis (Fig. 1F; 4A, E, F; 9C). The presence of strictly simple trichomes is also found in these two genera (Fig. 3I; 8F; 11C). However, the numerous simple trichomes of *Sandwithia* are arranged in pairs (Fig. 8F, 11C). Floral morphology between the two genera is remarkably different, i.e., *Sagotia* has free sepals, no nectary glands (in male flowers) and sessile or subsessile stamens (Fig. 4; 5), while *Sandwithia* has fused sepals in bud, nectary glands and stamens with long, twisted and folded filaments (Fig. 9 ;10). The fusion of sepals in bud in *Sandwithia* is also found in several genera in the subfamily Crotonoideae, e.g., *Anomalocalyx* Ducke , *Grossera* Pax, *Tapoïdes* Airy Shaw and *Tannodia* Baill (Radcliffe-Smith, 2001; Webster, 2014), explaining why it was classified as a member of the tribe Aleuritideae subtribe Grosserinae in pre-molecular classifications (Webster, 1975, 1994b; Radcliffe-Smith, 2001). Molecular phylogenetic reconstruction found that those genera now belong to the C2 clade while

Sandwithia is in the C1 clade (Wurdack et al., 2005). Previous studies stated that there is no nectary in both flower sexes of *Sagotia* (Radcliffe-Smith, 2001; Webster, 2014; Silva et al., 2020). However, with thin sectioning and micro-computed tomography data, we found that there is a narrow ring of (possibly) secreting tissue inside the calyx in female flower of *Sagotia* (Fig. 7I, J). However, further field data are needed to confirm the nectary function of this ring. Despite different stamen structures in both genera, high stamen number is a shared character between them (Fig. 4J, K; 5J; 10L-N). Moreover, the outermost stamen whorl of both genera is arranged alternate with petals which could be considered as a synapomorphy between them. However, in *Sandwithia,* the outermost stamens may arise in pairs (Fig. 10F-J). Pollen from *Sagotia* and *Sandwithia* were reported to have similar sizes (approximately 50 and 55 μm respectively (Punt, 1962)), but our measurement in the non-acetolysis treated pollen of *Sandwithia* found that their diameter is about 37.5 μm. (Fig. 9N). Ektexine morphology is also different between the two genera with striate pila with attenuate tip in *Sagotia* versus smooth pila in *Sandwithia* (Secco, 1987).

For the AABC clade, the internal relationship between the four genera in this clade is still inconclusive, whether there are two subclades (topology 1) (Berry et al., 2005b; Wurdack et al., 2005; van Welzen et al., 2020) or all genera form a grade (topology 2) (Silva et al., 2020), but the common shared character is the thyrse/raceme inflorescence (except *Brasiliocroton*) and a generally lower stamen number than in the SS clade (except *Acidocroton* section *Ophellantha* and many *Croton*). In this clade, nearly all genera (except *Acidocroton*) also share the presence of stellate trichomes (Fig. 23F; 24B, D; 26C, E; 28K, L; 29B, C; 31B; 34B; 38A, C, D). In male flowers, petals are found to be smaller than sepals at anthesis (except *Acidocroton*). Stamen structures are varied in this clade with inflexed filaments found in *Astraea* and *Croton*, while stamens in *Acidocroton* and *Brasiliocroton* are curved toward the floral centre but are not inflexed (Fig. 14L; 17K; 30L). Inflorescence and flower of *Astraea* and *Croton* are highly similar with the presence of thyrses with condensed cymules (Fig. 23A, B; 36A-D). Their flowers are also superficially similar with the presence of the outermost stamen whorl opposite petals in male flowers (Fig. 24K; 25J; 38K; 39D, E) (Baillon, 1858; Gandhi and Thomas, 1983; De-Paula et al., 2011) and female flowers

with reduced petals (Fig. 26H-J; 40A, D, E)(Nair and Abraham, 1962; Webster, 1993; Radcliffe-Smith, 2001; Caruzo and Cordeiro, 2007; De-Paula et al., 2011). However, several characters were found to help separate these two genera, e.g., lobed leaf (*Astraea*) vs. mostly simple (*Croton*), chromosome number n=9 (*Astraea*) vs. n=10 (many *Croton*), moniliform trichomes in the lower part of petals (*Astraea*) vs. simple trichomes (*Croton*). In addition, we found that inflorescences of *Astraea* are determinate (Fig. 23C), different from the mostly indeterminate ones in *Croton* (Fig. 36A, G). Pedicels of male flowers of *Astraea* also have a joint from recaulescent growth (Fig. 23E) but do not have uplifting bracteoles as in *Sagotia* and *Sandwithia* (Fig. 3A, B, G; 8B, C), which is different from pedicels in male flowers of *Croton*. Flowers of some species of *Astraea* also show the presence of red patches which have never been reported in any *Croton* (Silva and Cordeiro, 2020). Therefore, segregation of *Astraea* and *Croton* are supported by both morphology and molecular data.

Previous studies identified some shared characters between *Brasiliocroton* and *Croton*, e.g., the presence of petiolar glands, tree habit, and stellate and derived trichomes (de Sá-Haiad et al., 2009; Riina et al., 2014). We found that *Brasiliocroton* has petals densely lined with simple trichomes on the margin which could be found in some *Croton* (Fig. 29E-F; 31E-F; 38G) supporting the close relationship between *Brasiliocroton* and *Croton*. Two species of *Brasiliocroton* also have an interesting vascular bundle branching pattern. We found that bundles supplying petals in male and female flowers further extend and fork to supply lateral veins of two adjacent sepals (Fig. 30D-E; 32E-F; 33E-F; 35C). This branching pattern is not found in other genera in the tribe Crotoneae. In young female flowers of *B. muricatus*, we found two primordia adjacent to reduced petals, sometimes with a primordium located between petals (Fig. 34E-H). Two primordia on two sides of petals are similar to primordia of nectary. However, evidence from the micro-computed tomography images found that these structures are coexist with nectary glands (Fig. 35E, H). In the micro-computed tomography image, those structures are brighter than surrounding tissue from high cell density which suggesting their glandular nature (Fig. 35 E-G). From previous records, colleters could be found in the same position as filamentous structures/reduced petals in female flowers of *Astraea* and *Croton* (De-Paula et al., 2011; Feio et al., 2016).

However, we do not have more mature flower to verify the identity of these structures. Moreover, we found glandular structures on an old nectary gland in a young fruit of *B. mamoninha*. In this case, we think that they may be from anomalous development since that the colleter is found in an old nectary structure.

Acidocroton is like an outcast of the AABC clade. Its flowers superficially resemble flowers from the SS clade with the presence of strictly simple trichomes, large petals at anthesis and a ring of nectary lobes in both flower sexes. This genus also has distinctive stamens with a connective appendage with unknown function (Fig. 14M, N; 17A, K, L). From the previous phylogenetic studies and the present study, *Acidocroton* is most closely related to *Astraea* (Fig. 2; 42) (Berry et al., 2005a; Wurdack et al., 2005; Riina et al., 2014; Silva et al., 2020). The presence of colleters, glandular structures secreting a mucilaginous substance to protect young part from desiccation, microbes and herbivores (Thomas, 1991; de Sá-Haiad et al., 2009; Machado et al., 2015), on sepals of female flowers is the only shared character between them (Fig.19A-D; 21C, F; 26D; 27J, M; De-Paula et al., 2011). It was thought that the presence of colleters in flowers is unique to *Astraea* (De-Paula et al., 2011), but our study and other literature found that colleters could be found in flowers of nearly all genera in the tribe Crotoneae, except *Sandwithia* (Machado et al., 2015; Feio et al., 2016). Colleters on sepals are found in some species of *Croton*, but their occurrence is highly variable. However, sepaline colleters are specifically found in female flowers of *Acidocroton* and *Astraea* (also in female flowers of *Sagotia* and male flowers of *Ac. oligostemon*). Interestingly, the presence of sepal colleters and also petals bigger than sepals at anthesis are two characters that support the second hypothetical relationship of the tribe Crotoneae (Fig. 2B). However, other characters outweigh these two characters. In addition, styles of *Acidocroton* show a high branching pattern ranging from tetrafid to multifid similar to *Astraea* with multifid styles. *Astraea* also shows a reduction from stellate trichomes to simple trichomes which are the only kind of trichomes in *Acidocroton*. Floral structures of *Acidocroton* are unique among other genera in the AABC clade and may be an extreme variation from the ancestor that resembles *Croton* and *Astraea*. The presence of petals larger than sepals may be an adaptation to a similar condition to the SS clade

Table 3. *Comparison of morphological characters of all six genera in the tribe Crotoneae*.

Characters	*Sandwithia*[1]	*Sagotia*	*Acidocroton* (include *Ophellantha*)	*Astraea*	*Brasiliocroton*	*Croton*
Habit[1]	tree	tree or shrub	shrub	subshrub or herb	tree	tree, shrub, herb
Plant reproductive system[1]	dioecious or monoecious	monoecious	monoecious	monoecious	monoecious	monoecious, rarely dioecious
Indumentum	simple	simple	simple	simple, stellate, moniliform in petals	stellate	simple, stellate and variations to lepidote
Inflorescence form	botryoid or determinate thyrse	botryoid or determinate thyrse	raceme	determinate raceme, thyrse with condensed cymule	panicle	indeterminate raceme, partial thyrse, thyrse with condensed cymules
Inflorescence reproductive system	unisexual or bisexual	unisexual or bisexual	unisexual	bisexual	unisexual or bisexual	unisexual (rarely bisexual)
Nectary in male flower	2-4 lobes	absent	numerous lobes or annular covering receptacle	5 lobes	5 lobes	5 lobes
Stamen number	Ca. 30	20-45	16-50+	8-15	14-30	(1-)11-16(-100+)
Stamen inflexed in bud	no	no	no	yes	no	yes (rarely no)
Colleters in female sepals	absent	present	present	present	absent	absent/present
Petals in female flower	reduced	absent	absent or reduced	absent or reduced	absent or reduced	absent or reduced (rarely fully developed)
Nectary in female flower	annular	annular	annular	5 lobes	5 lobes	5 lobes or annular
Branching of stigma	bifid	bifid	tetrafid	multifid (rarely bifid)	bifid	bifid to multifid (few simple)

[1] (Radcliffe-Smith 2001; Webster 2014)

2.5.2 Relationship between Crotoneae and Jatropheae

The tribe Crotoneae belongs to the clade C1 of inaperturate crotonoids with Jatropheae as a sister group (Wurdack et al., 2005). Both tribes share the presence of inapeturate pollen grains with croton-pattern and petals in at least male flowers, which are also found in the clade C2 (Wurdack et al., 2005). All genera in clade C1 also have a distribution mainly in North and South America except *Jatropha*, *Astraea* and *Croton*, while most genera from the clade C2 are present in Africa and Asia (Wurdack et al., 2005). Infloresences of Jatropheae and Crotoneae are generally different. Inflorescences of *Jatropha* are described as racemose-paniculate in general (compound dichasium) (Dehgan and Webster, 1979). However, since a female terminal flower is present, and each floral unit is based on a dichasial cyme, they should be described as determinate thyrses according to Claßen-Bockhoff and Bull-Hereñu (2013). This type of inflorescence is also found in two other genera in the tribe, i.e., *Joannesia* and *Vaupesia* (Velloso, 1798; Schultes, 1955). For the tribe Crotoneae, inflorescence form is highly diverse, ranging from raceme, botryoid, thyrse and panicle. Diversity and evolution of inflorescences in the tribe Crotoneae will be discussed in the next section. However, the common inflorescence morphology in the tribe Crotoneae is the presence of strictly male flowers on the upper part of the main axis of the inflorescence. Occurrence of petals in female flowers is the main character to distinguish the two tribes, i.e., present in Jatropheae (Velloso, 1798; Schultes, 1955; Dehgan and Webster, 1979; Dehgan and Schutzman, 1994; Dehgan, 2012; Webster, 2014) versus reduced or absent in Crotoneae (Silva *et al.* 2020). In the protologue, female flowers of *Sandwithia* were described as having petals; however, our examination found only fully developed petals mixed with other reduced forms (Fig. 11D, E). Therefore, we could confirm that reduction or absence of petals in female flowers is present in all genera in the tribe Crotoneae. Reduction of petals in female flowers is also present among many groups in the clade C2, e.g., subtribe Codiaeinea of the tribe Codiaeae, subtribe Cocconeriinae and Beryinae of the tribe Ricinocarpeae and subtribe Neoboutoninae of the tribe Aleuritideae (Webster, 2014). The androecia of tribes Jatropheae and Crotoneae are highly different. Stamen morphology is highly stable in the tribe Jatropheae with erect stamens in bud, while stamen morphology in

the tribe Crotoneae is highly variable ranging from sessile to folded, to erect, to inflexed in bud (present study; Secco 1987; Radcliffe-Smith 2001; Berry, *et al.* 2005b; van Ee *et al.* 2011; Webster 2014; Riina *et al.* 2014; Silva *et al.* 2020). Stamens in the Jatropheae could be free or fuse together (Schultes, 1955; Dehgan and Webster, 1979; Dehgan and Schutzman, 1994; Dehgan, 2012), while stamens in the Crotoneae are always free (present study; Webster 2014). Tribe Jatropheae also has a relatively stable stamen number with the presence of generally eight to ten stamens (Velloso, 1798; Ducke, 1922; Schultes, 1955; Dehgan and Webster, 1979; Dehgan, 2012). In tribe Crotoneae, stamen number is extremely diverse but generally higher than 10 (Table 3). Stamen number is also linked with the arrangement of stamens in the flower. In the Jatropheae, the five outermost stamens are opposite petals (Nair and Abraham, 1962; Venkata-Rao and Ramalakshmi, 1968; Liu et al., 2015). In Crotoneae, the outermost antepetalous stamen whorl is found in *Astraea*, *Brasiliocroton* and *Croton* (Fig. 25H; 30H-J; 32I-K; 39D, E) (Baillon, 1858; Marchand, 1860; Nair and Abraham, 1962; Gandhi and Thomas, 1983; De-Paula et al., 2011), while *Sagotia*, *Sandwithia* and *Acidocroton* have the outermost stamens arranged alternate with petals (Fig. 15H; 16H; 18F-L). From a literature review, we found that pollen size is different between these two tribes. Diameter of pollen grains from the tribe Jatropheae (*Joannesia* and *Vaupesia* 70 µm (Punt, 1962), *Jatropha* 50-100 µm (Punt, 1962; Dehgan and Webster, 1979; de Souza et al., 2016)) is slightly bigger than pollen in the tribe Crotoneae (*Sagotia* 50 µm, *Sandwithia* 55 µm, *Acidocroton* 36-45 µm (*Ac. adelioides*, Punt 1962; *Ac. spinosus*, the present study), *Brasiliocroton* 30-39.4 µm (de Souza et al., 2019), *Astraea* 33-51.9 µm (Silva et al., 2020)) except *Croton* with greatly variation in pollen size (35-135 µm) (Punt, 1962; Carreira et al., 1996; de Lima et al., 2007; Park and Lee, 2013; de Souza et al., 2016). Ancestors of *Croton* probably have pollen sizes similar to other genera. However, most of the pollen size data of *Croton* are obtained from species in subgenus *Geiseleria* with few data from subgenus *Adenophylli* and subgenus *Croton*; therefore, pollen grains from more species need to be examined to verify our hypothesis.

2.5.3 Inflorescence diversity and evolution in Crotoneae

In the SS clade, inflorescences of *Sagotia* and *Sandwithia* are generally botryoids (determinate raceme) with spirally arranged flowers arising acropetally and ending with a terminal flower that is older than lower flowers (Fig. 1F, I; 3A, B, E). In both genera, bracteoles are present on the upper part of the pedicel instead of at the base of pedicel as in other genera (Fig. 3A, B; 6A; 8C), which could be described as recaulescent growth .The recaulescent growth of the petiole in those genera is a sign of reduction from dichasial cymules as present in determinate thyrses of some samples of *Sagotia* (Fig. 3F) (Berry et al., 1999) and in the illustration of Sandwithia (Fig. 1I) (Lanjouw, 1932). Changing from cyme to a solitary flower is relative easily as observed in *Astraea* and *Croton*. Genetic experiments in *Petunia* found that mutation in gene *extrapetal* (*exp*) or *hermit* (*her*) change monochasial cyme inflorescence into a single flower (Rebocho et al., 2008; Castel et al., 2010). However, there is no genetic study demonstrate transition from thyrse (raceme with cyme) to raceme and vice versa.

In the AABC clade, *Acidocroton*, *Astraea* and *Croton* have inflorescences that are derived from the raceme type. The inflorescence of *Astraea* and *Croton* are highly similar with the presence of condensed cymules on the raceme axis (thyrse), reflecting the old classification that recognised *Astraea* as a section of *Croton* (Berry et al., 2005b; Wurdack et al., 2005). However, our examination found that thyrses of *Astraea* end with a terminal flower (determinate) (Fig. 23C; 47), while thyrses of *Croton* are indeterminate (Fig. 36A, G; 47). In *Arabidopsis*, expression of the wild type of *TERMINAL FLOWER LOCUS 1* gene (*TFL1*) controls the maintain of indeterminate inflorescences while the recessive form (*tfl1*) induces formation of a terminal flower (Shannon and Meeks-Wagner, 1991; Benlloch et al., 2007). This mechanism is also found in other plants (reviewed in Benlloch et al., 2015). Apart of the genetic cause, it is found that there are several morphological constraint of the inflorescence merism that dictate the type of inflorescence to develop, e.g., space, shape and enlargement of the meristem (Bull-Hereñu and Claßen-Bockhoff, 2011 b). However, transition from determinate to indeterminate and vice versa are easily acquired as they could occur in the same genus or the same species (examples in Bull-Hereñu and Classen-Bockhoff,

2011a; Claßen-Bockhoff and Bull-Hereñu, 2013). Therefore, diverse inflorescence type in the Crotoneae could be explained by relatively easily transformation between determinate and indeterminate inflorescences.

Inflorescences of *Croton* are highly diverse but could generally be classified as indeterminate racemes or thyrses because of the presence of solitary flowers in the former and cymules in the latter. Cymules of *Croton* are diverse with the presence of only male, only female or both flower sexes in the same cymule (Fig. 36). However, there are many intermediate forms (partial thyrses) between thyrse and raceme present in the genus. Inflorescences of *Croton* generally produce high numbers of flowers. However, in some species of *Croton*, a small number of flowers are produced (Fig. 36H, I). Those reduced inflorescences generally end with a male flower that is younger than lower flowers (Fig. 36H, I), contrary to an older terminal flower found in botryoid inflorescences of *Sagotia* and *Sandwithia* (Fig. 1F, H, I; 3A, B, E). Therefore, we think that they should be described as reduced indeterminate inflorescences to reflect their different structure from the classic determinate inflorescence.

Acidocroton section *Ophellantha* and many *Croton* have indeterminate racemes (Fig. 47). *Acidocroton* is also a sister to *Astraea* which has thyrsoid inflorescences. Transition between cyme and a solitary flower is common in *Astraea* and *Croton*. Therefore, we think that racemes in *Acidocroton* section *Ophellantha* are derived from ancestral thyrses as present in *Astraea* and *Croton*. Within *Acidocroton*, species from the section *Acidocroton* produce flowers in clusters. In each cluster, flowers are arranged in a helical pattern as in raceme-derived inflorescences. Therefore, floral clusters in *Acidocroton* section *Acidocroton* should be described as highly condensed racemes (Fig. 13J; 47). In addition, previous literature described inflorescences of *Acidocroton* to be borne axillary to the stem (Radcliffe-Smith, 2001; Webster, 2014). However, we found that inflorescences of *Acidocroton* section *Ophellantha* are borne terminally (Fig. 13B) (Fernández-Alonso and Jaramillo-Mejía, 1995; Hanan-Alipi and Steinmann, 2013). For the section *Acidocroton*, clusters of flowers appear to be axillary of two spines. However, each cluster is subtended by several leaves which are possibly the result of short shoot formation. Therefore,

inflorescences of *Aidocroton* should be described as terminal as in other genera in the tribe Crotoneae. The condensation of vegetative and reproductive shoots occurs in other Euphorbiaceae distributed in Mexico, Central America and the Caribbean islands, e.g., *Adelia*, *Enriquebeltrania, Jatropha* section *Loureira* subsection *Neopauciflorae* and *Jatropha* section *Mozinna* (Dehgan and Webster, 1979; De-Nova et al., 2006, 2007; Dehgan, 2012; Webster, 2014). Short shoot formation, which is related to the production of smaller leaves, was reported to be adaptation for drought tolerance at least in the Mediterranean area (Westman, 1981; De Micco and Aronne, 2009). Moreover, species from *Acidocroton* section *Acidocroton* have xeromorphic characters, e.g., bigger spines, smaller and tougher leaves than the non-serpentine counterpart group, *Acidocroton* section *Ophellantha*, reflecting their adaptation to grow in the serpentine soil of the Great Antilles (Borhidi, 1991; Iturralde, 1997).

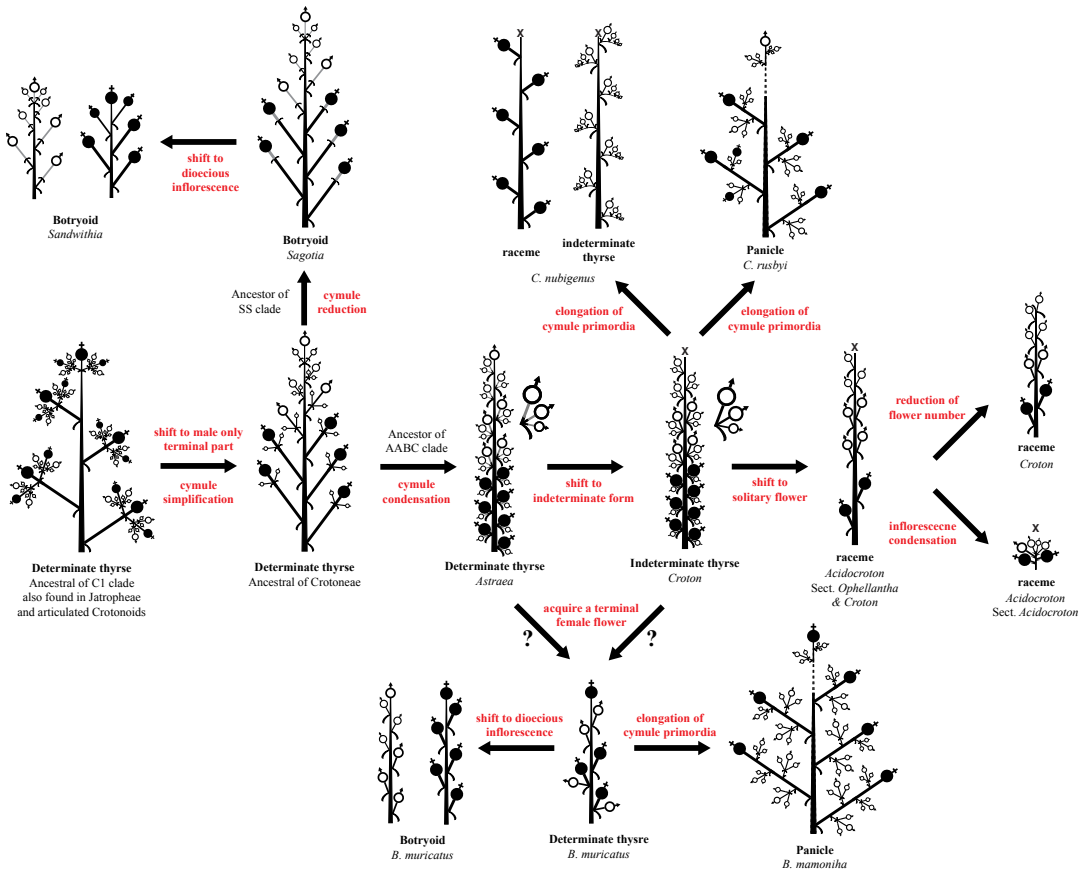

Figure 47. A diagram shows hypothetical evolutionary pathway of inflorescence in the tribe Crotoneae. The position of inflorescence diagrams of all genera and representative species follows the phylogenetic relationship (Fig. 42). Taxon name below each diagram represents the genus that the inflorescence is found.

Brasiliocroton has a unique inflorescence structure among other genera as a panicle with pistillate flowers located on the distal part and male flower cymules on the proximal part, while pistillate flowers in other genera are generally found on the proximal side (Fig. 47). In the protologue describing *Brasiliocroton*, there was mention that this plant is misidentified as *Micrandra* from the articulated crotonoid clade which has a similar inflorescence structure (Berry et al., 2005a). Paniculate inflorescences are also present in many genera in the articulated Crotonoids and C2 clade, e.g., *Aleurites*, *Annesijoa*, *Hevea* (Stuppy et al., 1999; Radcliffe-Smith, 2001; van Sam and van Welzen, 2004; Webster, 2014). It is found that panicles of *Brasiliocroton* are nested within thyrses and thyrse-derived inflorescences of other genera in the Crotoneae in all topologies (Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2011; Sun et al., 2016; Silva et al., 2020; van Welzen et al., 2020). Therefore, we think that the panicle of *Brasiliocroton* may be derived from the elongation of cymule primordia of ancestors with thyrses. This hypothesis is supported by the presence of elongation of cymule primordia in *Astraea* and *Croton*. Thyrse inflorescences with elongation of cymule primordia are found in *C. nubigenus* (Fig.37A-C). Another case is the panicle found in *C. rusbyi*, which is highly similar to inflorescences of *Brasiliocroton.* However, it belongs to section *Cyclostigma* subgenus *Adenophylli* which is nested within the majority taxa bearing thyrses on the phylogenetic tree (van Ee et al., 2011). Furthermore, we found one specimen of *C. rusbyi* with short lateral branches resembling a possibly transitional form of the transformation from thyrses to panicles (Fig. 31I). Inflorescences with long lateral branches are also reported in some species of *Astraea* but were later found that they develop from thyrsoid forms as a result of phytoplasma infection (Silva et al., 2017). Some inflorescences of *B. muricatus* have determinate thyrses (Fig. 28H, I) which may represent an ancestral form of panicle. However, a weak point of our hypothesis is that thyrses in the Crotoneae only have male flowers present on the distal part contrary to panicles that end with a female flower. Thus, our hypothesis could not explain how male-only parts change into bisexual part. There are several genetic and developmental studies explaining the transition from determinate and indeteminate inflorescences (and vice versa) (Bull-Hereñu and Classen-Bockhoff, 2011 a; 2011 b). However, those studies were conducted in taxa with bisexual flowers. Further studies using

comparative developmental genetics and ontogeny between determinate and indeterminate inflorescences of Jatropheae and Crotoneae would clarify this problem.

2.5.4 Floral trichome diversity and evolution in Crotoneae

Diversity of trichome structure is well known in *Croton* which has a wide range of trichome structures from simple, stellate to lepidote and dendritic shaped (see in Webster *et al.* 1996). Webster (1996) suggested that stellate trichomes with porrect (a central projection) is the most primitive type in *Croton* (including *Astraea* at that time) and simple trichomes are a derived form as a reduction of rays in the stellate type. However, on the present phylogenetic evidence, it is found that simple trichomes which were reported in *Sagotia*, *Sandwithia* as well as in Jatropheae and other lower Crotonoideae (Webster, 2014) are likely precursory of stellate trichomes in *Astraea, Brasiliocroton* and *Croton* (Fig. 43; 48). Our observation found that simple trichomes are strictly present in *Sagotia*, *Sandwithia* and *Acidocroton* (Fig. 43). Interestingly, we found that in *Sandwithia* pairs of trichomes are commonly found (Fig. 8E; 48). In the clade C2 of the core-crotonoideae, malphigaceous trichomes (T-shaped) were reported in some other genera, e.g, *Anomalocalyx*, *Dodecastigma*, *Pausandra* and *Leeuwenbergia*, (Webster, 2014). However, trichome pairs in *Sandwithia* are different since both trichomes in the pair do not have a common stalk (Fig. 8E). Pairs of trichomes transformed from solitary trichomes are reported in *Arabidopsis thaliana* with a mutation of *SIAMESE* gene (Walker et al., 2000). Interaction of this gene together with other genes, i.e., *glabra3* and *triptychon*, could create clusters of trichomes. Interestingly, bifurcate (V-shaped) trichomes are found mixing with stellate and fasciculate types in *Quercus* (Tschan and Denk, 2012).

We hypothesise that the grouping of several simple trichomes is the transitional state between simple and stellate trichomes (Fig. 48). Stellate trichomes are scattered in many taxa of Crotonoideae (*Micrandopsis* [articulated crotonoids], tribe Ricinocarpeae and some taxa in tribe Aleurithideae (Webster, 2014)) but they are embedded in clades possessing simple trichomes (Wurdack et al., 2005). *Acidocroton* have simple trichomes but is nested within a clade with compound trichomes as

supported by all topologies (Fig. 2). Floral stellate trichomes in *Astraea* show a gradient of reductions from stellate trichomes into simple trichomes which may explain the occurrence of simple trichomes in *Acidocroton*. Reduction from stellate to strictly simple trichomes is also found in the abberant South East Asian *C. nanus* (Esser and van Welzen, 2001). The strict presence of simple trichomes together with non-inflexed stamens in bud induced previous taxonomist to classify them into a separate genus *Colobocarpos* which was later phylogenetically shown to be an extreme variant of *Croton* (Wurdack et al., 2005). A similar scenario may have happened in the common ancestor of *Acidocroton*.

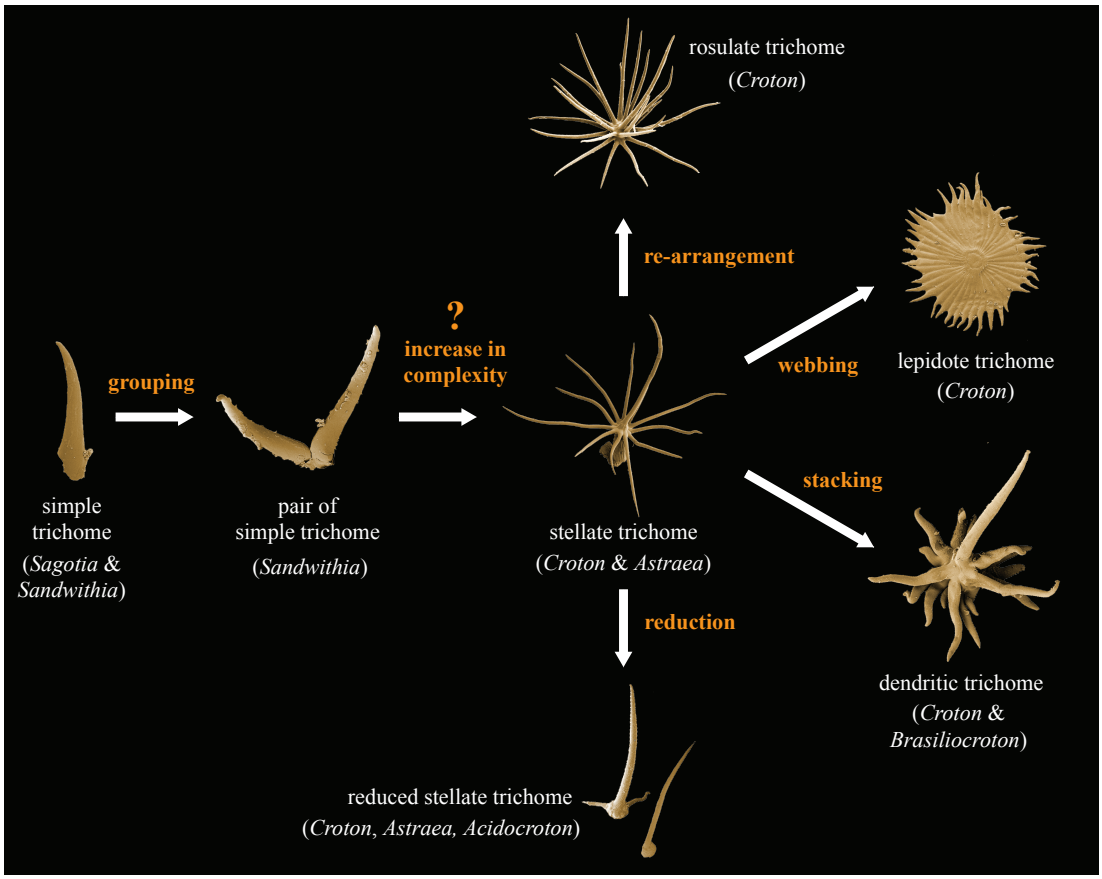

Figure 48. A diagram shows the hypothetical evolutionary pathway of floral trichomes in the tribe Crotoneae. The position of inflorescence diagrams of all genera and representative species follows the phylogenetic relationship (Fig. 42; 43). Taxon name in bracket below each diagram represents the genus where the trichomes are found. Images are not to scale.

In the AABC clade, *Brasiliocroton* and *Croton* share the presence of dense simple trichomes along petal's margin. *Astraea* and *Croton* also share the presence of long trichomes on the lower part on the adaxial side of petals. In *Croton*, these hairs

are simple trichomes, while in *Astraea*, they are moniliform trichomes, which is unique among all genera in the tribe Crotoneae. In both genera, trichomes on the lower part of petals cover nectary glands which arrange alternate with petals. The presence of densed trichomes on patals in *Astarea*, *Brasiliocroton* and *Croton* correlates with the presence of trichomes on the nectary glands. In those three genera, nectary glands are glabrous. Therefore, we think that the presence of hairs may help nectar accumulation and possible to reduce nectar evaporation. In *Sagotia*, *Sandwithia* and *Acidocroton*, which do not have densed trichomes on petal's margin, their nectary glands have pubescent surface. For moniliform trichomes in *Astraea*, it is reported that this type of hair is associate with region that pollinating bees settle in flower (Endress, 1994), but field observation is needed to confirm this occurrence in *Astraea*.

2.5.5 Origin of petal in Crotoneae and core-Crotonoideae

The presence of petals in at least male flower is found to be a synapomorphy of core Crotonoideae (inaperturate crotonoids clade C1 and C2) (Wurdack et al., 2005; Tokuoka, 2007). All taxa in Crotoneae also exhibit petals in male flowers (except in *Croton* section *Drepadenium* and *Eremocarpus* - Chapter 4; van Ee et al., 2011), while female flowers generally present various reductions of petals ranging from miniature petals, filamentous structures or total loss (The present study; De-Paula et al., 2011). However, fully developed female petals are present in *C. alabamensis*, many species from section *Eluteria* and some African and Madagascar species (Chapter 3; Berry et al., 2005b; van Ee et al., 2006, 2011; Friis and Gilbert, 2008; Berry et al., 2017). Apart of Crotoneae, female petals are present in many genera in the inaperturate crotonoids clade (Radcliffe-Smith, 2001; Webster, 2014). Interestingly, tribe Jatropheae, *Grossera, Cavacoa, Leeuwenbergia* and *Dodecastigma*, found to be close to the base of the inaperturate crotonoids clade, have petals present in their female flowers (Ducke, 1932; Leonard, 1955; Letouzey and Hallé, 1974; Radcliffe-Smith, 2001; Wurdack et al., 2005; Barberá et al., 2014; Webster, 2014; van Welzen et al., 2020), suggesting that the presence of petals in both flower sexes is likely to be the ancestral state of the inaperturate crotonoids clade. It is found that some clades of Crotonoideae and the majority of other Euphorbiaceae are characterised by a complete absence of a

corolla (Wurdack et al., 2005; Webster, 2014). Species from the articulated crotonoids clade, a sister group of the core-crotonoideae, lack petals in their flowers but they have petaloid tepals (sepals) instead. It was proposed that petals could be derived from staminodes (andropetals) or sepal/bract (bracteopetals) (review in Ronse De Craene 2007). However, a discussion about the origin of petals in Crotonoideae has not been done before. We will discuss three possible hypotheses explaining the origin of petals and bipartite perianth in the core-Crotonoideae taxa. The first two hypotheses are drawn from a phylogenic study by Wurdack and colleages (2005) that the petalous core crotonoideae clade is nested within a grade of apetalous Gelonieae and the articulated crotonoids clade. Thus, the presence of petals may be a derived character. The third hypothesis is based on the presence of petals in many separate groups within the Euphorbiaceae and related families which may imply the presence of petals as plesiomorphic character.

1) Staminodial origin

This hypothesis implies that ancestors of the core-crotonoideae lost petals then re-acquired petals through transformation of stamens or staminodes. Petals in a few groups of eudicots, e.g., Caryophyllales and Rosales, are clearly andropetal (Ronse De Craene, 2007). From an ontogenetic point of view, andropetals are generally delayed in their initiation being part of the androecium with narrow primordia, often resulting in petals with narrow base (Ronse De Craene, 2007, 2008). In the Crotoneae, slender petals are found in *Astraea*, *Brasiliocroton* and *Croton*, while other genera have broad petals. Floral ontogenetic studies done in several genera from core-Crotonoideae, i.e., *Aleurites*, *Astraea*, *Croton, Garcia, Jatropha* and *Vernicia*, found that petal primordia are broad and have an early initiation separated from stamen primordia (Chapter 3; McCann, 1942; Moncur, 1988; Singh, 2005; Liu et al., 2008, 2015; De-Paula et al., 2011; Claßen-Bockhoff, 2016; Mao et al., 2017) suggesting a bracteopetal nature. However, petal primordia of *Croton* have a broad base but their slightly narrow base in mature stages may be the result of pressure from growth of two adjacent nectary glands (Chapter 3). Therefore, petals in Crotoneae and core-Crotonoideae are probably not derived from a stamen transformation. Ronse De Craene (2007) stated that

andropetals are rare among the core eudicots and probably derived secondarily if present.

2) Secondary sepal origin

This hypothesis is deduced from the molecular phylogenetic studies which found that the articulated crotonoids is the sister group of the inaperturate crotonoids clade (core Crotonoideae) (Wurdack et al., 2005; Tokuoka, 2007). The articulated crotonoids clade comprises economically important genera, *Hevea* and *Manihot*. Flowers from taxa in this clade have only one whorl of perianth (tepals) with petaloid features suggesting that the incorporation of bracts and/or bracteoles (maybe in the form of epicalyx) in asepalous flowers could generate a bipartite perianth with the inner whorl as petals and the outer whorl as sepals as in Loranthaceae, Portulacaceae and Didiereaceae (Ronse De Craene, 2007). Uplifting bracteoles were observed in flowers from three genera in the tribe Crotoneae, e.g., *Sagotia* (male and female) and *Sandwithia* (male). Moreover, sepals from several species in the Crotoneae have leaf-like appearance (Fig. 6A-C, F; 13B). However, there is no epicalyx present in flowers from the articulated crotonoid clade and core Crotonoideae. It is found that petaloidy of sepals in the eudicots is generally linked with petal reduction or loss (Ronse De Craene, 2007). There is evidence that petaloid sepals still retain their sepal genetic identity (as in *Marcgravia* and *Impatiens* - Geuten *et al.* 2006). Therefore, gene expression study of tepals in flowers of articulated crotonoids may help testing our hypothesis. Furthermore, many genera in the articulated crotonoid have filamentous structures in female flowers, which could be remnants of petals. However, a recent floral anatomical study in *Hevea* and *Manihot* suggest that those filamentous structures are staminodes (Zardini, 2019). Ontogenetic studies are needed to confirm whether they are derived from a stamen or petal whorl.

3) *Retention of petals*

This hypothesis deduces that ancestors of the subfamily Crotonoideae may have petals and the core Crotonoideae clade retains the character while other clades, e.g., articulated crotonoidae and Gelonieae, lost their petals independently. Despite the

majority of genera in the Euphorbiaceae lacking petals in their flowers, petals are present in flowers from many genera scattered throughout the subfamily Acalyphoideae and Crotonoideae (Wurdack et al., 2005). This raises the question whether the ancestor of Euphorbiaceae had petals in flowers or not? From literature reviews, flowers of the Cheilidoideae, which is the sister to all other Euphorbiaceae, lacks petals (van Welzen, 1994; Wurdack et al., 2005; Xi et al., 2012; Webster, 2014). However, petals are present in two out of five genera in Peraceae (*Clutia* and *Pogonophora*) (Wurdack et al., 2005; Webster, 2014). In Rafflesiaceae, the closest relative of Euphorbiaceae s.s. (Wurdack and Davis, 2009), floral ontogeny and floral organ identity genes expression studies found that petals are present in all three genera (modification into a diaphragm in *Rafflesia* and hybrid organ origin perianth tube in *Rizanthes*) (Nikolov et al., 2013). However, comparison of floral structures between Rafflesiaceae and Euphorbiaceae has to be handled with care because flowers from these two families are extremely different with the presence of unisexual flowers as an only confirmed common character (de Olivera Franca and De-Paula, 2017). Moreover, petals are also present in Ixonanthaceae and Linaceae from the biovular clade of Euphorbioids clade of Malpighiales (Xi et al., 2012), but Phyllanthaceae and Picrodendraceae rarely have petals (Radcliffe-Smith, 2001; Endress et al., 2013; Webster, 2014). Therefore, it is possible that the ancestors of Euphorbiaceae and possibly the subfamily Crotonoideae had petals in their flowers. The tribe Crotoneae shows a reduction of petals in female flowers independently from other groups in the clade C2, supporting the scenario of multiple parallel reductions of petals. Loss of petals may be associated with suppression of B-genes which control the identity of both androecium and corolla whorls (Bowman et al., 1991; Litt and Kramer, 2010; Rijpkema et al., 2010). However, the loss of petals could not always be explained by gene expression pattern alone since other factors, e.g., time, pressure and meristem size, also affect floral morphology (Ronse De Craene, 2018). Heterochrony is found to be another mechanism involved in the development of filamentous structures in female flowers of *Croton* (and possibly in *Astraea* as well) (Chapter 3). De-Paula and colleagues (2011) found that in female flowers of *C. fuscescens* vascular bundles supplying petals/filamentous structures are visible but there is no corolla present in the flowers, but there is massive nectary tissue present instead. This may be result of an

expansion of nectary tissue engulfing the petals/filamentous structures primordia. Michaelis (1924) also reported the case of several filamentous structures protruding from nectary tissue in female flower of *C. tiglium*. In the present study, the presence of a long filamentous structure within the nectary ring in female flowers of *Ac. gentryi* may be a similar phenomenon (Fig. 19I). Therefore, several mechanisms may help explain the possible loss of petals in Gelonieae and articulated crotonoid clade. However, the recent phylogenetic topology of the subfamily Crotonoideae is mostly unresolved especially in the relationship in the clade C2 (Wurdack et al., 2005). Few phylogenomic studies were carried out, but with few taxa from the family, producing even more confuse topologies (e.g., polytomy of Crotonoideae taxa) (Xi et al., 2012; Li et al., 2017, 2019; de Santana Lopes et al., 2018; Liu et al., 2018; Wang et al., 2019; Xin et al., 2019; Zhou et al., 2019; Jiang et al., 2020). Therefore, broad sampling phylogenomics studies are needed to produce well-supported relationships for ancestral character state reconstruction in testing this hypothesis.

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2.5.6 Nectary diversity and evolution

Nectaries are presence in both flower sexes from all genera in the tribe Crotoneae (except male flowers of *Sagotia* – table 3). In most genera, nectaries are arranged opposite to sepals. Number of nectary glands in a flower is generally equal to the number of sepals or petals, e.g., three in *Sandwithia* or five in *Astraea*, *Brasiliocroton* and *Croton* (Fig. 49). Sometimes nectaries are fused together forming a ring but number of lobes could indicate number of glands. Nectary is totally lost in male flowers of *Sagotia* which is highly unusual since most taxa from subfamily Crotonoideae have nectary in theirs flowers (Radcliffe-Smith, 2001; Webster, 2014). In female flower of *Sagotia*, there is a narrow nectary ring without distinct lobes (Fig. 7F-I; 49). This structure is overlooked in previous description (Radcliffe-Smith, 2001; Webster, 2014) due to its small size and shrinkage from herbarium preparation. The genus *Acidocroto*n post unique and unusual nectary within the tribe Crotoneae. In both flower sexes of *Ac. gentryi*, there are many nectary glands (>10) arranged surrounding androecium (Fig. 19F-I; 20G; 49), while female flowers of *Ac. oligostemon* have a nectary ring (Fig. 21F; 22G; 49). Male flowers of *Ac. spinosus* and *Ac. oligostemon*

have honeycomb-like nectary surrounding and engulfing filaments (Fig. 17J; 18J; 49). Interstaminal nectaries that expand covering whole receptacle are rare in angiosperm. There are some reported in few genera in Euphorbiaceae, e.g., *Leeuwenbergia* (Letouzey and Hallé, 1974), *Blumeodendron* (Ottens-Treurniet and van Welzen 2016), and *Acidoton* (Planche XVIII, figure 10 in Baillon, 1858), and several genera in Salicaceae (from former Flacourtiaceae), e.g., *Dovyalis* (L.P. Ronse De Craene, unpublished data; Thom, 1971; Sleumer, 1972), *Azara* (L.P. Ronse De Craene, unpublished data), *Xylosma* and *Flacourtia* (irregular grooves surround filaments - Bernhard and Endress 1999). Within the Crotoneae, fusion of nectaries was reported in female flowers from several species of *Croton* (De-Paula et al., 2011). However, the present study found that fusion of nectary is more widespread in the tribe as in female flowers of *As. comosa* and *Ac. oligostemen*. Fusion of nectary also occur in male flowers as well as in male flowers of *Ac. spinosus*, *Ac. oligostemon* and *C. alabamensis*. Non-fusion nectary is found in *Brasiliocroto*n and *Sandwithia*, the tribe Jatropheae and several genera in the clade C2 of inaperturate crotonoids and likely to be a pleisiomorphic state (Radcliffe-Smith, 2001; Webster, 2014). From ontogenetic point of view, nectary are found to be originated separately and post-genital fusion occur when they expand and touch each other (De-Paula et al., 2011). However, the origin of ring nectary in *Sagotia* is still obscure; the ring may arise congenitally since there is no distinct lobe observed. Ontogenetic study is needed for our better understanding of this structure.

Intra-floral nectary is abundant in flowers of Euphorbiaceae (Radcliffe-Smith, 2001; Wurdack et al., 2005; Webster, 2014); however, their origin is poorly known. In the Crotonoideae, floral disk are reported to presence in nearly all genera except few taxa, e.g., subtribe Cocconeriinae (Webster, 2014). In *Croton* and *Astraea*, nectary is thought to be derived from staminodial origin (De-Paula et al., 2011; Gagliardi et al., 2017) or receptacular origin (Caruzo and Cordeiro, 2007). The 'staminodial origin' hypothesis is supported by antesepalous position and vascularisation of nectary despite nectaries supplied by bundles extend from sepal's bundle (De-Paula et al., 2011). However, nectary of *Croton* is vascularised, while nectary in *Astraea* is non-vascularised (De-Paula et al., 2011).

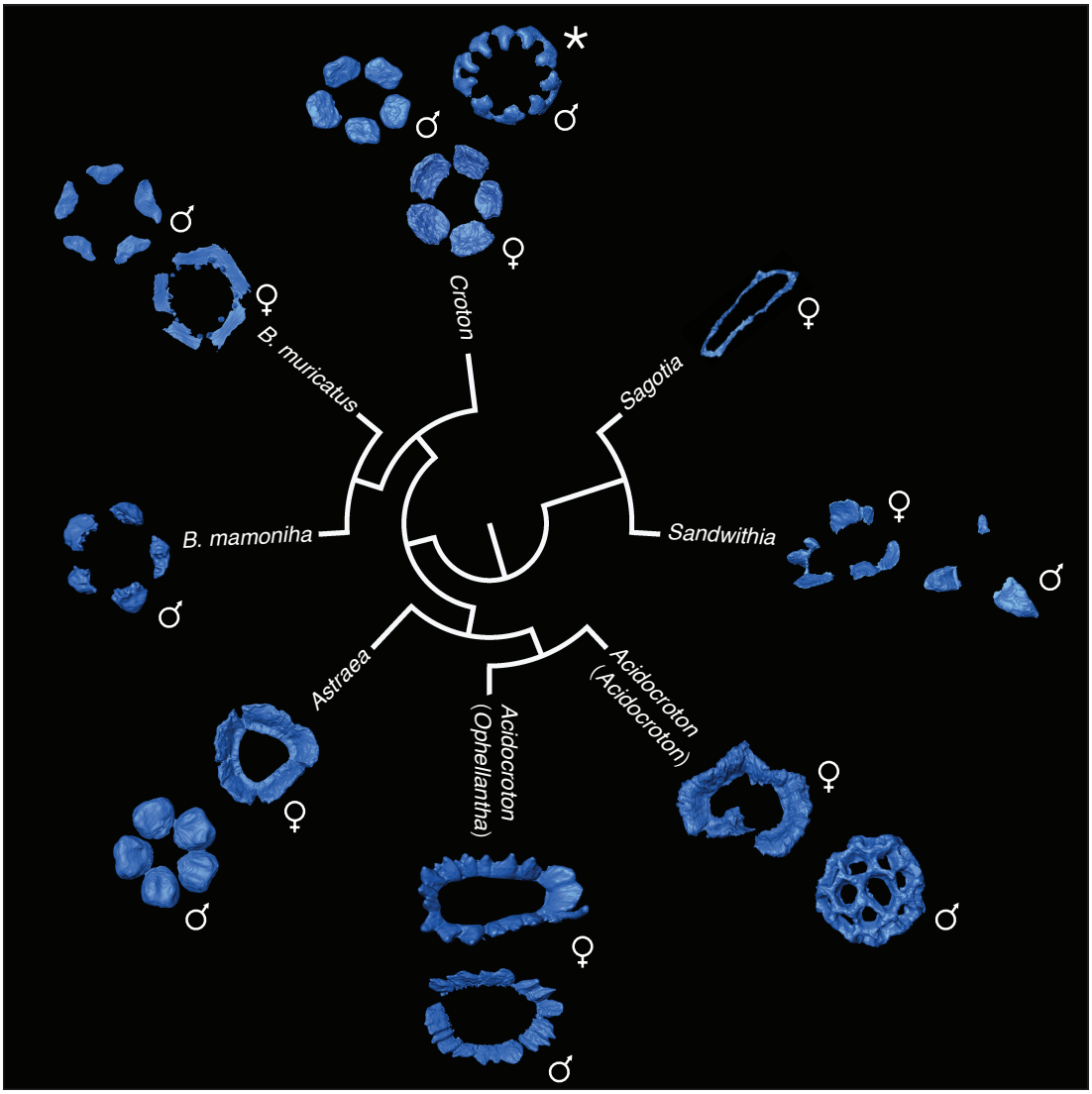

Figure 49. A diagram shows diversity of floral nectaries in the tribe Crotoneae. Surfaces of floral nectary were reconstructed from 3D objects from the following species, i.e., *Sag. racemosa*, *San. guyanensis*, *Ac. oligostemon* (sect. *Acidocroton*), *Ac. gentryi* (sect. *Ophellantha*), *As. comosa, B. mamoninha*, *B. muricatus*, *C. polyandrus* and *C. alabamensis* (asterisk). Images are not to scale.

In the present study, we found no traces of vasculature supplying the nectary in most of our samples. The brightness of the nectary may obscure the presence of vascular strands within the tissue. However, if those nectaries are vascularized, the vascular strands should be visible entering from the ground tissue but we did not see this. From the sectioning generated from micro-computed tomography images, we found that nectary glands are locate above the division of sepal's strand into veins which may give impression of vascularized nectary (as in Fig. 5K in De-Paula et al., 2011). Some anatomical study found non-vascularised nectaries in some species of *Croton*

(Venkata-Rao and Ramalakshmi, 1968; Freitas et al., 2001). However, we could confidently state that if presence, those vasculatures do not extend from the floral stele. However, we found that nectary glands in male flowers of *C. alabamensis* are supplied by lateral branch of sepal's strands (Fig. 41I). Nectary of *Jatropha* was reported to be vascularised but source of bundles could be from sepal or petal (Venkata-Rao and Ramalakshmi, 1968). Labile origin of vasculature in Crotoneae and Jatropheae suggest opportunistic origin supporting the 'receptacular origin' hypothesis since staminode-derived nectaries are generally supplied by stele's bundles (Ronse De Craene and Smets, 2001). Moreover, previous studies found that nectary in female flowers originate from two primordia locate adjacent to reduced petals but each nectary lobe is supplied by a vascular bundle (Chapter 3; De-Paula et al., 2011), which could be explained by the 'receptacular origin' hypothesis that those vascular bundles are opportunistic. In female flower of *B. muricatus*, two primordia with supplied bundles arise adjacent to each petal similar to reported nectary primordia in female flowers of *Astraea* and *Croton* (Chapter 3; De-Paula et al., 2011). However, they grow into short filamentous structures instead of nectary with is visible as five bright flat structures on the receptacle without supplied vasculature (Fig. 35E-H). Nectaries of Crotoneae are found to be highly variable in position relate to position of stamens since the last floral structure to developed (De-Paula et al., 2011). Shift of outermost stamens position from antepetalous position (found in *Astraea* and most of *Croton* - Baillon, 1858; Marchand, 1860; Michaelis, 1924; Nair and Abraham, 1962; Venkata-Rao and Ramalakshmi, 1968; Gandhi and Thomas, 1983; De-Paula et al., 2011; Gagliardi et al., 2017) to antesepalous position (found in *Acidocroton* and *C. alabamensis*) force nectaries to arise in available empty space resulting in number increase leading to further modification as discussed in the above section about nectary of *Acidocroton*. Moreover, the honeycomb-like nectary found in *Ac. spinosus* and *Ac. oligostemon* also emphasise lability of the nectary morphology. Apart of Euphorbiaceae, a receptacular nectary is found to be common in the order Malpighiales (Bernardello, 2007) and perhaps throughout the Rosids clade (Smets and Cresens 1988).

2.5.7 Stamen diversity and evolution

Within the Crotoneae, stamen number is extremely diverse ranging from one to more than 100 (Webster, 2014). The SS clade have relatively high stamen numbers with about 30 stamens (Table 3). In the AABC clade, stamen number are generally ranging from 12 to 25. *Acidocroton* section *Ophellantha* have high stamens numbers which may be the result from stamen increase. In *Croton*, stamen number is extremely variable but generally numbers are about 11-16 (Table 3). *Croton maestrensis* has the lowest stamen number in the whole genus with one to three stamens (Fig. 39F) (Alain, 1960; van Ee et al., 2008), while some *Croton* species from the section *Cyclostigma* could have more than 100 stamens (Chapter 4; Riina et al., 2009). As mentioned before, the presence of generally more than 11 stamens is one major difference between Crotoneae and its sister tribe Jatropheae (generally 8-10 stamens) (Wurdack et al., 2005; Webster, 2014). Moreover, stamens in Jatropheae are commonly partially fused (Nair and Abraham, 1962; Dehgan and Webster, 1979; Dehgan, 2012) contrary to the usually free stamens in Crotoneae (present study; Webster 2014).

Stamen shapes in bud are highly diverse among Crotoneae. In the SS clade, male flowers of *Sagotia* have erect anthers with sessile or very short filaments (Fig. 4L, M), contrary to *Sandwithia* which have long filaments twisting and folding inside the flower buds (Fig. 9M) (Secco, 1987, 1988; Secco et al., 2019). Sessile or subsessile anthers as in *Sagotia* are rare among Euphorbiaceae with records from *Ditta* (Adenoclineae clade) (Leon and Alain, 1953), *Plukenetia* (clade C2, Crotonoideae) (Gillespie, 1993, 2007), *Sampantaea* (Acalyphoideae) (van Welzen and Chayamarit, 2007), *Claoxylon* (Acalyphoideae) (McPherson, 2019), *Mabea* (Euphorbioideae) (Vieira and de Carvalho-Okano, 1996), and *Nealchornea* (Euphorbioideae) (Secco, 2005). Within the order Malpighiales, there are few observed cases of sessile or subsessile anthers in flowers, e.g., *Rhizophora* (Rhizophoraceae) (Tomlinson et al., 1979), *Paradrypetes* (Rhizophoraceae) (Levin, 1992), some *Garcinia* (Clusiaceae) (Sweeney, 2008), *Euthemis* (Ochnaceae) (Kanis, 1972; Kubitzki and Amaral, 1991), *Rhabdophyllum* (Ochnaceae) (Sosef, 2008), *Idertia* (Ochnaceae) (Sosef, 2013), and *Campylospermum* (Ochnaceae) (Amaral and Bittrich, 2014). In Ochnaceae, sessile or subsessile anthers co-occur with poricidal anthers linked with a buzz pollination

syndrome (Kubitzki and Amaral, 1991); however, anthers with longitudinal slits of *Sagotia* are clearly not linked with buzz pollination (Fig. 4M). In *Arabidopsis,* elongation of filaments is controlled by proteins produced by auxin response genes, *Aux/IAA19*, which could be activated with an induction of the plant hormone Auxin via the *AUXIN RESPONSE FACTOR* genes (*ARF*) (Liscum and Reed, 2002; Tashiro et al., 2009; Ghelli et al., 2018). Mutations of these genes generate shorter filaments in the mutant plants (Nagpal, 2005; Tashiro et al., 2009; Varaud et al., 2011; Ghelli et al., 2018) which may explain the shortened filaments in *Sagotia*. However, there is no 'sessile anther' phenotype observed in *Arabidopsis* to date. Thus, we could not pinpoint any possible genetic cause of this trait.

In the AABC clade, species from both sections of *Acidocroton* have generally curved stamens in bud (Fig. 14L; 15K; 17K). The presence of connective appendages on anthers of *Acidocroton* is unique among all genera in the tribe. Connective appendages were reported in many angiosperms with various functions, e.g., improve visibility of flowers, facilitate pollinator gripping, control anther dehiscence and provide reward (Renner, 1989; Luckow and Grimes, 1997; Hermann and Palser, 2000; Han et al., 2008). However, there are no ecological data to verify these functions of connective appendage in *Acidocroton*. Unexpectedly, species of *Brasiliocroton* have different stamen forms in buds; *B. mamoninha* has curved stamens (Fig. 29H; 30L) while *B. muricatus* has erect stamens (Fig. 31K, L). Male flower buds of *B. muricatus* included in our examination may be too young, so the filaments do not elongate yet. However, illustrations of male flowers from protologues of both species support our observation (Berry et al., 2005a; Riina et al., 2014). *Astraea* and *Croton* have inflexed filaments in bud (Fig. 24M, N: 38K) (Webster, 1993, 2014; Radcliffe-Smith, 2001; Berry et al., 2005b; van Ee et al., 2011). There are some exceptions in *Croton* with *C. alabamensis* having curved stamens in bud while most species in section *Moacroton* have erect stamens in bud (Fig. 38L) (Chapter 3, 4; Radcliffe-Smith, 2001; Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2008, 2011). Our results show that each genus has its own way of packing stamens in flower buds to minimize conflict with the maximum number of stamens (generally >10) in a limited space. Endress (1994) pointed out that there are two patterns of packing floral organs in floral bud, i.e.,

stuffing (irregular three-dimensional arrangement) and stacking (regular three-dimensional arrangement), which corresponds to stamen packing in Crotoneae. The 'stuffing' strategy for packing occurs in male flowers with irregular organ number of *Sagotia* and *Sandwithia* (SS clade). Stamens in *Sandwithia* have an irregular shape by twisting and folding resulting from the mutual pressure from growth expansion and elongation. In *Sagotia*, the orientation of stamens is irregular from the pressure from nearby stamens. However, the androecium of *Sagotia* develops a star shape due to spatial pressure from petals. For the AABC clade, stamen number is relatively more stable than in the SS clade, which is suitable for the 'stacking arrangement'. The well-known case of the 'stacking' pattern in the Crotoneae is the presence of stamens with inflexed filaments in male flower buds of *Croton* that are highly organised. Male flowers of *Brasiliocroton,* the close genus of *Croton*, have the potential to produce five pairs of stamens in the outermost whorl but the androecial whorl still follows a 'stacking pattern'. In taxa with a very high stamen number, such as *Acidocroton* section *Ophallantha*, the 'stacked' packing strategy may be broken down into 'stuffing' due to increased mutual pressure. Interestingly, stamens in male flowers of *Astraea* are arranged in several whorl of five as in the 'stacking' pattern. However, filaments are slightly twisted comparable to the 'stuffing' pattern. From an evolutionary point of view, the 'stacking' pattern is likely to be an ancestral state since it also occurs in the sister tribe Jatropheae and in some articulated crotonoid taxa, e.g., *Manihot*, *Micrandra*, while the 'Stuffing' pattern seems to be derived and linked with a stamen increase.

Inflexed filaments in bud were thought to be a unique character of *Croton* inducing some taxonomists to exclude taxa with non-inflexed stamen as separate genera, e.g., *Moacroton* (Radcliffe-Smith, 2001) and *Colobocarpos* (Esser and van Welzen, 2001). However, phylogenetic data revealed that those taxa with non-inflexed filaments in bud belong to *Croton*, while *Astraea* which have inflexed filaments in bud should be treated as a separate genus (Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2011; Silva et al., 2020). Apart of *Croton* and *Astraea*, 'inflexed filaments in bud' occasionally appear in angiosperms. In Euphorbiaceae, this character is found in *Micrandra* (articulated crotonid) and most genera in the tribe Epiprinae

(Acalyphoideae) which are distantly related to the Crotoneae (Webster, 2014). 'Inflexed filaments in buds' are found in different groups of flowering plants, e.g., several families in the order Ericales (Kubitzki, 2004), Apiales (Kadereit and Bittrich, 2018) and Myrtales (Kubitzki, 2007, 2011). Most of them have a convex receptacle with inferior ovary, while in Myrtales the receptacle is concave (hypanthium). Speculation on the origin of 'inflexed filaments in bud' in the Crotoneae is complicated due to the uncertain position of *Brasiliocroton* on the phylogeny (Fig. 2). The presence of 'inflexed filaments in bud' in *Astraea* and *Croton* may have a single origin (less parsimonious in the first topology and equivocal in the third topology) or evolved two time independently (more parsimonious in the first topology and equivocal in the third topology) (Fig. 2; 46). However, stamens of *B. mamoninha* are curved similar to inflexed filaments in *Croton*. It is possible that the ancestors of *Brasiliocroton* had inflexed stamens in bud, but these were later lost. In *Croton*, loss of inflexed filaments occurs in species from section *Moacroton* (except *C. maestrensis* and *C. poecilanthus*) because their filaments are very short (similar to young *B. muricatus*) (Radcliffe-Smith, 2001; Webster, 2014). In male flowers of *C. alabamensis*, stamens are non-inflexed in bud which may relate to hypanthium formation (shift from a convex receptacle to concave receptacle), but stamens are curved toward the centre of the flower (Fig. 38L; Chapter 3).

Androecial architecture is highly diverse in the tribe. In the SS clade, the outermost stamens in *Sagotia* and *Sandwithia* are arranged alternate with petals (Fig. 4J, K; 5F-I; 10G-J). In *Sagotia*, only the outermost five stamens form a clear whorl, each of them is adjacent to two inner stamens. Evidence from sectioning found that there are five groups of bundles (stamen fascicles) that develop centripetally. There is evidence of splitting of a vascular bundle to supply two stamens (Fig. 5G-I). This unusual bundle pattern may be the result of a secondary increase of stamens. In the trimerous *Sandwithia*, unequal number of stamen and unequal size of the nectary are observed. There are two outermost stamens located opposite to the big nectaries while there is one stamen located opposite the small nectary. The cause of this different stamen number may be the unequal size of petal. There is a smaller space available on the receptacle between the first-formed petal and second petal than spaces between the

first petals or second petals and third petals. Therefore, there is enough space for two stamens primordia and a big nectary in the larger space while the smaller space can only fit one stamen and small nectary.

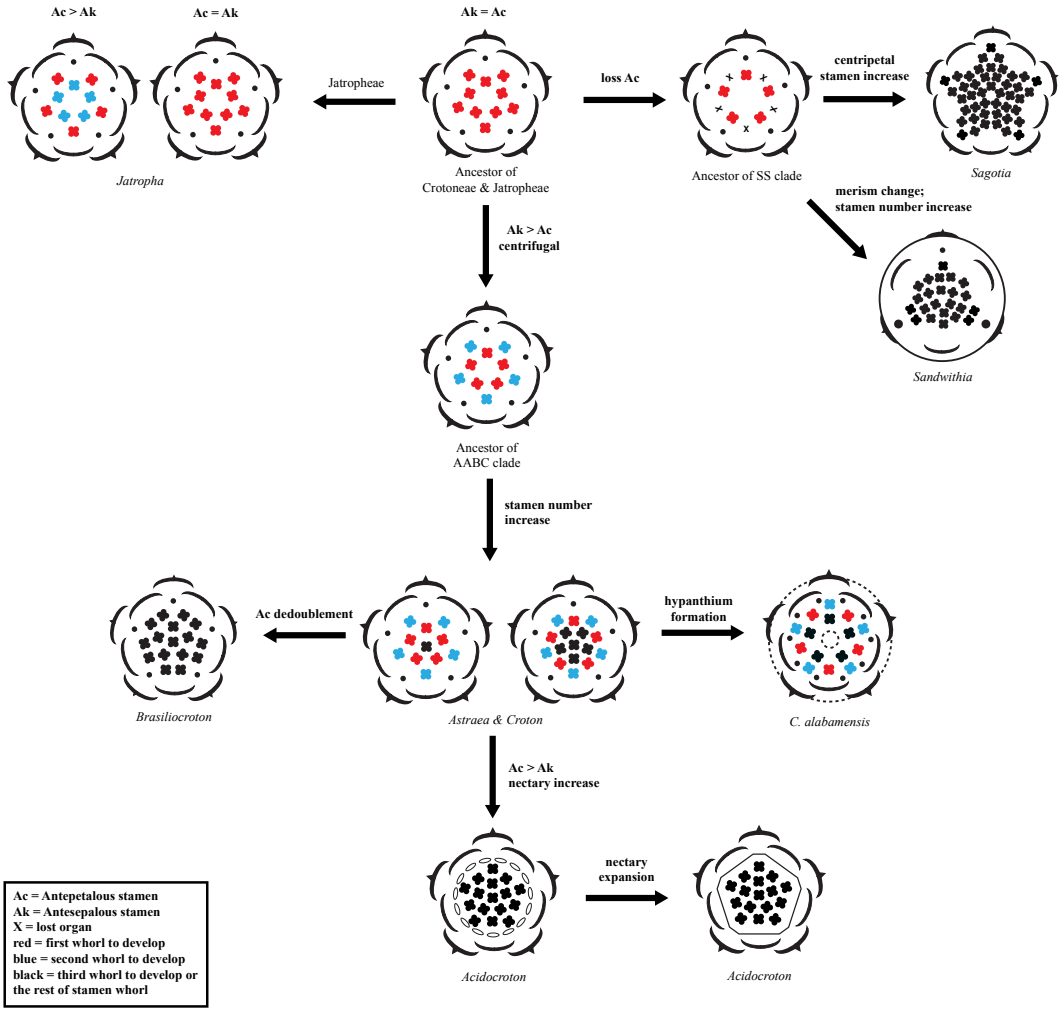

Figure 50. A diagram shows hypothetical evolutionary pathways of the androecium in the tribe Crotoneae. The position of inflorescence diagrams of all genera and representative species follows the phylogenetic relationship (Fig. 42; 44). Taxon name below each diagram represents the genus where the floral diagram is found.

For the AABC clade, *Astraea* and *Croton* are reported to have the outermost stamen whorl generally arranged opposite petals (Fig. 45) (Baillon, 1858; Marchand, 1860; Michaelis, 1924; Nair and Abraham, 1962; Gandhi and Thomas, 1983; De-Paula et al., 2011). Within the AABC clade, *Brasiliocroton* also has the outermost stamen whorl arranged opposite petals. In *B. muricatus*, there are two stamens arranged opposite each petal (Fig. 32I-L), while in *B. mamoninha*, there is one stamen

(occasionally two) opposite each petal (Fig. 30H-J). We thought that the single stamen opposite each petal is an ancestral character since it also occurs in *Astraea* and *Croton* (Fig. 25H; 39D, E; 45), while the stamen pair opposite a petal is derived via dédoublement of stamen primordia (Ronse De Craene and Smets, 1996). The presence of double stamens is found to be correlated with obdiplostemony and centrifugal stamen development (Ronse De Craene and Smets, 1995), as in *Brasiliocroton*, the outermost stamen whorl is arranged opposite petals like in obdiplostemoneous flowers. However, a developmental study is needed to verify if they have centrifugal stamen development as in *Astraea* and *Croton* or not (Chapter 3; De-Paula et al., 2011). *Acidocroton* is the only genus in the AABC clade with the outermost stamen whorl arranged alternate with petals (Fig. 15L; 16H ;18F-L). Since the genus is sister to *Astraea* and it is also nested within other genera with outermost stamens opposite petals (Fig. 2; Berry et al., 2005b; Wurdack et al., 2005; van Ee et al., 2011; Silva et al., 2020), we speculate that the stamen architecture in *Acidocroton* is secondarily derived.

In *Astraea* and *Croton*, although the outermost stamens are opposite to petals, but their androecium could not be described as 'obdiplostemony/obhaplostemony' (as done in Gandhi and Thomas 1983), because there are one to five central stamen in their flowers. However, we will continue to compare their stamen architecture with other taxa with obdiplostemoneous stamens since they share the same character with the presence of outermost stamen opposite petals. The presence of the outermost stamen whorl opposite petals (described as obhaplostemony and obdiplostemony) is an unusual character among angiosperm (Ronse De Craene and Smets, 1987). However, this arrangement of stamens is common in the Euphorbiaceae and reported in several genera of Euphorbiaceae, e.g., *Jatropha* (Baillon, 1858; Beille, 1901; Michaelis, 1924; Nair and Abraham, 1962; Singh, 2005; Liu et al., 2008, 2015; Ronse De Craene, 2010), *Caperonia*, *Manniophyton*, (Michaelis, 1924), *Vernicia* (McCann, 1942; Mao et al., 2017), *Aleurites* (Moncur, 1988), *Ditaxis*, *Argythamnia*, *Chiropetalum*, *Ricinocarpus* and *Dimorphocalyx* (*Tritaxis*) (Baillon, 1858). Moreover, in many genera that lack of petals, there are reports of outermost stamen whorl alternate with tepals (sepals?), e.g., *Mabea*, *Sapium* (Baillon, 1858), *Siphonia* (Eichler,

1878), *Excoecaria* (Baillon, 1858; Venkata-Rao and Ramalakshmi, 1968), *Acalypha*, (Eichler, 1878; Michaelis, 1924), *Sebastiania* (Baillon, 1858; Eichler, 1878), *Hevea*, (Baillon, 1858; Beille, 1901; Zardini, 2019), *Claoxylon* (Michaelis, 1924), *Manihot* (Beille, 1901; Zardini, 2019), *Micrandra* (Schultes, 1979), *Alchornea* (*Caelebogyne*) (Le Maout and Decaisne, 1873; Gama et al., 2019). Interestingly, different publications reported different stamen arrangements in *Chrozophora*, e.g., outermost stamen opposite to petals (Payer, 1857; Baillon, 1858), outermost stamen alternate to petals (Beille, 1901) or outermost stamens arranged in five pairs opposite petals (Venkata-Rao and Ramalakshmi, 1968). In *Astraea* and *Croton*, floral ontogenetic studies, however, found that the antepetalous outermost stamen whorl develops centrifugally after the first alternipetalous whorl, which is unusual among unisexual oligandric and low polyandric flower (Chapter 3; De-Paula et al., 2011). Since we could not obtain flowers from other genera in different stages, we could not confirm if this pattern of development occurs in other genera of the tribe Crotoneae. Obdiplostamony is considered to be a derived character and is a transitional state toward other stamen arrangements, i.e., haplostemony and obhaplostemony (Ronse De Craene and Smets, 1995; Ronse De Craene and Bull-Hereñu, 2016). The centrifugal antepetalous whorl in *Astraea* and *Croton* could be considered as a derived character from diplostemonous ancestors (Corner, 1946; Ronse De Craene and Smets, 1995). In some species of *Jatropha*, there are reports that the antepetalous stamen whorl is the first whorl to develop following by the alternipetalous whorl (Fig. 50)(Singh, 2005; Liu et al., 2008, 2015). However, in some Jatropha species, both antepetalous and alternipetalous whorls develop simultaneously (Fig. 50)(Liu et al., 2015). We speculate that the simultaneous development of two outermost stamen whorls would be an ancestral character of the clade C1 (both Jatropheae and Crotoneae) (Fig. 50). This helps explain the labile stamen arrangement within the tribe Crotoneae. In the SS clade, the outermost alternipetalous stamen whorl may be the result of a reduction of the antepetalous whorl (Fig. 50). In the AABC clade, the ancestor may have a shift to an earlier development of the alternipetalous whorl; therefore, the outer antepetalous whorl appear to develop centrifugally (Fig. 50). Loss of the antepetalous whorl may have happened in the ancestor of *Acidocroton* resulting in the presence of an outermost anternipetalous whorl (Fig. 50).

2.5.8 Note on pollination biology

There are some field observations of pollination biology in *Croton*, while there is no similar representative study carried out in other genera in the Crotoneae. Various pollination modes were observed in *Croton*, e.g., anemophily (Domínguez et al., 1989), entomophily (Reddi and Reddi, 1985; Armbruster et al., 1999; Freitas et al., 2001; Pires et al., 2004; Narbona and Dirzo, 2010) or mixed pollination (Novo et al., 2010). Secco (1987) suggested that the short stamens in *Sagotia* which produce pollen with echinate ornamental is an adaptation for anemophily and long stamens of *Sandwithia* is an adaptation for wind pollination. However, the presence of nectary in male flowers of *Sandwithia* suggest entomophily. Therefore, with in the Crotoneae, we could imply that insect pollination (entomophily) is the common phenomenon because most of them have nectary in their both flower sexes (except male flowers of *Sagotia* with may offer pollens instead). Different groups of insects are found to visit flowers of *Croton*, e.g., ants, bees, butterflies, beetles, flies, thrips and wasps (Reddi and Reddi, 1985; Armbruster et al., 1999; Freitas et al., 2001; Pires et al., 2004; Narbona and Dirzo, 2010; Novo et al., 2010). In *C. suberosus*, there are more insects visits during the male blooming phase than the female blooming phase, possibly because of pollen-gathering insects and the presence of petals (Narbona and Dirzo, 2010). However, petals in flowers from most genera in the Crotoneae are green, yellow or white. Interestingly, *Sagotia* is found to have a colour shift in flowers. Male flowers of *Sagotia* have white petals, receptacle and stamens at anthesis that later turn to red after anthesis (Fig. 1F; 4A). In female flowers, only the style and stigma change colour from white to pink or red (Fig. 1G; 6A). In contrast to *Sagotia*, some species of *Astraea* have anthetic male flowers with a red colour patch on the petals and anthetic female flowers with red styles that later change to white (Fig. 1C)(Silva and Cordeiro, 2020). The phenomenon of shifting colour is reported in at least 21% of all angiosperms with zoophilous pollination (Weiss, 1991). Colour change in flower is considered to be a signal to pollinators to distinguish pollinated and non-pollinated flowers and indicate floral rewards (Weiss, 1991; Schaefer et al., 2004). *Combretum indicum* (L.) DeFillps (Combretaceae) change flower colour from white to red and shift the pollinator system from hawk-month (white flowers) to a generalist syndrome (red flowers) (Eisikowitch

and Rotem, 1987). In *Tibouchina pulchra* Cogn. (Melastomataceae), the flower change from white to pink related to the age of flowers (Brito et al., 2015). It is reported that pink/red colour is less attractive to bees (Endress, 1994) and shifting to red after anthesis may guide insects to anthetic flowers. From the perspective of bees, both white and pink colour could be seen as a spectrum of blue-green colour, but our perception of red colour may look like a green background to the bees (Brito et al., 2015). However, it is also possible that colour-change in flowers may be the result of other processes, e.g., chemical, light etc., not related to plant-animal interaction (Weiss, 1995; Weiss and Lamont, 1997; Thairu and Brunet, 2015; Yan et al., 2018). The sister tribe Jatropheae also have variation of floral colour, ranging from green to yellow to red and a combination of these (Velloso, 1798; Ducke, 1922; Schultes, 1955; Dehgan and Webster, 1979; Dehgan, 2012). However, both male and female flowers of Jatropheae have big showy petals to attract pollinators contrary to Crotoneae where well-developed petals are mostly restricted to male flowers.

In *Astraea* and most *Croton*, there are long trichomes located in the lower part of petals on the adaxial side (Fig. 24H, I; 38H). Trichomes are moniliform and unicellular in *Astraea* which is rarely found in angiosperms (Endress, 1994). Moniliform trichomes were found lining the entrance of the corolla tube or stamen-petal tube of some taxa in eudicots (Endress and Matthews, 2006; Florentin et al., 2016). Moniliform trichomes with unknown function are also found on petals and stamens of flowers from many genera of Commelinaceae (Hardy and Stevenson, 2000; Hardy and Ryndock, 2012; Pellegrini and Faden, 2017). Moniliform trichomes are often found on areas associated with bee activities where they might provide a signal (Endress, 1994). There are several reports mentioning various other possible functions of moniliform trichomes. In some stingless bee pollinated orchids, i.e., *Polystachya* Hook., *Maxillaria* Pabst & Dungs, *Xylobium* Lindl. and *Teuscheria* Garay (Orchidaceae), multicellular uniseriate moniliform trichomes present on the labellum fragment into pseudopollen (Davies, 2002; Davies et al., 2003; Davies and Stpiczynska, 2006). Endress and Rapini (2014) suggested that unicellular moniliform trichomes on petals may be associated with a secondary pollen presentation in flowers of *Emmotum* (Metteniusaceae). In *Petenaea cordata* Lundell (Petenaeaceae), tuffs of

pink moniliform trichomes replace the position of lost petals (Christenhusz et al., 2010) and may have a function of visual attraction. In *Astraea*, moniliform trichomes are transparent and cover the nectary similar to *Croton*. We speculate that these trichomes may function as sponges to absorb nectar and help prevent nectar evaporation especially in the dry open environment in which many *Croton* and *Astraea* grow.

2.6 Conclusion

The tribe Crotoneae possesses a heterogenous floral morphology contrary to the tribe Jatropheae which is its sister group. The presence of inflorescences with male terminal parts, reduced petals in female flowers, and more than 10 free stamens are synapomorphies of the tribe. Two clades were recognised within the tribe. For the SS clade, it is found that *Sagotia* and *Sandwithia* share the presence of botryoid inflorescences with recaulescent petioles. Their male flowers also show a derived stamen increase. For the AABC clade, thyrsoid/racemose inflorescences with condensed or reduced cymules are predominant. Paniculate inflorescences of *Brasilicroton* may be derived from a secondary elongation of cymule primordia. In *Astraea*, *Brasiliocroton* and *Croton*, it is found that the outermost stamens are arranged opposite petals (in pairs in *Brasiliocroton*). *Acidocroton* is like an outcast of the clade with the presence of condensed inflorescences, petals bigger than sepals at anthesis, an increase and expansion of the nectary, anternipetalous outermost stamens and anther connective appendages. Stellate and other derived trichomes in *Astraea*, *Brasiliocroton* and *Croton* are interpreted as outcomes from a reorganisation of simple trichomes found in *Sagotia* and *Sandwithia*. Stamens are the most diverse organs in the tribe with a diversity in number (1 to >100), form (erect, curved, sessile, folded, inflexed in bud), and arrangement (antepetalous outermost stamens and alternipetalous outermost stamens). The origin of different stamen arrangements may come from an ancestor with simultaneously developed stamens. Floral biology of the tribe is also interesting with colour-changing in floral parts of *Sagotia* and *Astraea* and different types of trichomes on petals which may be linked to insect pollination and nectar conservation. Apart from *Astraea* and *Croton*, the detailed morphology of

reproductive structures in other genera is poorly known. Our morphological comparative study combined with a phylogenetic framework help explain the diversity and evolution of several inflorescence and floral structures in the tribe, e.g., a thyrse as ancestral inflorescence character, shift from determinate to indeterminate inflorescence, origin of petals, receptacular origin of nectary and possible simultaneous stamen development as ancestral character. We hope that our study will be an important step exploring the great morphological diversity in the family Euphorbiaceae.

Appendice

Source of DNA regions used for reconstruction of phylogenetic tree.

Species	ITS	Reference	*trnL-F*	Reference
Joannesia princeps		(van Welzen et al., 2020)	AY794686	(Wurdack et al., 2005)
Jatropha integerrima	AY971261	(Berry et al., 2005b)	AY794685	(Wurdack et al., 2005)
Sagotia. racemosa	AY971264	(Berry et al., 2005b)	AY794687	(Wurdack et al., 2005)
Sanwithia. guyanensis	MN566187	(Silva et al., 2020)	AY794688.	(Wurdack et al., 2005)
Acidocroton trichophyllus	EF421766	(van Ee et al., 2008)	EF408087	(van Ee et al., 2008)
Ac. spinosus (= *Ophellantha spinosa*)	AY971263	(Berry et al., 2005b)	AY971344	(Berry et al., 2005b)
Astraea lobata	EF421720	(van Ee et al., 2008)	EF408089	(van Ee et al., 2008)
As. surinamensis	EU497727	(van Ee and Berry, 2009b)	EU497699	(van Ee and Berry, 2009b)
As. comosa	MN566153	(Silva et al., 2020)	MN566256	(Silva et al., 2020)
Brasiliocroton mamoninha	EU586944	(Riina et al., 2009)	EU586998	(Riina et al., 2009)
B. muricatus	KF208628	(Riina et al., 2014)	KF208631	(Riina et al., 2014)
Croton abutiloides	EU586903	(Riina et al., 2009)	EU586957	(Riina et al., 2009)
C. alabamensis var. *alabamensis*	DQ227513	(van Ee et al., 2006)	DQ227545	(van Ee et al., 2006)
C. andinus	FJ614761	(van Ee, 2010)	FJ614801	(van Ee, 2010)
C. argentinus	EU497729	(van Ee and Berry, 2009b)	HM564210	(van Ee et al., 2011)
C. argyranthemus	HM564074	(van Ee et al., 2011)	HM564211	(van Ee et al., 2011)
C. billbergianus	EU477998	(van Ee et al., unpublished)	EU478148	(van Ee et al., unpublished)

C. bonplandianus	AY971185	(Berry et al., 2005b)	AY971276	(Berry et al., 2005b)
C. cascarilloides	AY971191	Berry et al., 2005b)	AY971282	Berry et al., 2005b)
C. celtidifolius	EU586920	(Riina et al., 2009)	EU586975	(Riina et al., 2009)
C. chilensis	EU586905	(Riina et al., 2009)	EU586959	(Riina et al., 2009)
C. chimboracensis	AY971204	(Berry et al., 2005b)	AY971293	(Berry et al., 2005b)
C. conduplicatus	EU477957	(van Ee et al., unpublished)	EU586963	(Riina et al., 2009)
C. cordiifolius	EU586917	(Riina et al., 2009)	EU586971	(Riina et al., 2009)
C. corinthius	EF421751	(van Ee et al., 2008)	EF408110	(van Ee et al., 2008)
C. corylifolius	EU497734	(van Ee and Berry, 2009b)	EU497709	(van Ee and Berry, 2009b)
C. cuneatus	EU478005	(van Ee et al., unpublished)	EU497710	(van Ee and Berry, 2009b)
C. cupreatus	HM564077	(van Ee et al., 2011)	HM564214	(van Ee et al., 2011)
C. dioicus	AY971203	(Berry et al., 2005b)	AY971292	(Berry et al., 2005b)
C. discolor	EU497736	(van Ee and Berry, 2009b)	EU497711	(van Ee and Berry, 2009b)
C. echinoideus	EU586907	(Riina et al., 2009)	EU586967	(Riina et al., 2009)
C. flavens	EU477905	(van Ee et al., unpublished)	EU478134	(van Ee et al., unpublished)
C. floribundus	HM564080	(van Ee et al., 2011)	-	-
C. fluviatilis	KP878385	(van Ee et al., unpublished)	KP878447	(van Ee et al., unpublished)
C. fuscescens	HM564081	(van Ee et al., 2011)	HM564217	(van Ee et al., 2011)
C. gratissimus	AY971214	(Berry et al., 2005b)	AY794696	(Wurdack et al., 2005)

C. hircinus	EU477889	(van Ee et al., unpublished)	EU478127	(van Ee et al., unpublished)
C. hirtus	EU478071	(van Ee et al., unpublished)	EU497715	(van Ee and Berry, 2009b)
C. humilis	HM564083	(van Ee et al., 2011)	HM564218	(van Ee et al., 2011)
C. insularis	AY971220	(Berry et al., 2005b)	AY971308	(Berry et al., 2005b)
C. linearis	EU477933	(van Ee et al., unpublished)	EU478138	(van Ee et al., unpublished)
C. luetzelburgii	HM564087	(van Ee et al., 2011)	HM564222	(van Ee et al., 2011)
C. lundellii	EF421733	(van Ee et al., 2008)	EF408099	(van Ee et al., 2008)
C. macrobothrys	EU586928	(Riina et al., 2009)	EU586984	(Riina et al., 2009)
C. maestransis	EF421753	(van Ee et al., 2008)	EF408127	(van Ee et al., 2008)
C. matourensis	EU478096	(van Ee et al., unpublished)	EU497720	(van Ee and Berry, 2009b)
C. megalobotrys	KP878357	(van Ee et al., unpublished)	KP878430	(van Ee et al., unpublished)
C. micans	AY971232	(Berry et al., 2005b)	AY971319	(Berry et al., 2005b)
C. michauxii var. elliptica	EU478004	(van Ee et al., unpublished)	HM564224	(van Ee et al., 2011)
C. monanthogynus	EU478113	(van Ee et al., unpublished)	EU478169	(van Ee et al., unpublished)
C. mutisianus	EU586930	(Riina et al., 2009)	EU586985	(Riina et al., 2009)
C. nobilis	HM044797	(Caruzo et al., 2011)	HM044778	(Caruzo et al., 2011)
C. nubigenus	EU478103	(van Ee et al., unpublished)	EF408121	(van Ee et al., 2008)
C. ovalifolius	AY971238	(Berry et al., 2005b)	AY971323	(Berry et al., 2005b)
C. pachypodus	EF421789	(van Ee et al., 2008)	EF408128.1	(van Ee et al., 2008)
C. perspeciosus	EU586931	(Riina et al., 2009)	EU586986	(Riina et al., 2009)
C. piptocalyx	EF421791	(van Ee et al., 2008)	EF408132	(van Ee et al., 2008)
C. polyandrus	HM564093	(van Ee et al., 2011)	HM564228	(van Ee et al., 2011)
C. priscus	EU586950	(Riina et al., 2009)	EU587002	(Riina et al., 2009)
C. punctatus	EU478022	(van Ee et al., unpublished)	EU478153	(van Ee et al., unpublished)
C. pungens	AY971241	(Berry et al., 2005b)	AY971326	(Berry et al., 2005b)

C. persimilis	AY971244.	(Berry et al., 2005b)	AY971329	(Berry et al., 2005b)
C. pulegiodorus	-	R. Riina (unpublished data)	-	R. Riina (unpublished data)
C. rusbyi	-	R. Riina (unpublished data)	-	R. Riina (unpublished data)
C. sampatik	EF421792	(van Ee et al., 2008)	EF408133	(van Ee et al., 2008)
C. sapiifolius	EF421754	(van Ee et al., 2008)	EF408150	(van Ee et al., 2008)
C. schiedeanus	EU478051	(van Ee et al., unpublished)	EU478156	(van Ee et al., unpublished)
C. sellowii	HM564095	(van Ee et al., 2011)	HM564230	(van Ee et al., 2011)
C. setiger	KX147520	(Iwanowicz, Vandergast et al., unpublished)	AY794697	(Wurdack et al., 2005)
C. socotranus	AY971250	(Berry et al., 2005b)	AY971333	(Berry et al., 2005b)
C. suaveolens	AY971252	(Berry et al., 2005b)	AY971334	(Berry et al., 2005b)
C. speciosus	AY971251	(Berry et al., 2005b)	AY794699	(Wurdack et al., 2005b)
C. spruceanus	HM044806	(Caruzo et al., 2011)	HM044785	(Caruzo et al., 2011)
C. suberosus	EU477979	(van Ee et al., unpublished)	EU478144	(van Ee et al., unpublished)
C. thorelii	KP878398	(van Ee et al., unpublished)	KP878453	(van Ee et al., unpublished)
C. tiglium	KP878399	(van Ee et al., unpublished)	KP878454	(van Ee et al., unpublished)
C. trigonocarpus	DQ227530	(van Ee, Jelinski, Berry et al., 2006)	DQ227562	(van Ee, Jelinski, Berry et al., 2006)
C. trinitatis	EU478092	(van Ee et al., unpublished)	HM564232	(van Ee et al., 2011)
C. urucurana	EU586937	(Riina et al., 2009)	EU586991	(Riina et al., 2009)

Matrix of character states for 81 Crotoneae species used in the ancestral character state reconstruction.

species	Floral trichomes	Stamen number	Stamen arrangement	Inflexed stamen in bud
Joannesia princeps	0	1/2	0	0
Jatropha curcas	0	1/2	0	0
Ja. integerrima	0	1/2	0	0
Sagotia racemosa	0	6	0	1
Sanwithia guyanensis	0	6	0	1
Acidocroton oligostemon	0	3	0	1
Ac. gentryi	0	6	0	1
Astraea comosa	1	2/3	1	0
As. lobata	0	2/3	1	0
As. surinamensis	0	2/3	1	0
Brasiliocroton mamoninha	1	4	0	0
B. muricatus	1	3	0	0
Croton piptocalyx	1	3	1	0
C. sampatik	1	2	1	0
C. sapiifolius	1	2/3	1	?
C. pachypodus	2	2/3	1	0
C. maestrensis	2	0	1	1
C. trigonocarpus	2	1	0	1
C. nubigenus	0/1	3	1	0
C. alabamensis	2	3	0	1
C. corinthius	1	3	1	?
C. corylifolius	1	3	1	0
C. cf lundellii	1	3	1	0
C. speciosus	1	6	1	2
C. celtidifolius	1	6	1	2
C. macrobothrys	1	3/4	1	0

C. aff mutisianus	1	3	1	0
C. perspeciosus	1	6	1	2
C. pulegiodorus	1	3	1	0
C. rusbyi	1	2/3	1	0
C. urucurana	1	3	1	0
C. chimboracensis	1	2/3	1	0
C. abutiloides	1	6	1	0
C. bonplandianus	1	2/3	1	0
C. chilensis	1	4/5	1	0
C. conduplicatus	1	3	1	0
C. echioides	1	3	1	0
C. flavens	1	3	1	0
C. humilis	1	3/4/5/6	1	?
C. leptostachyus	1	3	1	0
C. linearis	1	2/3	1	0
C. discolor	1	?	1	0
C. pungens	1	6	1	?
C. suaveolens	1	2/3	1	0
C. suberosus	1	3	1	0
C. cascarilloides	2	3	1	0
C. fluviatilis	1	2	1	0
C. gratissimus	2	3	1	0
C. insularis	2	2	1	0
C. megalobotrys	1	4	1	0
C. megalocarpus	2	5	1	0
C. nobilis	2	4	1	0
C. persimilis	2	2	1	0
C. socotranus	2	6	1	0
C. thorelii	1	2	1	0
C. tiglium	1	3	1	0
C. cupreatus	2	2	1	0

C. setiger	1	1	1	0
C. cuneatus	2	3	1	0
C. schiedeanus	2	2	1	0
C. michauxii	2	0/1	1	0
C. argyranthemus	2	1	1	0
C. dioicus	2	2	1	0
C. punctatus	?	2	1	0
C. priscus	1	3	1	0
C. andinus	1	2	1	0
C. argentinus	1	3	1	0
C. billbergianus	1	2/3	1	0
C. spruceanus	1	3	1	0
C. cordiifolius	1/2	2	1	0
C. polyandrus	1	2	1	0
C. luetzelburgii	1	3	1	0
C. hirtus	1	2	1	0
C. trinitatis	1	1	1	0
C. longibracteatus	1	2	1	0
C. ovalifolius	1	2	1	0
C. sellowii	2	2	1	0
C. matourensis	2	2	1	0
C. fuscescens	1	2	1	0
C. micans	2	2/3	1	0
C. monanthogynus	1	1	1	0

Character states information

1. trichome
    0 simple
    1 stellate
    2 lepidote
2. stamen number
    0 1-4
    1 5-9
    2 10-14
    3 15-19
    4 20-24
    5 25-29
    6 30+
3. stamen arrangement
    0 Outermost stamen whorl opposite to petals
    1 Outermost stamen whorl alternate to petals
    2 chaotic
4. inflexed stamen
    0 non-inflexed in bud
    1 inflexed in bud

Chapter 3: Divergent developmental pathways in dimorphic flowers of *Croton* L. (Euphorbiaceae) with special emphasis on petals

This chapter has been submitted for a special volume of the journal 'Frontier in Ecology and Evolution' entitled 'From Meristems to Floral Diversity: Developmental Options and Constraints'.

Data contributions: Experiments are designed by the author. Most of ontogenetic data are obtained from the work of Stuart Ritchie (MSc dissertation, Royal botanic Garden Edinburgh, UK) under the supervision of the author. Other observations were conducted by the author.

3.1 Chapter summary

Croton have highly dimorphic flowers with petals present in male flowers, contrary to filamentous structures in the same position in female flowers. However, well developed petals can be found in female flowers of some African species and two New World sections, i.e., sect. *Alabamenses* and sect. *Eluteria* subsect. *Eluteria*. Our aims are to compare ontogeny in male and female flowers of *Croton* which may elucidate the origin of petals and homology of the filamentous structures in comparison with petals and also explore floral diversity in male flowers. The development of flowers of *C. alabamensis* (sect. *Alabamenses)* and *C. schiedeanus* (sect. *Eluteria*, subsect. *Eluteria*) was studied and compared with *C. chilensis* (sect. *Adenophylli*). Ontogeny from both flower sexes of those three species were observed with scanning electron microscopy (SEM). In male flowers, petals develop in alternation with sepals and later the outermost stamen whorl develops opposite the petals. In female flowers, filamentous structures and petals share an early development in alternation with the sepals. However, the filamentous structures of *C. chilensis* become arrested in their growth, while petals of *C. alabamensis* and *C. schiedeanus* continue their growth similar to those of the male flowers. We suggest that the filamentous structures in female flowers represent paedomorphic forms of petals in

male flowers, and that well-developed petals in *C. alabamensis* and *C. schiedeanus* are derived via a developmental reversion. A staminodial origin of petals and nectaries is questioned. Additionally, male flower development shows unexpected diversity, including a floral cup in *C. alabamensis*. All investigated species have an unusual centrifugal initiation of the second stamen whorl. The outer stamen whorl is generally antepetalous, except in *C. alabamensis*, and differences in configuration are linked with different spatial constraints. Sections *Alabamenses* and *Eluteria* are far apart on the phylogeny and do not share any other unique characters. Therefore, petals in female flowers of both sections probably arose independently and represent a reversal in petal evolution of *Croton*.

3.2 Introduction

In flowers, "petal" is a term generally applied to the showy pigmented inner perianth whorl, in contrast to the term "sepal" which represents the protective green outermost perianth whorl. The presence of pigments, delicate texture, single vascular bundle and narrow base are common features found in petals (Endress, 1994; Ronse De Craene, 2007). However, it is not always easy to classify the perianth as sepals and petals especially in cases where there is only one perianth whorl present, since this may be a result of reduction of either whorl correlated with a functional change (Ronse De Craene, 2010). Petals play a major role in pollinator attraction via visual cues but sometimes they also facilitate scent dispersal, provide nectar, and function as landing platforms for insects (Endress, 1994; Whitney et al., 2011; Ojeda et al., 2016). The presence of both sepals and petals (biseriate perianth) in the flower is the most common pattern in angiosperms, at least in core eudicots (Ronse De Craene and Brockington, 2013). Evolutionary developmental genetic studies in the model plants *Arabidopsis* and *Antirrhinum* found that interaction between different gene classes gives rise to organ determination in each whorl of the flower (ABCDE model) (Litt and Kramer, 2010; Rijpkema et al., 2010). Co-expression of A- and B-class genes is found to determine petal identity in core eudicots (Pentapetalae) (Litt and Kramer, 2010; Rijpkema et al., 2010). However, Ronse De Craene (2007) argued that homology of petals should not be deduced based on gene expression pattern alone, but

should include historical, phylogenetic and ontogenetic perspectives since all floral organs are capable of becoming petaloid and the genetic difference between different types of petals was not studied. There are different sources of evidence that 'petals' from different groups of angiosperms may have different origins. A continuous transition in the perianth from bracts to petal-like perianth parts is observed in the ANA grade, many Magnoliids (Magnoliales, Laurales) and sometimes in core eudicots (e.g., Cactaceae, *Berberidopsis*, *Paeonia* and Theaceae), while in some Magnoliids and Monocots, petals may be derived by pigmentation of the inner tepals (Ronse De Craene, 2008). Petals derived from the transformation of sterile stamens (staminodes) is also reported in Ranunculales (Ronse De Craene, 2008). In the core-eudicots, the largest group of angiosperms, the presence of a perianth with clear distinction between sepals and petals is prominent (Ronse De Craene, 2008, 2010; Soltis et al., 2018). Two possible origins for petals in core eudicots have been proposed, i.e. andropetals (staminodial origin) or bracteopetals (bracteolar origin) (Ronse De Craene, 2007). Andropetals generally have a delayed development and small primordia, contrary to bracteopetals which generally have an early development and broad primordia (Ronse De Craene, 2008). However, Ronse De Craene (2007) suggested that in core-eudicots andropetals are rare, and petals are mostly derived from bracteopetals.

The family Euphorbiaceae belongs to the order Malpighiales, the largest order of the Rosid clade in the core-eudicots (The Angiosperm Phylogeny Group, 2016). The family has great diversity in floral morphology and is well-known for the sophisticated inflorescence-flower pseudanthial complex called cyathium which is found in the genus *Euphorbia* (Wurdack et al., 2005; Prenner and Rudall, 2007). However, in general little is known about flowers in the whole family. The presence of petals is generally an uncommon character in the Euphorbiaceae with a scattered distribution in distantly related internal groups (Wurdack et al., 2005). The inaperturate crotonoid clade (subfamily Crotonoideae), comprising about 60 genera, is one group in Euphorbiaceae where the presence of petals at least in male flowers is a key character in defining their affinity (together with inaperturate pollen grains) (Wurdack et al., 2005; Webster, 2014). The putative sister group of this clade is the articulated crotonoid clade, comprising the economically important rubber tree

(*Hevea*) and cassava (*Manihot*), which does not have petals but have petaloid tepals (sepals?) instead (Wurdack et al., 2005). So far, no developmental study has been conducted to investigate the origin of petals and their evolution in the inaperturate crotonoid clade, whether they represent andropetals or bracteopetals.

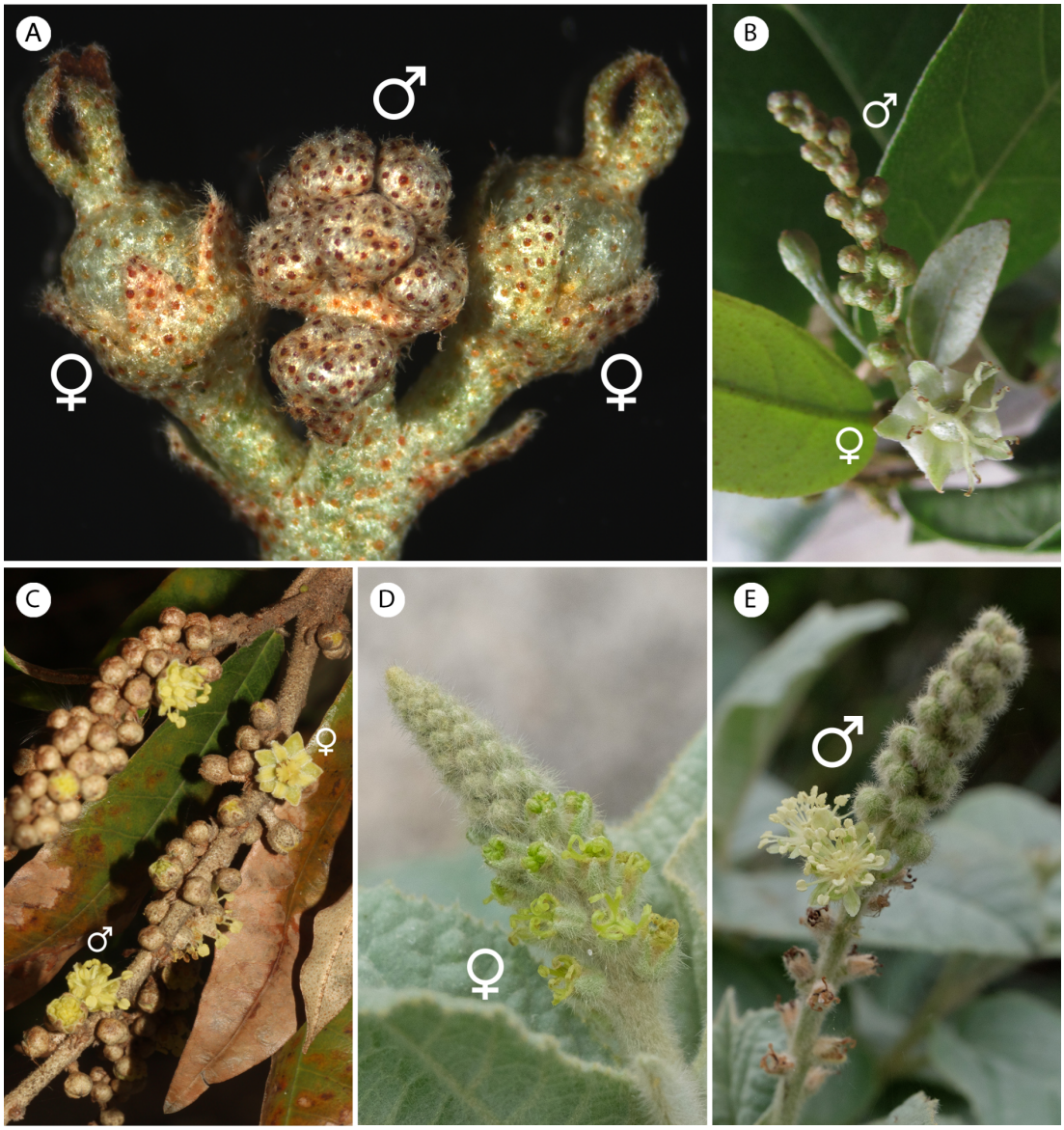

Figure 1. Inflorescence of *Croton* with/without petals in female flower (A) Inflorescence of *C. alabamensis* showing blooming female flowers while male flowers are still closed. (B) Inflorescence of *C. schiedeanus* with long-pedicellate female flower on the proximal part and many male flowers on the distal part. (C) Inflorescence of *C. gratissimus*, an African species with petals in female flowers. (D) Inflorescence of *C. chilensis* showing female stage. (E) Inflorescence of *C. chilensis* in the male stage (two open male flowers).

Chapter 3: Dimorphic flowers of *Croton*

Croton is a mega-diverse genus of more than 1,200 recognized species with a broad tropical to sub-tropical worldwide distribution in various habitats (Webster, 1993; Govaerts et al., 2000; Berry et al., 2005b; van Ee et al., 2011, 2015). Their large size, high morphological variation and wide distribution create great difficulties for taxonomic studies (van Ee et al., 2011). Flowers of *Croton* are unisexual with both sexes usually borne on the same indeterminate racemose-thyrsoid inflorescence with female flowers on the proximal part and male flowers on the distal part (Fig. 1; 2; Webster, 1993; Berry et al., 2005b; van Ee et al., 2011). Well-developed petals are normally present in male flowers, contrary to female flowers that usually lack petals or have filamentous structures present in the same position. Filamentous structures are not just present in *Croton* but also in other genera of the inaperturate crotonoid clade (Nair and Abraham, 1962; Venkata-Rao and Ramalakshmi, 1968). The origin of the filamentous structures of female flowers is controversial since they were interpreted as reduced petals (Nair and Abraham, 1962; Webster, 1993; Radcliffe-Smith, 2001; Caruzo and Cordeiro, 2007; De-Paula et al., 2011) or staminodes (Gagliardi et al., 2017). However, the presence of well-developed petals in female flowers was reported in two New World sections, i.e., sect. *Alabamenses* (Fig. 1A) and sect. *Eluteria* subsect. *Eluteria* (Fig. 1B) (van Ee et al., 2011), and some African species (Fig. 1C) (Friis and Gilbert, 2008; Berry et al., 2016). Our study aims to clarify how these petals develop compared with the filamentous structures from typical female flowers and petals from male flowers.

We conducted a comparative ontogeny in three *Croton* species with different floral morphologies to find answers for following questions: (1) origin of petals, (2) homology between petals of male and female flowers, (3) origin of filamentous structures in the female flowers, and (4) diversity in male flowers. A better understanding of petal origins and their evolution in *Croton* will help in understanding similar phenomena in other taxa of subfamily Crotonoideae and also in the family Euphorbiaceae.

3.3 Materials and Methods

3.3.1 Sample collection

Male and female flowers of *C. alabamensis* (sect. *Alabamenses*) (Fig. 1A) and *C. schiedeanus* (sect. *Eluteria*, subsect. *Eluteria*) (Fig. 1B), both of which have well-developed petals in female flowers, were studied and compared with flowers from *C. chilensis* (sect. *Adenophylli*) (Fig. 1D-E), which has filamentous structures in female flowers (Table 1). Samples were collected from cultivated plants and in the field and fixed in FAA solution (90% ethanol at 70%, 5% glacial acetic acid and 5% formalin solution at 40%), then stored in 70% ethanol.

Table 1. Origin of samples of *Croton* used in this study

Samples	Plant Code	Collection number	Source/ Origin
C. alabamensis var. *alabamensis* E.A.Sm. ex Chapm.	-	Pratt & Ferry PFA8	Cultivated plant from Kenneth Wurdack garden/ USA
C. alabamensis E.A.Sm. ex Chapm. var. *texensis* Ginzbarg	Grown from seeds at RBGE	LBJWC-1704 (seed code)	Lady Bird Johnson Wild Flower Center/ Texas, USA
C. chilensis Müll.Arg.	BGHMR53 (2009.0241A)	1099 Led	RBGE/wild collected in Chile
C. schiedeanus Schltdl.	-	1441/865 La	Wild collected/ Belize
.	-	1331 La	Wild collected/ Belize
	-	1093 La (Herb. L. Stoddart 02 under name *C. glabellus*)	Wild collected/ Belize

3.3.2 Morphological and ontogenetic observation

Inflorescence and floral morphology were observed using light microscopy (Zeiss Stemi 2000-C) and photos were captured with an AxioCam MRc 5 (Zeiss). For the developmental and detail morphology studies, flowers from different stages were dissected under a light microscope (Zeiss Stemi SV6), dehydrated in an alcohol gradient, dehydrated in acetone, dried at CO_2 critical point in a K850 critical-point dryer (Quorum Technologies), further dissected, mounted on aluminum stubs, coated with platinum (Emitech K575X) and observed with a scanning electron microscope (SEM) (Leo Supra 55-VP). All pictures were later edited in Photoshop program (Adobe Inc.). Floral and inflorescence diagrams were draw in Illustator program (Adobe Inc.).

3.4 Results

3.4.1 *Croton chilensis*

General morphology

The inflorescence of *Croton chilensis* is generally a thyrse (an indeterminate inflorescence with lateral cymes) with the presence of solitary female flowers or bisexual cymules (a female flower associated with several male flowers) on the lower part and solitary male flowers or male cymules on the upper part (Fig. 1D-E; 2A). Inflorescences are produced terminally on the stem. The female flowers reach maturity before male flowers (Fig. 1D). Both male and female flowers are subtended by a bract and two bracteoles. Male flowers are borne on a slender pedicel while female flowers are nearly sessile (Fig. 2A). Flowers from both sexes are pentamerous (Fig. 2D; G).

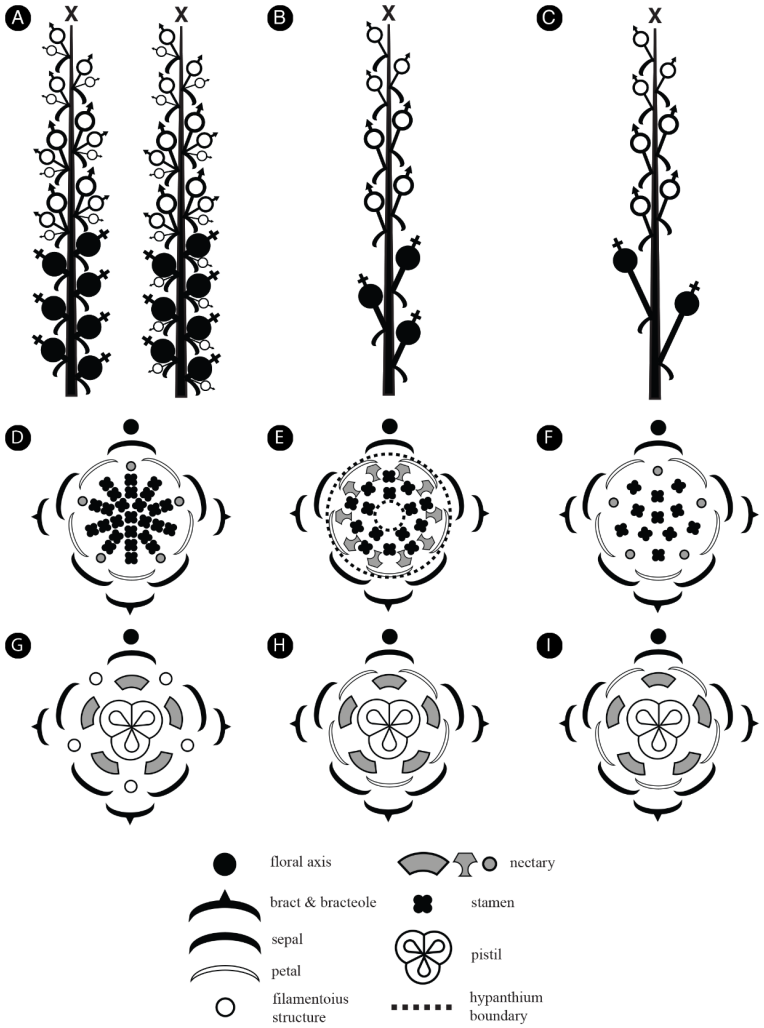

Figure 2. Diagrams showing morphology of inflorescence and flower at anthesis from the three species of *Croton* used in this study. (A, D, G) *C. chilensis*; (B, E, H) *C. alabamensis*; (C, F, I*) C. schiedeanus*. (A) Inflorescence of *C. chilensis* with thyrse type and solitary female flowers or bisexual cymules on the lower part and many male cymules on the upper part. (B) Inflorescence of *C. alabamensis* as a raceme type with few solitary female flowers on the lower part and some solitary male flowers on the upper part. (C) Inflorescence of *C. schiedeanus* as a raceme type with one or two female flowers with long pedicel on the lower part and many solitary male flowers on the upper part. (D) Male flower of *C. chilensis* with 25 stamens arranged in several whorls. Note, stamens from the outermost whorl arranged opposite petals. (E) Male flower of *C. alabamensis* with 15 stamens arranged in cup- shape flower (hypanthium) surrounding central empty space. The outermost stamens alternate with petals and have a nectary clasping their filament. (F) Male flower of *C. schiedeanus* with 11 stamens arranged in two whorls with the outermost whorl arranged opposite to petals and a central stamen. (G) Female flower of *C. chilensis* with presence of five filamentous structures arranged alternate with sepals and nectaries. (H) Female flower of *C. alabamensis* with fully developed petals present alternating with sepals and nectary lobes. (I) Female flowers of *C. schiedeanus* with five petals present. Floral diagrams were drawn following Ronse De Craene (2010).

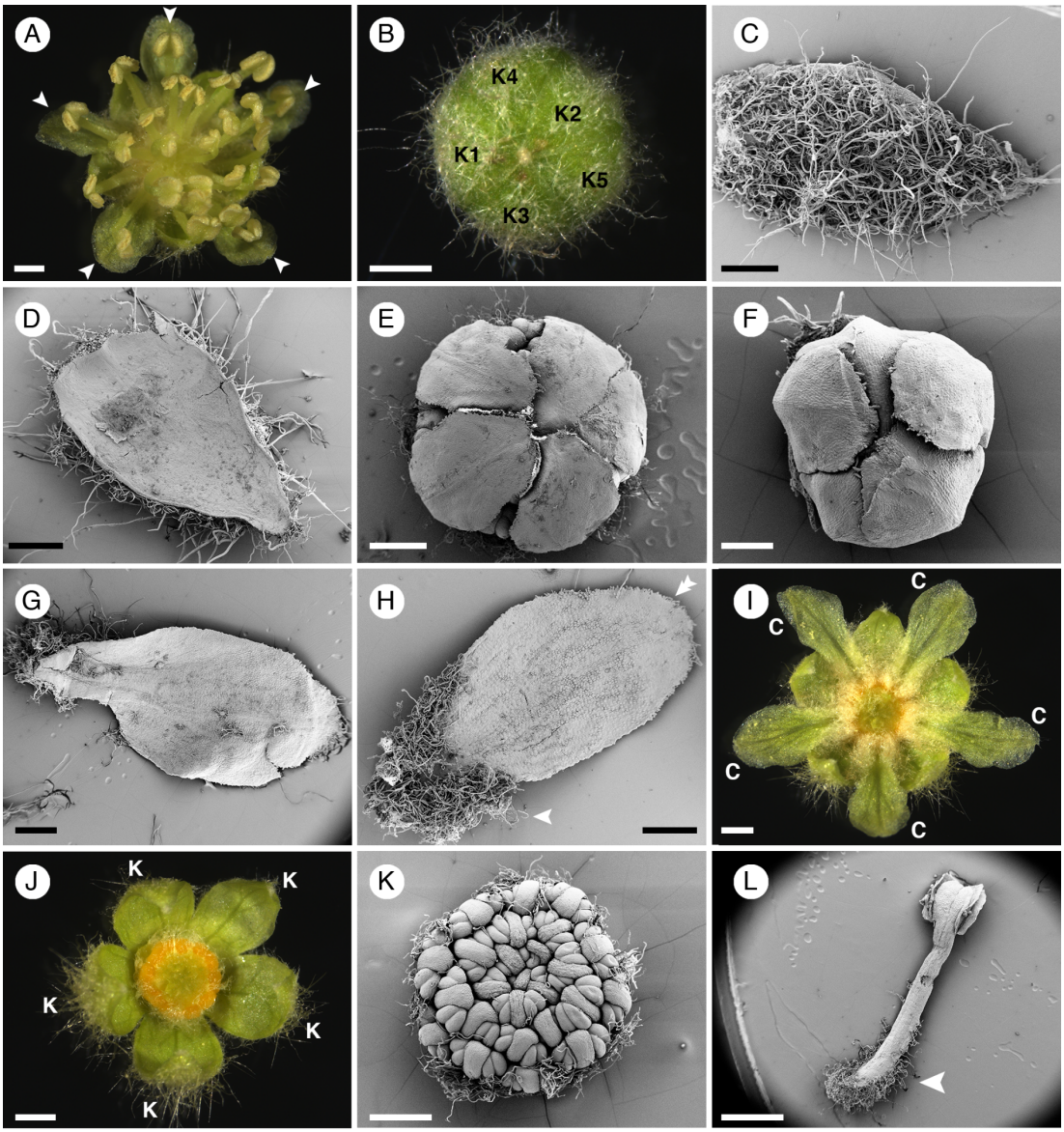

Figure 3. Morphology of male flower of *Croton chilensis*. (A) An anthetic flower. Note, the outermost stamens are opposite petals (arrowheads). (B) A flower bud near anthesis with quincuncial aestivation. (C) Abaxial surface of a sepal covered with long stellate trichomes. (D) Adaxial surface of a sepal with glabrous surface. (E) Corolla with cochlear aestivation. (F) Corolla with quincuncial aestivation. (G) Abaxial surface of a petal with few stellate trichomes on the surface. (H) Adaxial surface of a petal with long simple trichomes covering the lower part (arrowhead). Note, there are papillae on the apex's margin (double arrowhead). (I) A flower with stamens removed showing trichomes on the lower part of petals covering nectary glands. (J) A flower with petals and stamens removed showing many yellow nectary glands. (K) Stamens in flower bud. Note, anthers are inflexed making them look like facing outward. (L) A stamen with lower part of filament covered with many simple trichomes (arrowhead). K, sepal; C, petal. Scale bars: (A, B, I, J, L) = 1,000 µm; (C-E, G, H, K) = 500 µm; (F) = 200 µm.

The perianth of male flowers is differentiated into outer sepals and inner petals, both with green colour (Fig. 2D; 3A). Aestivation of sepals is quincuncial (Fig. 3B). Sepals are ovate in shape with a wide base and acute apex (Fig. 3C, D). The abaxial surface of sepals is covered with stellate trichomes (Fig. 3C) while the adaxial surface is glabrous (Fig. 3D). Petals are arranged in cochlear or quincuncial aestivation (Fig. 3E, F). Petals are obovate in shape with a narrow base (no claw) and obtuse apex (Fig. 3G, H). Both surfaces of petals are generally glabrous except for the lower area of the adaxial side which is covered with many simple trichomes (Fig. 3G, H). The apex of the petals is covered with papillae (Fig. 3G, H). Inside the corolla whorl, there are five bilobed nectary glands alternating with the petals (Fig. 3I, J). In the middle of the flowers, there are about 20 (19-24) stamens present on a convex receptacle (could be interpreted as a short androphore by some authors) (Fig. 3A). Anthers are inflexed in bud (Fig. 3K) before expanding at anthesis (Fig. 3A, L). The outermost stamen whorl is opposite the petals (Fig. 3A). There are several simple trichomes at the base of each filament (Fig. 3L). The receptacle is pilose. There is no evidence of a gynoecial structure in the male flowers (Fig. 3A).

In female flowers, the five sepals are present (Fig. 2D; 4A). Aestivation of sepals is quincuncial (Fig. 3B). Sepal shape is ovate with a wide base, which makes it look triangle shaped (Fig. 4B, C). The apex is acute (Fig. 4B-C). Within the calyx and alternating with the sepals, there are generally filamentous structures (Fig. 4D), occasionally petals (Fig. 4A) or colleters (Fig. 4E). Opposite to the sepals, there are five nectary glands present, alternating with the filamentous structures (Fig. 4D-F). Petals, if present, have a narrowly ligulate shape with acute apex (Fig. 4A, F). Stamens are completely absent in the female flowers. At the centre of the flower, there is an ovary comprising three carpels (Fig. 4G). The surface of the ovary is covered with dense stellate trichomes (Fig. 4G-H). There are three styles with bifid stigmatic tips (total of six tips) (Fig. 1D, 4A).

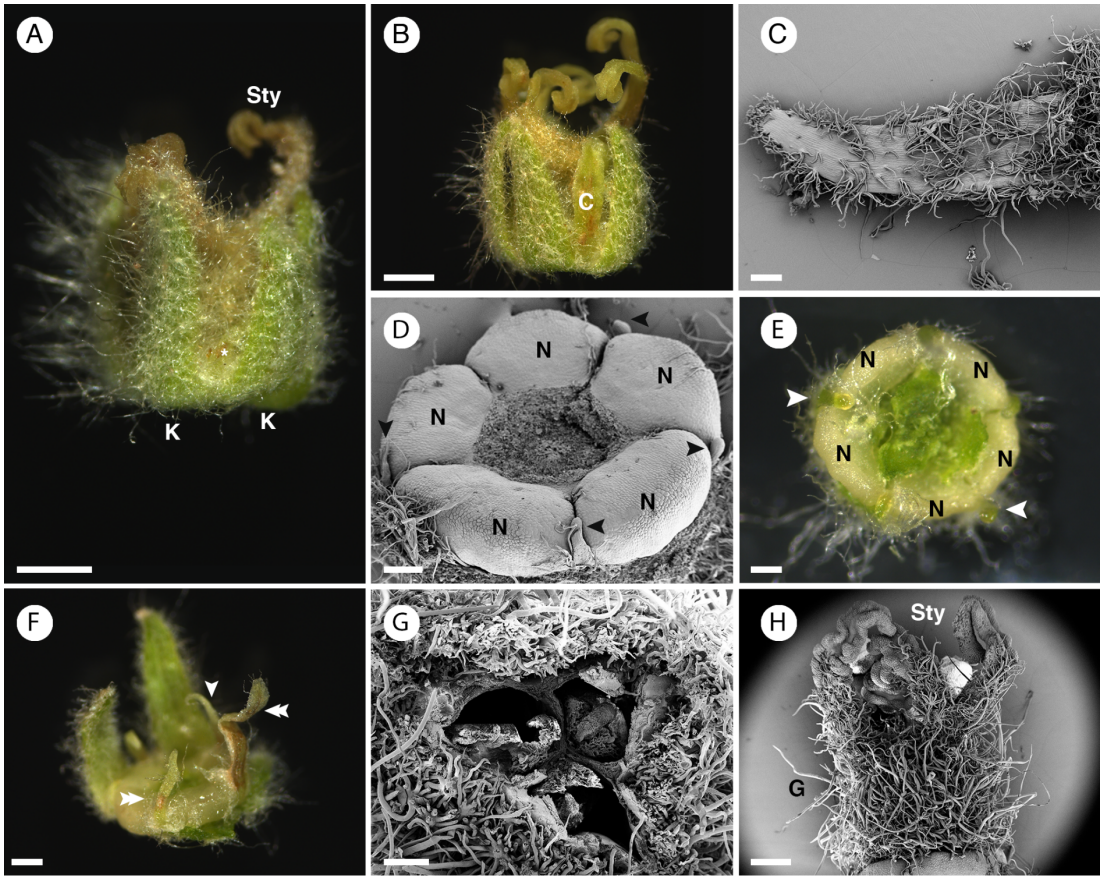

Figure 4. Morphology of female flower of *Croton chilensis*. (A) A flower at anthesis. Sepals are conspicuous while petals are inconspicuous (asterisk). Note, the abaxial surface of sepals is covered with long stellate trichomes. (B) An anthetic flower with a petal present. (C) Adaxial surface of a sepal covered with variably reduced stellate trichomes. (D) Five nectary glands alternating with small filamentous structures arranged externally. Note, filamentous structures are heteromorphic in the same flower with some becoming glandular (arrowhead). (E) Filamentous structures and nectary glands at anthesis. Some filamentous structures become glandular (arrowhead). (F) Heteromorphic organs alternating with nectaries. Some have a filamentous form (arrowhead), while some resemble petals (double arrowhead). (G) An ovary consisting of three carpels with axile placentation. (H) There are three styles on top of an ovary. The style and stigma have a papillate surface. K, sepal; Sty, style; C, petal; N, nectary; G, ovary. Scale bars: (A, B) = 1,000 µm; (C-E, G) = 200 µm; (F, H) = 500 µm.

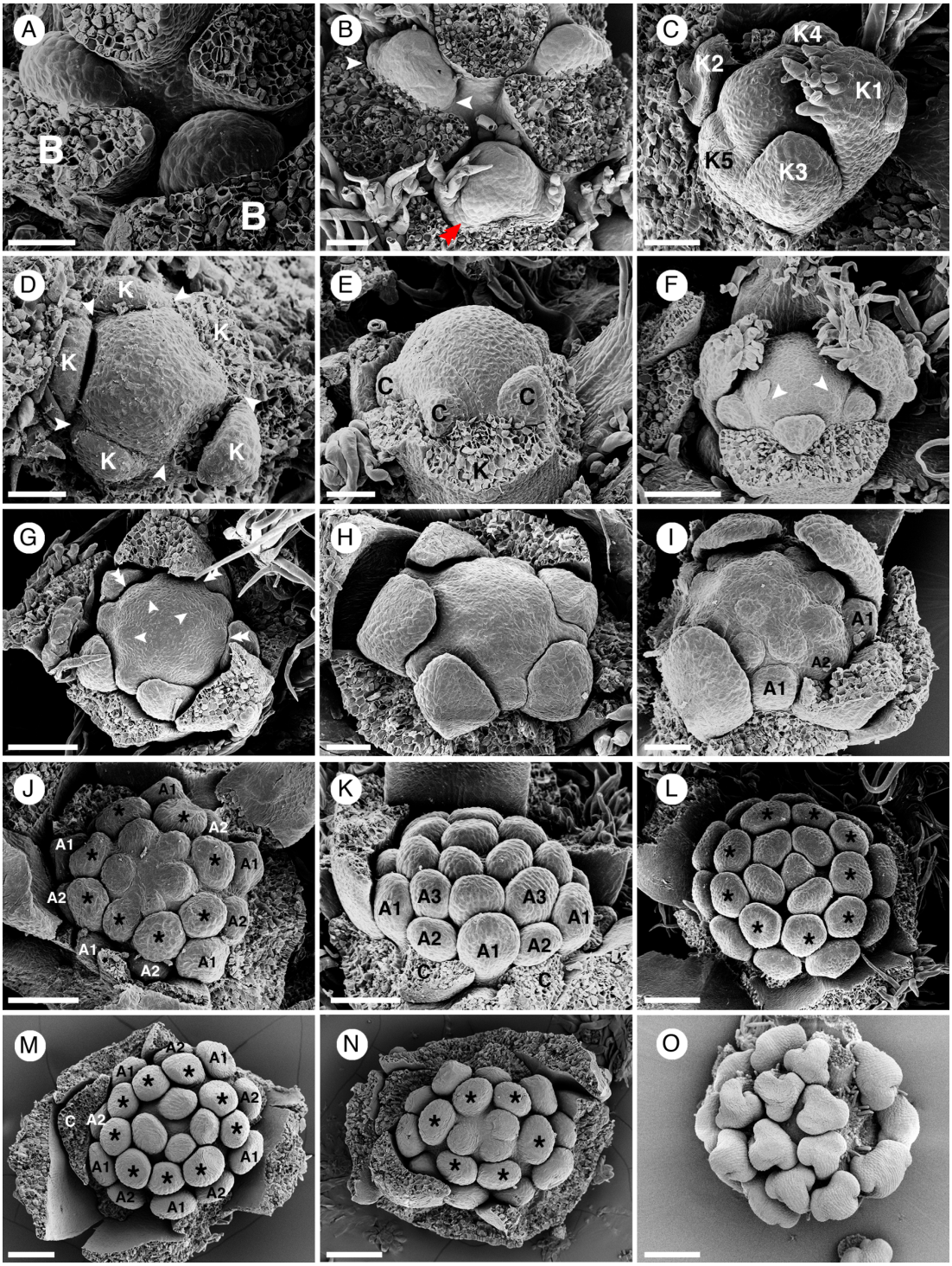

Figure 5. Ontogeny of male flowers from *Croton chilensis*. (A) Young floral primordia subtended by a bract. Note, the floral apex has a convex shape. (B) Sequence of early floral development with formation of bracteoles (arrowhead) and a first sepal (double arrowhead). (C) An older bud shows unidirectional growth of sepals with the initiation of petal primordia. (D) Apical view of initiation of petal primordia (arrowheads)of a young flower bud.

Figure 5 continued. (E) In later stage, young petals grow and expand surrounding the central floral apex. (F) While petals are growing, the first whorl of stamens develops alternating with petals (arrowhead). (G) After that, the second whorl of stamens develops centrifugally opposite petals, while the third whorl develops centripetally alternate with the first stamen whorl. (H) Later, the fourth stamen whorl initiates. Only first, third and fourth whorls are visible. The second whorl is covered by expanding petals which have unidirectional growth. (I) Next, more stamens develop but they could not complete a full whorl. (J) Anomalous stamen number (eight) in the third and fourth whorls (asterisks). Five stamens are present in the central part forming a complete whorl. (K) The antepetalous second stamen whorl is pushed by petals making the antesepalous stamen whorl the outermost whorl. (L) Nine stamens are present in the third and fourth whorls (asterisks). Five central stamens are present but do not form a complete whorl of five. (M) The outermost stamens during young development stage are stamens from the first whorl. An anomalous stamen number of nine is observed in the third and fourth whorls (asterisk). (N) A flower with anomalous low stamen number (total of 19 stamens). Boundary between third and fourth whorls could not be observed (asterisks). (O) Later, young stamens curve toward the floral apex to become inflexed at later stages. B, bract; K, sepal; C, petal; A, stamen. Scale bars: (A-E, H, I) = 50 μm; (F, G, K, M, N) = 100 μm; (I, J) = 90 μm; (L) = 120 μm; (O) = 200 μm.

Ontogeny of male flowers

Male floral primordia arise spirally on the inflorescence axis in the axil of a bract. A pair of bracteoles develops later laterally of the floral primordium (Fig. 5A-B). The first sepal develops in the position furthest from the axis on the convex floral primordium (Fig. 5C). Sepals then develop spirally in a 2/5 pattern (Fig. 5C-D). The first sepal is large and covers almost half the entire bud during the development (Fig. 5C). Five petal primordia emerge alternating with the sepals (Fig. 5D-I). Petals later develop unidirectionally with the abaxial petal more rapidly (Fig. 5H). Following initiation of all five petals, the first whorl of stamens initiates alternating with the petals (Fig. 5F). The second whorl of stamens develops next outside of the first whorl. However, in the early stage it is covered by the expanding petals (Fig. 5G-I, K, N). The third and fourth stamen whorls later develop almost simultaneously and centripetally on the remaining floral meristem (Fig. 5G-J). Arrangement and number of stamens are observed to be variable among flowers (Fig. 5K-L). It is difficult to distinguish between the third and fourth whorls. A final set of three to five stamens develops at the centre of flowers, sometimes the last initiated stamen is shifted towards the middle of the floral apex giving the false impression of a central stamen (Fig. 5J,

L-N). During development, stamens curve and fold inwardly, giving thecharacteristic *Croton* inflexed anthers in bud (Fig. 5O). Five nectary glands emerge much later in alternation with the petals.

Ontogeny of female flowers

Female floral primordia emerge on the proximal to approximately middle part of the inflorescence where there is a transition to the male flower zone (Fig. 1D-E; 2A). Each floral primordium is subtended by a bract and sided by two bracteoles. We could not observe the sepal initiation because of the presence of intertwining stellate trichomes obscuring the view. However, we could imply a 2/5 pattern as their arrangement follows a quincuncial aestivation in the mature flowers. Five petal-like primordia develop between the sepals (Fig. 6A-D). Different sizes of petal-like organs are observed which may be the result of a non-simultaneous initiation or unequal development (Fig. 6C-D). Three congenitally fused carpels arise rapidly, while petals are still in an early developmental stage (Fig. 6A-B). The ovary occupies the rest of the floral primordium. As the gynoecium develops, petal development is mostly arrested (Fig. 6A-H). Two nectary primordia emerge adjacent to the petals (they could also be interpreted as a pair of primordia between two petals) (Fig. 6G). The nectary primordia later expand and two nectaries on the same side coalesce into five bilobed nectary glands which are later joined together as a nectary ring (Fig. 6H-I). A bilobed style develops on top of each carpel (total of three styles/six stigmatic tips) (Fig. 6H). In closed buds, some petal-like organs are elongated and develop a colleter at the apex (Fig. 4E; 6J, K). Some petal-like organs develop into miniature petals with various shapes and sizes resulting in a highly heterogeneous corolla morphology (Fig. 4A, E-F; 6J-L).

Figure 6 (next page). Ontogeny of female flowers of *Croton chilensis*. (A) A young flower shows initiation of petal-like primordia (arrowhead) and formation of an ovary by fusion of three carpels. (B) Petal-like primordia (asterisks) are arranged alternating with sepals. (C) Petal-like organs show slight differences in growth. (D) An anomalous ovary. Petal-like organs clearly show unequal development (asterisk). E) Later, three carpels start to close while petal-like organs development is arrested (arrowhead). Nectary primordia start to initiate at the base of the ovary (double arrowhead).

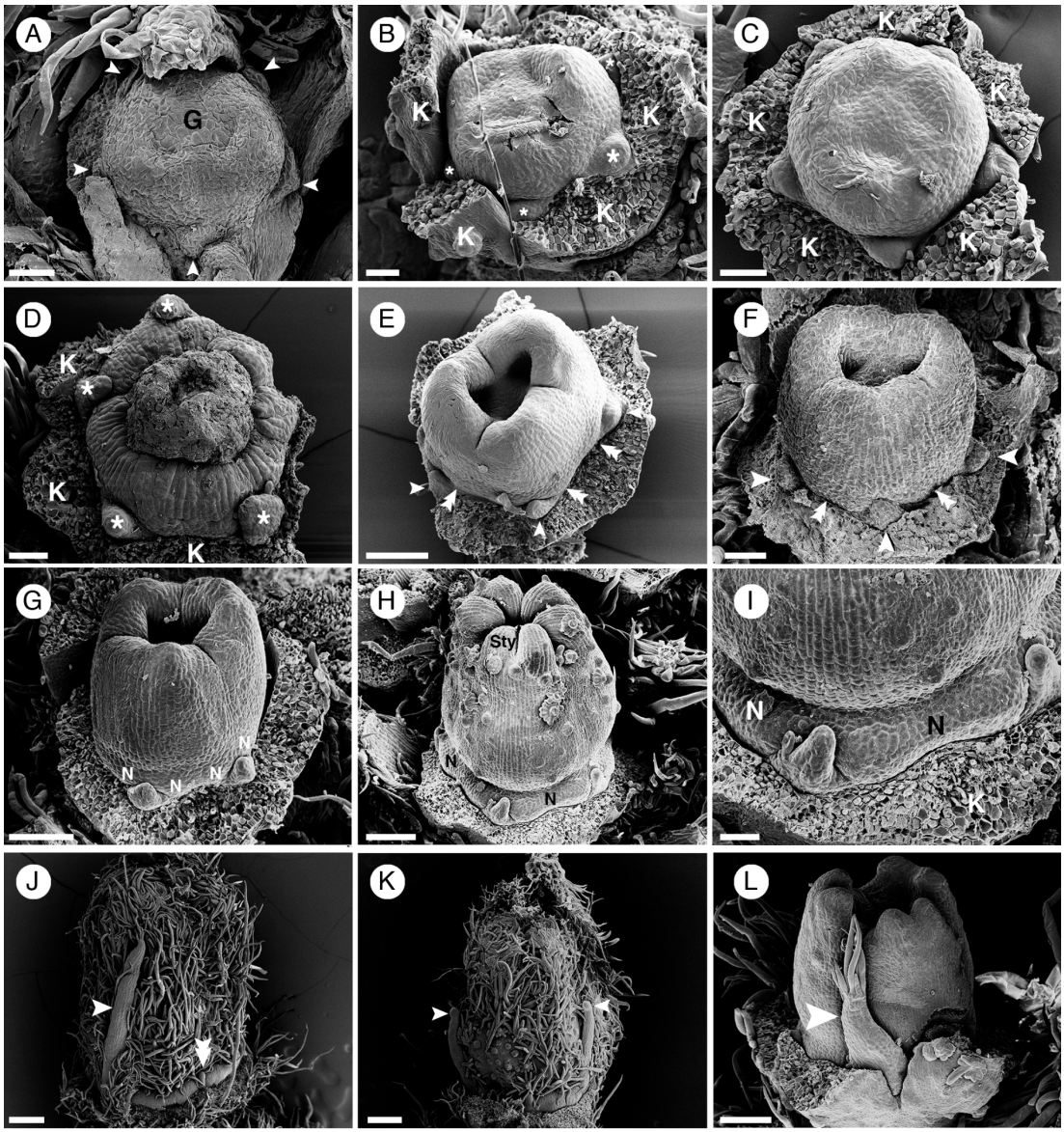

Figure 6 continued. (F) Petal-like organs stop their development while the ovary grows and expands in size (arrowheads). Nectary glands start to form at the base of the ovary opposite to sepals (double arrowheads). (G) Later stage: a bilobed style emerges on top of each carpel. Bilobed nectary glands are forming from fusion of two expanded nectaries. (H) Next, a bifid style is differentiated on top of each carpel. Nectariferous tissue expands into a ring. (I) Magnified view of the previous picture shows nectary ring expansion while petal-like organs are still minute. (J) Later, some petal-like organs grow into filamentous structures (arrowhead) while some are still arrested in their development (double arrowhead). (K) Some filametous structures have a tendency to become colleters (arrowhead). (L) A miniature petal is sometime present from extension of development of the petal-like organs (arrowhead). G, ovary; K, sepal; N, nectary; Sty, style. Scale bars: (A, B, I) = 40 μm; (C, D, F) = 50 μm; (E, G, L) = 100 μm; (H) = 120 μm; (J, K) = 200 μm.

3.4.2 *Croton alabamensis*

General morphology

Flowers of *Croton alabamensis* are borne on racemose inflorescences with up to three female flowers on the lower part and more numerous male flowers distally (Fig. 1A; 2B). Previous literature found that there are more female flowers (two to six) in late flowering inflorescences (Ginzbarg, 1992). Inflorescences are produced terminally on shoots. The female flowers mature much earlier than male flowers (Fig. 1A). Both male and female flowers are subtended by a bract and two bracteoles and both male and female flowers are pedicellate (Fig. 1A; 2B). Flowers from both sexes are pentamerous (Fig. 2E, H).

The perianth of male flowers is biseriate (Fig. 2E; 7A-B). Sepals have quincuncial aestivation (Fig. 7A) and an ovate shape with wide base and acute apex (Fig. 7B-C). The corolla also has quincuncial aestivation (Fig. 7D). Petals have an ovate shape with narrow base and obtuse apex (Fig. 7E-F). The abaxial side of sepals is covered with lepidote trichomes (Fig. 7A, B). On the adaxial side, sepals bear stellate trichomes on their margins (Fig. 7C) while petals bear simple trichomes on their margins (Fig. 7F). Petals have stellate-lepidote trichomes covering the abaxial surface (Fig. 7D, E). Margins and apex of petals are fimbriate comprising simple trichomes (Fig. 7E-F). Inside the corolla whorl, five pairs of ten nectary glands arranged alternating with petals and outermost stamens (Fig. 7G). In each pair, two nectary glands form a horse-shoe shape clasping the base of the filament (Fig. 7G). There are 15-18 stamens present in the male flowers with the outermost whorl alternating with petals (Fig. 7H). There are some stellate trichomes present on the lower part of the filaments (Fig. 7I). In the middle of the flower, there is an empty space without trace of carpel (Fig. 2E; 7H). Stamens are incurved toward the centre but the anthers do not inflex (Fig. 7H). The receptacle is pilose, covered with simple trichomes.

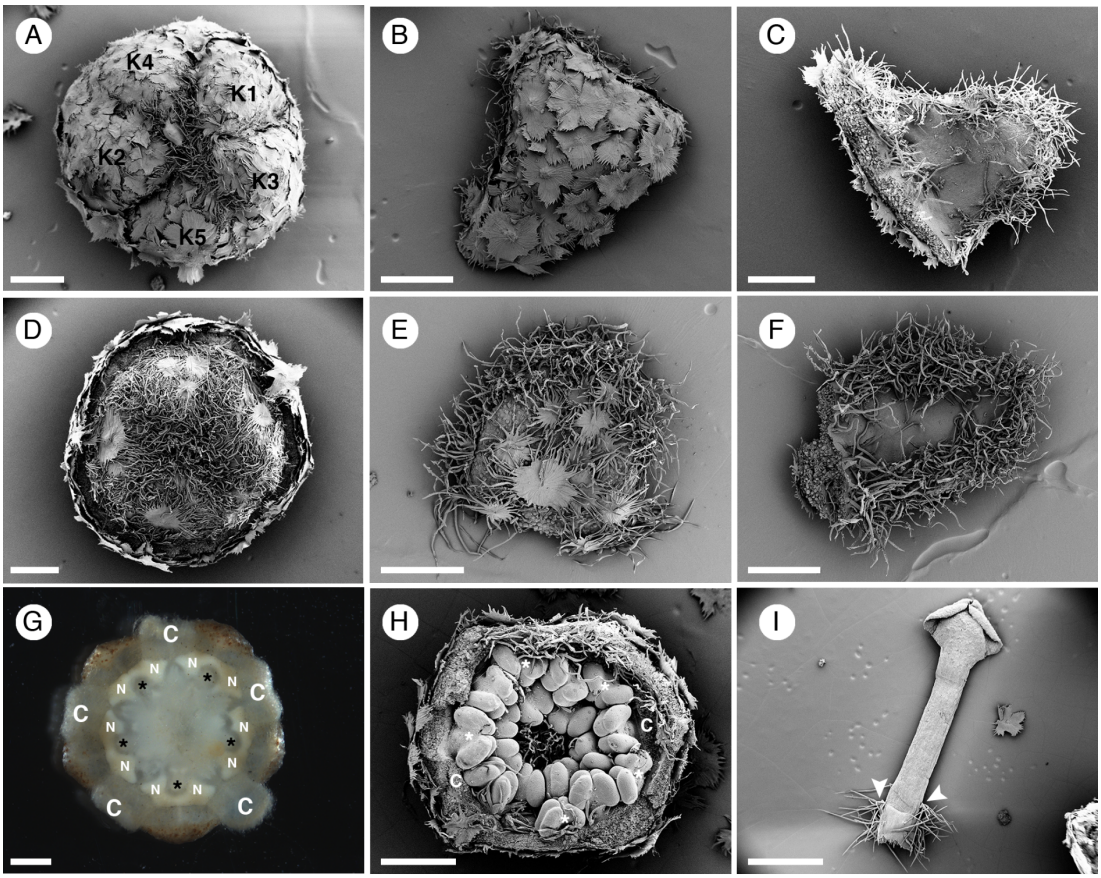

Figure 7. Morphology of male flowers from *Croton alabamensis*. (A) Calyx in the flower bud with quincuncial aestivation. Note, interlocking trichomes on the floral apex. (B) Abaxial surface of a sepal covered with lepidote trichomes. (C) Adaxial surface of a sepal covered with some stellate trichomes on the lower part. There are many stellate trichomes on the margin. (D) Corolla in a flower bud with quincuncial aestivation. The calyx lobes were removed. (E) Abaxial surface of a petal covered with stellate-lepidote trichomes. There are many trichomes on the margin. (F) Adaxial surface of a petal. The central area is glabrous but there is a tuft of simple trichomes on the margin. (G) Ten nectary glands paired to form a horse-shoe shape alternating with petals. Note, two adjacent nectaries clasping the base of filaments from the outermost stamen whorl (asterisks). (H) Stamens in the cup-shape floral bud. The outermost stamens arranged alternating with petals (asterisks). Note, stamens are not inflexed in bud. (H) A stamen with some stellate trichomes on the filament base (arrowhead). K, sepal; C, petal; N, nectary. Scale bars: (A-F, H) = 500 µm; (G, I) = 1,000 µm.

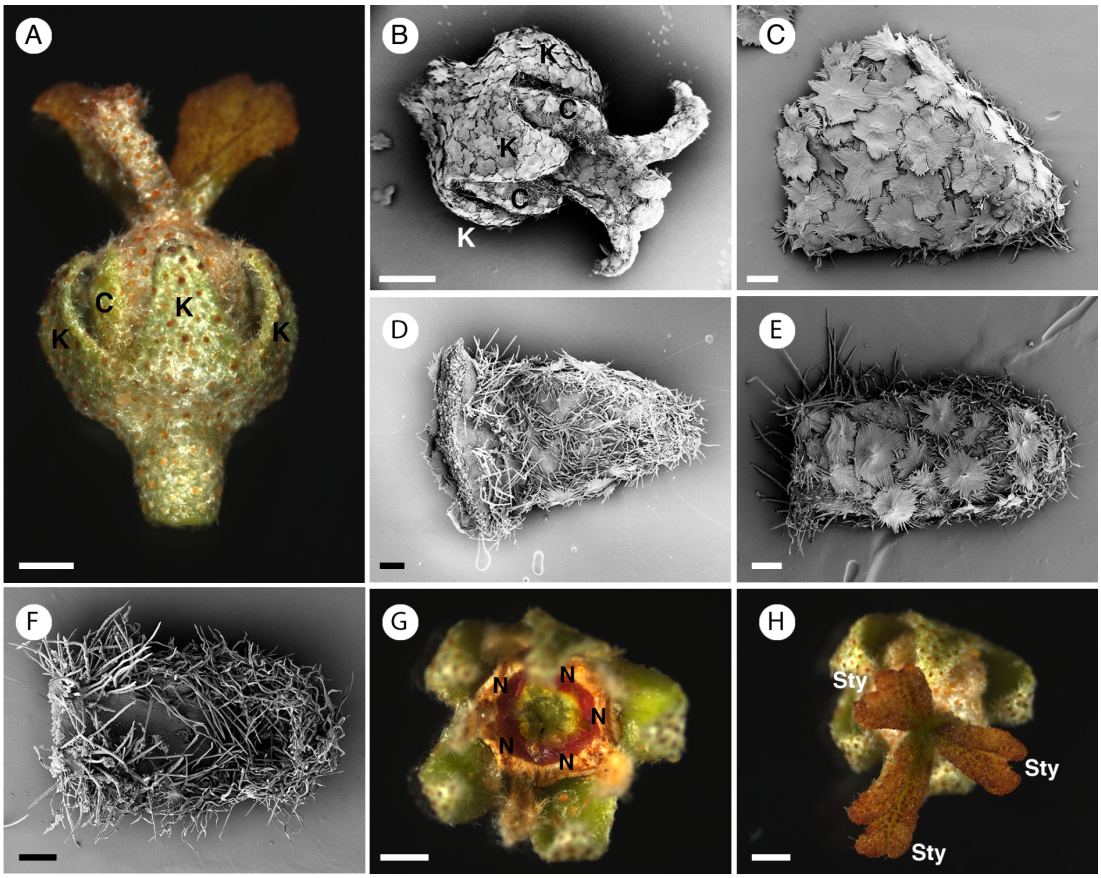

Figure 8. Morphology of female flowers from *Croton alabamensis*. (A) A flower near anthesis. Fully developed petals are present. Styles are bilobed but do not fork. (B) A flower near anthesis with visible sepals and petals. Styles are slightly bifid in this flower. Note that the outer surface of all organs is covered with lepidote trichomes. (C) Abaxial side of a sepal covered with lepidote trichomes. (D) Adaxial side of a sepal. There are many stellate trichomes covering the surface. (E) Abaxial side of a petal covered with lepidote and stellate-lepidote trichomes. (F) Adaxial side of a petal. The margin is densely covered with simple trichomes. (G) A nectary ring inside the corolla with initial yellow colour that later changes to red. (H) Three styles and stigmatic surfaces. Note, stigmatic lobe number vary from two to four. K, sepal; C, petal; N, nectary; Sty, style. Scale bars: (A, B, G, H) = 1,000 µm; (C-F) = 200 µm.

In female flowers, there are two perianth whorls (Fig. 2H; 8A-B). Sepal aestivation is quincuncial. In the sepals, the abaxial surface is covered by lepidote trichomes (Fig. 8A-C). Sepals have an ovate shape with a wide base and acute apex (Fig. 8A-D). The adaxial surface of the sepals is glabrous on the lower part but covered with some stellate and simple trichomes on the upper part (Fig. 8D). Petals of female flowers are similar to those of male flowers but there is a mix of lepidote and stellate-

lepidote trichomes present along the midrib on the abaxial side (Fig. 8E). Petals have an oblong shape with narrow base and obtuse apex (Fig. 8E-F). The petals are arranged in alternation with nectary glands (Fig. 8G). Inside the nectary, there is a tricarpellate ovary covered with lepidote trichomes (Fig. 8A). On top of the ovary, three styles are present with simple style/stigma, but sometimes a slightly divided stigma is present (Fig. 8H).

Ontogeny of male flowers

Only male flowers from *C. alabamensis* var. *alabamensis* were used in the developmental study. Male flowers develop spirally on the upper part of the racemose inflorescence (Fig. 2B). Each floral primordium is subtended by a bract and two bracteoles (Fig. 9A). The first sepal emerges on the abaxial side of the flower (Fig. 9A-B), followed by additional sepals in a 2/5 pattern (Fig. 9B). Floral primordia are initially convex shaped (Fig. 9A-B) but later shift vertically to a concave shape due to hypanthium formation during petal initiation (Fig. 9C). Five petal primordia emerge alternating with sepals (Fig. 9C). Petals develop unidirectionally towards the abaxial side of flower (Fig. 9C-E). Following initiation of petals, two whorls of five stamens emerge, with the antepetalous stamen primordia developing more rapidly in early stages (Fig. 9D). Antepetalous stamens are more developed than the outer anlternipetalous whorl, so we deduce that it is the first whorl to develop (Fig. 9D-E). Stamen development then follows the unidirectional pattern as a third whorl of five stamens is initiated centripetally (Fig. 9E), resulting in fifteen stamens arranged in three whorls of five on the slope of the hypanthium (Fig. 9F-G). No stamen, nor any other floral organ develops at the centre of the flower (Fig. 9F-I). During maturation, stamens curve towards the centre but are never inflexed (Fig 9G-I). Much later after stamen initiation, five nectary glands develop alternating with petals and opposite the outermost antesepalous stamen whorl. Nectary glands expand to surround the base of the filaments of antesepalous stamens, clearly visible at anthesis (Fig. 7G).

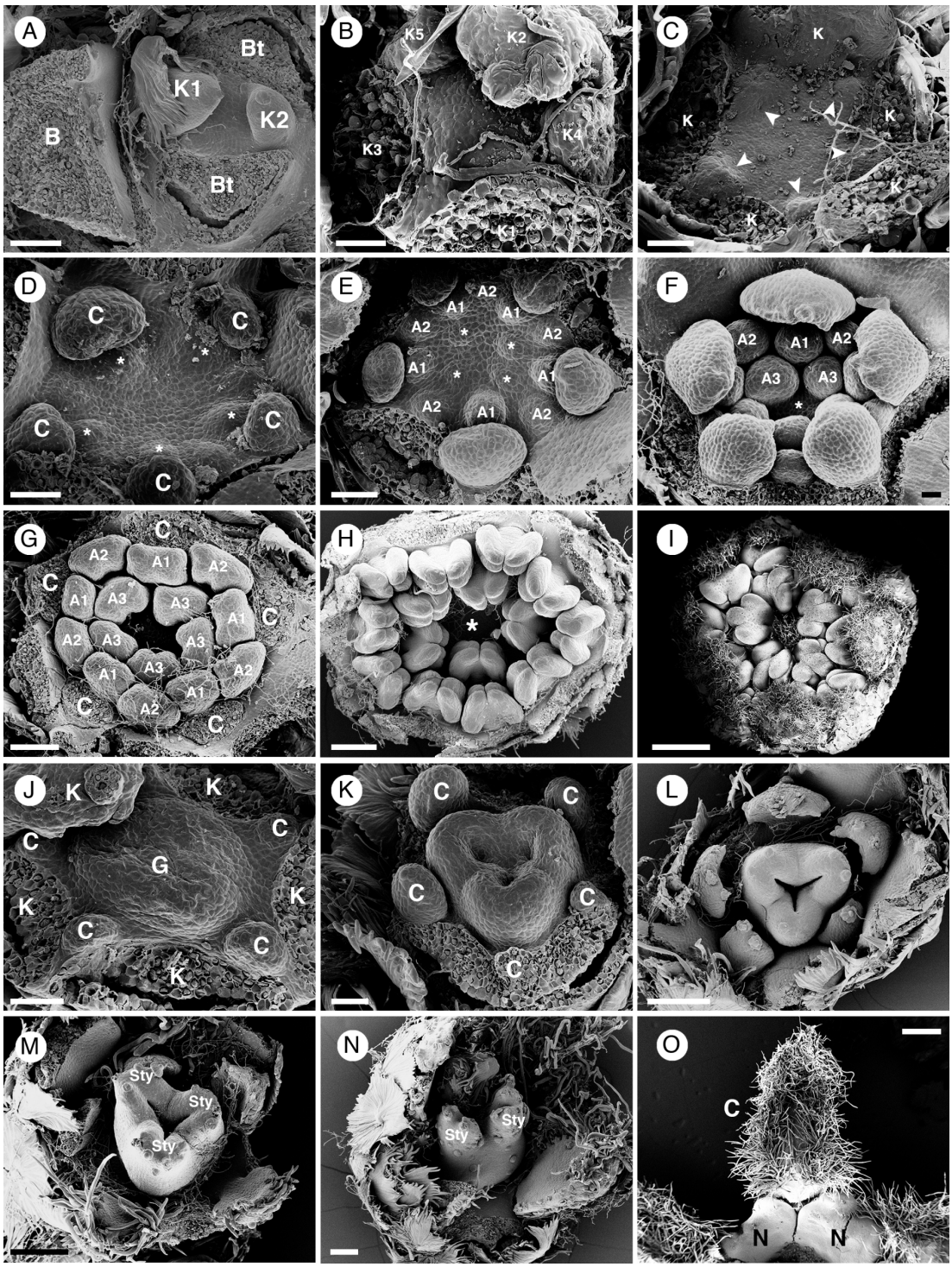

Figure 9. Ontogeny of male (A-I) and female (J-O) flowers from *Croton alabamensis*. (A) A young convex floral primordium subtended by a bract and two bracteoles. The first and second sepals are visible. (B) Soon, more sepal primordia initiate and grow unequally in a quincuncial (2/5) pattern. Note, the floral primordium is still convex in shape. (C) After that, a hypanthium starts to form while five petal primordia initiate alternating with sepals (arrowhead).

Figure 9 continued. (D) Later, petals continue to grow and clearly show unidirectional growth. Two whorls of stamens are visible. Stamens from the antepetalous whorl (asterisks) is bigger than stamen from the alternipetalous whorl. Note, the hypanthium is well developed making the flower become cup-shape. (E) Next, the third whorl of stamens develops centripetally (asterisk). (F) The hypanthium starts to grow with petals and three whorls of stamens are visible. There is an empty space in the middle of the flower (asterisk). (G) Flower in later stage with all perianth parts removed to show three whorls of stamens. The outermost whorl alternates with petals. (H) Apical view of older flower bud showing central empty space (asterisk). (I) Later, stamens grow and bend toward the centre but never become inflexed. (J) Female flower in an early stage shows five petal primordia and a tricarpellate ovary formation. (K) In later stage, petal primordia continue to grow unequally. Three fused carpels are clearly seen. (L) Next, petals continue expanding and the ovary start closing. (M) A style starts to form on top of each carpel. (N) Three bilobed styles are visible on the ovary. (O) Much long after that, five bilobed nectary glands are formed alternating with petals. K, sepal; C, petal; A, stamen; G, ovary; Sty, style; N, nectary. Scale bars: (A, G, N) = 100 μm; (B, F) = 30 μm; (C-E, J, K) = 50 μm; (H) = 200 μm; (I) = 1,000 μm; (L, M) = 150 μm; (O) = 400 μm.

Ontogeny of female flowers

Ontogeny of the female flower was observed in *C. alabamensis* var. *alabamensis*. Female flowers are located on the lower part of the inflorescence (Fig. 1A; 2B). A bract and two bracteoles subtend the flower. Five sepals are present with quincuncial aestivation implying a 2/5 initiation pattern. Five petals emerge alternate to sepals (Fig. 9J). A congenitally fused tri-carpellate ovary develops rapidly, even when petal primordia are still young (Fig. 9J). Petals grow unidirectionally with the abaxial petals developing faster (Fig. 9J-L). Contrary to *C. chilensis*, all petals in female flowers continue to expand until they resemble petals in male flowers at anthesis (Fig. 9J-O). A bifid style develops on top of each carpel (Fig. 9N). However, some samples have a simple style at the mature stage, while some flowers retain a bifid style (Fig. 9N). Much later, nectary glands develop at the base of the ovary in an antesepalous position (Fig. 9O).

3.4.3 *Croton schiedeanus*

General morphology

In *Croton schiedeanus*, flowers are produced spirally on racemose inflorescences (Fig. 1B; Fig. 2C). Inflorescences are axillary borne on the main axis. One to two female flowers are present on the proximal part and bloom earlier than male flowers on the distal part (Fig. 1B). Flowers from both sexes are pedicellate and subtended by a bract and two bracteoles (Fig. 1B; 2C). The pedicels of male flowers are slender while pedicels of female flowers are bulky and longer (Fig. 1B). Flowers from both sexes are pentamerous.

There are two whorls of perianth parts in male flowers (Fig. 10A, D; 2F). Sepal shape is ovate with a wide base and acute apex (Fig. 10B-C). The arrangement of sepals follows a quincuncial pattern (Fig. 10A). The abaxial side of sepals is covered by lepidote trichomes (Fig. 10B), while on the adaxial side there are many simple trichomes present near the margin and the apex (Fig. 10C). Petal shape is ovate with a narrow base and obtuse apex (Fig. 10E-F) arranged in cochlear aestivation (Fig. 10D). The abaxial surface of petals is mostly glabrous with one or two lepidote trichomes present (Fig. 10E). The adaxial surface of petals is also mostly glabrous and sparsely covered with simple trichomes (Fig. 10F). Margin and apex of petals are fimbriate, lined with simple trichomes (Fig. 10E-F). Outside the androecium whorl, there are five bilobed nectary glands alternate with the petals (Fig. 10F). Centrally, about 11 stamens are arranged on a convex receptacle with the outermost whorl opposite to petals (Fig. 2F; 10H). Anthers are inflexed in bud (Fig. 10F-H). Filaments are generally glabrous but sometimes a stellate trichome may be present (Fig. 10H). There is no indication of an ovary.

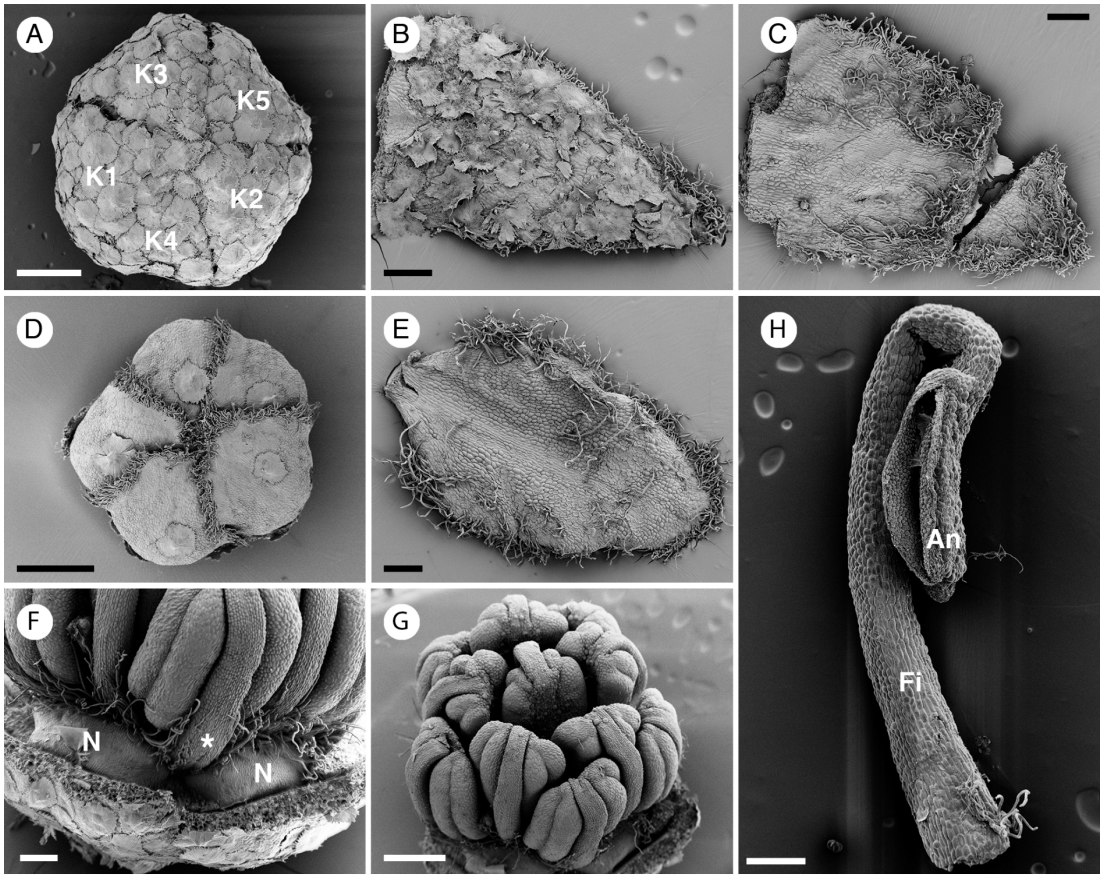

Figure 10. Morphology of male flowers from *Croton schiedeanus*. (A) Calyx in flower bud with quincuncial aestivation. The surface is covered with lepidote trichomes. (B) Abaxial surface of a sepal with lepidote trichomes. (C) Adaxial surface of a sepal with many simple trichomes on the margin and apex. (D) Corolla in a flower bud with cochlear aestivation. There is a lepidote trichome visible on each petal. Note, fimbriate margin of petals. (E) Adaxial side of a sepal with few simple trichomes on the surface. There are many simple trichomes lining the margin and apex. (F) Nectaries are located alternating with petals and outermost stamens (asterisk). (G) There are 11 stamens arranged in two whorls surrounding a central stamen. The outermost stamens are opposite the petals. Note, stamens are inflexed in a flower bud. (H) A stamen with a stellate trichome on the base of filament. K, sepal; N, nectary; Fi, filament; An, anther. Scale bars: (A, D, G) = 500 µm; (B, C, E, F, H) = 200 µm.

Figure 11 (next page). Morphology of female flowers from *Croton schiedeanus*. (A) An inflorescence with a female flower on the lower part and several male flower buds. The female flower has a longer and thicker pedicel than male flower buds. (B) A female flower bud with some perianth removed. There are petals present inside. (C) Abaxial side of a sepal completely covered with lepidote trichomes. (D) Adaxial side of a sepal with simple and stellate trichomes present.

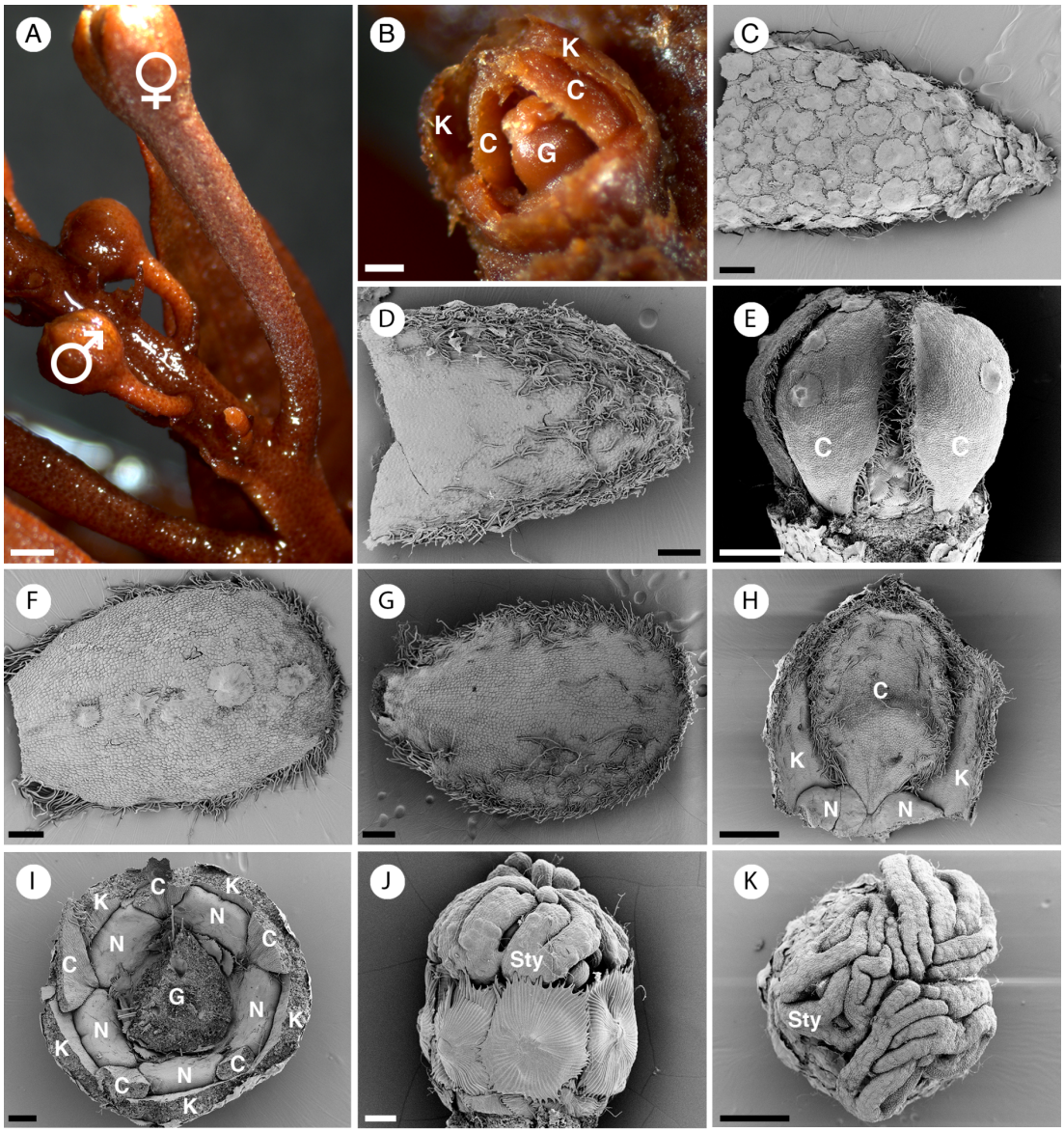

Figure 11 continued. (E) A female flower bud which calyx and nectary glands removed. Petals show narrow base. (F) Abaxial side of a petal. There are few lepidote trichomes along the midrib. The margin is fimbriate. (G) Adaxial side of a petal. The fimbriate margin consists of crushed stellate trichomes and dense simple trichomes. (H) A female petal arranged alternating with sepals and nectaries. Note, the base looks like a claw due to nectary expansion during development. (I) A flower bud in which ovary and part of perianth were removed. There are five bilobed nectary glands located around the ovary. (J) Ovary with surface covered with lepidote trichomes. There are styles on top of it. (K) Styles on top of an ovary with multifid stigmatic tips. K, sepal; C, petal; G, ovary; N, nectary; Sty, style. Scale bars: (A) = 1,000 µm; (B-D, F, G, I) = 200 µm; (E, H, K) = 500 µm; (J) = 100 µm.

Female flowers are borne on longer and thicker pedicels than male flowers (Fig. 11A). In female flowers, both sepals and petals are present (Fig. 1B; 2I; 11B). Sepals have an ovate shape with wide base and acute apex (Fig. 11C-D) arranged in quincuncial aestivation. On the abaxial side of the sepals, there are many lepidote trichomes covering the whole surface (Fig. 11C). On the adaxial side, there are stellate trichomes on the upper part, but the lower part is glabrous (Fig. 11D). Petals have an obovate shape with narrow base and obtuse apex and margins of petals overlap in a quincuncial aestivation only in the upper part (Fig. 11C-H). Petal lobes alternate with nectary glands (Fig. 2I; 11H-I). The abaxial surface of petals is mostly glabrous with a few lepidote trichomes on the midrib (Fig. 11E-F). The adaxial surface is also mostly glabrous with some simple trichomes near the margin (Fig. 11G). The margin of petals is fimbriate, densely covered with crushed stellate and simple trichomes (Fig. 11E-H). Within the nectary, there is a tricarpellate ovary covered with lepidote trichomes (Fig. 11J). Three styles are present with twice bifid (Fig. 1B) or multifid stigmatic tips (Fig. 11K).

Ontogeny of male flowers

Male flowers develop on the distal part of the inflorescence (Fig. 1B; 2C). Flowers are subtended by a bract and two bracteoles. Five sepals are present (Fig. 2F). The shape of floral primordia is convex, similar to *C. chilensis* (Fig. 12A-I). Five petals emerge alternating with sepals (Fig, 12B-C). Later they grow in a unidirectional way with the abaxial petal developing faster (Fig. 12C-F). The first whorl of five stamens emerges alternating with petals (Fig. 12E-F). Next, the second stamen whorl emerges outside the first whorl (centrifugally) opposite the petals (Fig. 12F-G). The last stamen to develop is a single stamen occupying the central area of the flower (Fig. 12H-I). During maturation, stamens curve and fold resulting in an inflexed anther appearance (Fig. 10H). Nectary glands initiate much later after the stamen development in alternation with petals (Fig. 10G).

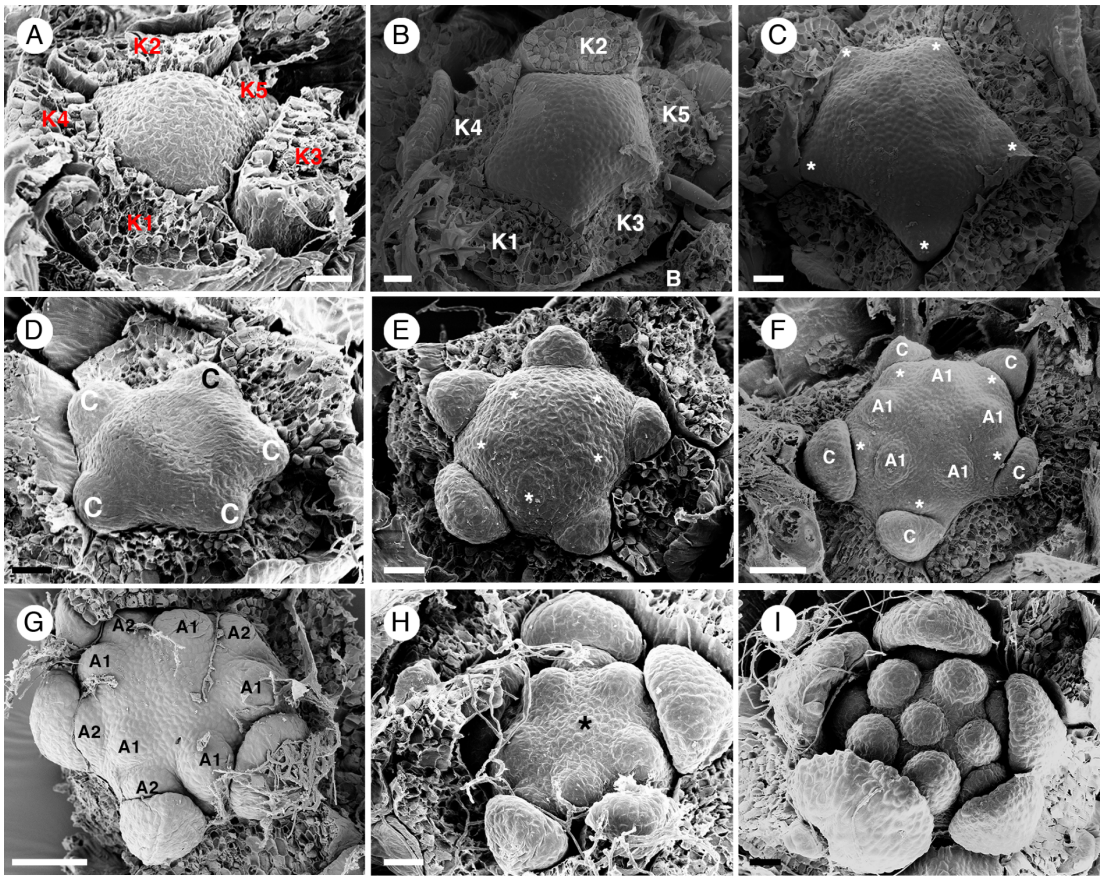

Figure 12. Ontogeny of male flowers of *Croton schiedeanus*. (A) A very young flower bud with five sepals initiating first. The rest of the floral primordium has a convex shape. (B) Apical view of a similar stage, petals initiate alternating with sepals. (C) Next, petal primordia grow and expand (asterisks). (D) Petals show a unidirectional growth pattern. (E) After that, the first stamen whorl starts to develop on the convex receptacle alternating with petals (asterisk). (F) Soon, the second whorl of stamens develops centrifugally outside of the first whorl opposite petals. (G) Apical view of slightly older stage; two stamen whorls are visible with the outermost whorl opposite petals. (H) After that, a stamen starts to initiate on the central empty space (asterisk). (I) Apical view of a young flower shows completion of stamen development. K, sepal; C, petal; A, stamen. Scale bars: (A-E, I) = 20 µm; (F, G) = 50 µm.

Ontogeny of female flowers

There are few female flowers of *C. schiedeanus* located on the proximal part of the inflorescence (Fig. 1B; 2C). A female flower is borne in the axil of a bract and sided by two bracteoles. Sepals initiate spirally in a 2/5 pattern (Fig. 13A). Five petal primordia develop alternate to them (Fig. 13B-C). Petals also show unidirectional growth similar to other species (Fig. 13D-E). Similar to *C. alabamensis*, petals

continue to grow and expand throughout the floral development (Fig. 13C-F, I). Three congenitally fused carpels initiate at the centre of flower (Fig. 13E-F). The ovary continues to develop and enlarge in size (Fig. 13F-I). Later three styles arise on top of the ovary. Many lobes appear on the style (Fig. 13H) which later become multifid (Fig. 11K) and are derived from a bifid pattern (Fig. 13I). Antesepalous nectaries develop in the late ontogeny (Fig. 11I).

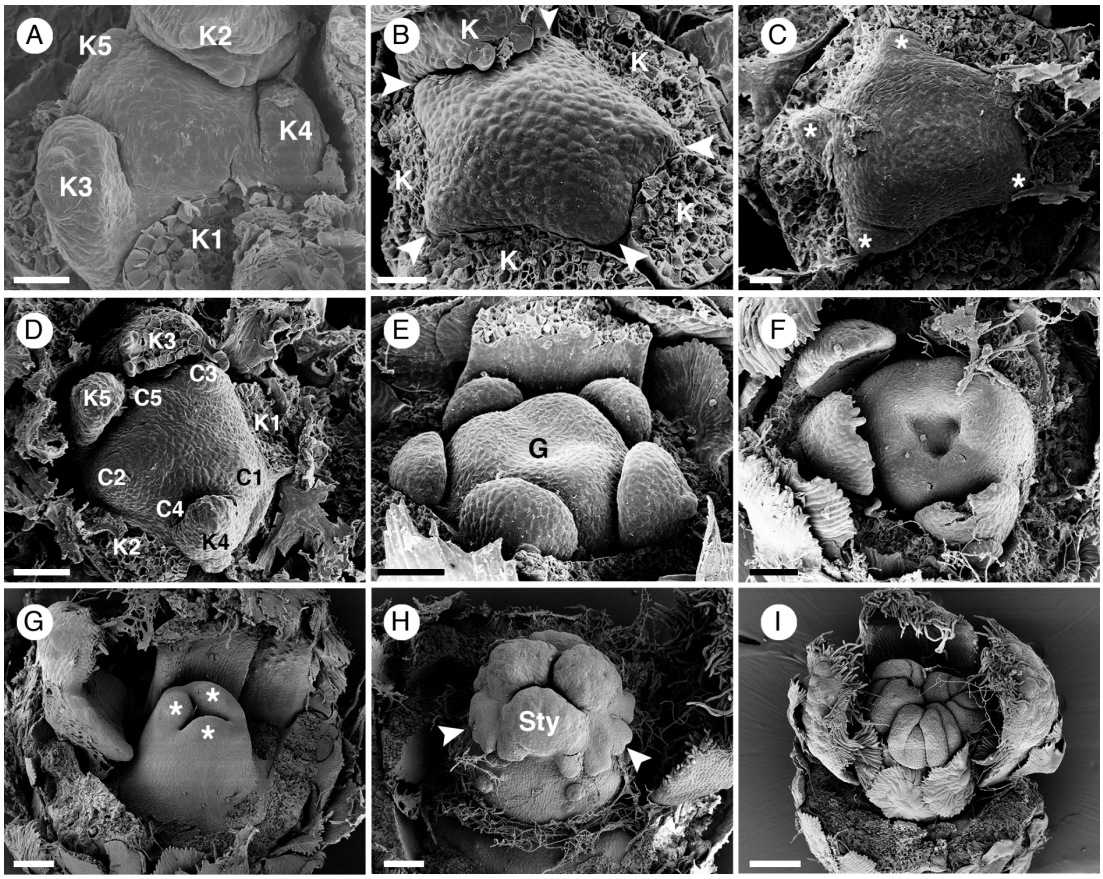

Figure 13. Ontogeny of female flowers from *Croton schiedeanus*. (A) An early stage of a flower shows unequal sepals on convex floral primordium. (B) After that, petal primordia start to form alternating with sepals (arrowheads). (C) Apical view of next stage; petal primordia start to expand surrounding a convex receptacle (asterisks). (D) View from similar stage shows unequal growth of petals. (E) Next, an ovary initiates with three fused carpels. Note, petals continue to grow in a unidirectional pattern. (F) An old flower bud shows expansion of petals and ovary. (G) Next, the ovary starts to close. The lobe on top of each carpel starts to form a style (asterisks). (H) Three styles are formed on top of the ovary. Note, there are many lobes visible (arrowheads). (I) Later, styles are dissected into many parts. K, sepal; C, petal; G, ovary; Sty, style. Scale bars: (A-C) = 20 µm; (D-F) = 5 0µm; (G) = 90 µm; (H) = 100 µm; (I) = 150 µm.

3.5 Discussion

3.5.1 Petal evolution in *Croton*

Petals are generally found in male flowers of *Croton* (Webster, 1993; Berry et al., 2005b; van Ee et al., 2011). Filamentous structures are reported to occur in female flowers in the same position as petals in male flowers (De-Paula et al., 2011; Gagliardi et al., 2017). The filamentous structures have been interpreted either as reduced petals or as staminodes in the past (Michaelis, 1924; Nair and Abraham, 1962; Webster, 1993; Radcliffe-Smith, 2001; Caruzo and Cordeiro, 2007; De-Paula et al., 2011; Gagliardi et al., 2017). Fully developed petals are found in two New World sections, i.e., section *Alabamenses* and section *Eluteria* subsection *Eluteria* (van Ee et al., 2011), and some African species (Friis and Gilbert, 2008; Berry et al., 2016). Our study demonstrates that, in the early developmental stages, petals of male flowers are similar to petals and filamentous structures of female flowers. They develop as broadly base structures in alternation with sepals before the androecium or gynoecium (Fig. 5A vs 6C: *C. chilensis*; 9C vs 9J: *C. alabamensis*; 12C vs 13C: *C. schiedeanus*). Therefore, it is highly likely that these represent homologous structures. Moreover, those mentioned developmental characters also suggest homology of petals with sepals of tepalar origin (bracteopetals) in *Croton*. In contrast, petals with a staminodial origin (andropetals) have a delayed initiation; they develop as part of the androecium, and primordia are characteristically narrow and morphologically comparable to stamens (Ronse De Craene, 2008).

Morphology of male petals and female filamentous structures are greatly different at maturity. However, we found that both structures share the same position and similar morphology in the young stage. Therefore, it is possible that heterochrony may be involved. Heterochrony is defined as the change in developmental time and rate resulting in evolutionary change, whichwas recognized as one of the major drivers of morphological evolution (Li and Johnston, 2000; Box and Glover, 2010; Ronse De Craene, 2018). After initiation, petals in male flowers continue to grow and expand throughout the whole development until maturity (Fig. 5; 9; 12), while growth of petals

in female flowers is arrested (Fig. 6; 9; 13) suggesting an early developmental blockage of petal development (paedomorphosis). The presence of fully developed petals in female flowers of *C. alabamensis* and *C. schiedeanus* suggests that in those species there is a reversal in the process. One could speculate that the presence of fully developed petals in female flowers resembles the ancestral condition. However, *C. alabamensis*, *C. schiedeanus* (sect. *Eluteria*) and some African species on the phylogenetic tree are nested in clades with other *Croton* species having no petals in female flowers (Berry et al., 2005b; van Ee et al., 2011, 2015), suggesting that the absence of fully developed petals is plesiomorphic. Most of the genera in tribe Crotoneae also lack fully developed petals in female flowers (Webster, 2014). However, among them only female flowers of *Sandwithia* are reported to have miniature petals (Chapter 2; Lanjouw, 1932; Secco, 1987). Outside Crotoneae, petals in female flowers are present in many groups. In the tribe Jatropheae, a sister tribe of the Crotoneae, all three genera, i.e., *Jatropha*, *Joannesia* and *Vaupesia*, have petals in both male and female flowers (Velloso, 1798; Ducke, 1922; Schultes, 1955; Dehgan, 2012; Webster, 2014). Moreover, many genera in lower position of another inaperturate crotonoid clade, e.g., *Grossera, Cavacoa, Leeuwenbergia and Dodecastigma,* are reported to have petals in female flowers as well (Ducke, 1932; Leonard, 1955; Letouzey and Hallé, 1974; Barberá et al., 2014; Webster, 2014; van Welzen et al., 2020). Therefore, the presence of fully developed petals may be an ancestral character of the inapeturate crotonoids. One could suggest ‘homoplasy’ as an explanation for the occurrence of fully developed petals in female flowers of some *Croton* species. However, combining both phylogenetic and ontogenetic perspectives, we conclude that this phenomenon is preferably explained as a case of ‘apomorphic tendency’ (or cryptic apomorphy). This represents a morphological character (fully developed petals in female flowers) that appears in some members but not in all species (*Croton*) because of deeply shared genetic attributes. As a result this character is not recognized as a synapomorphy on the phylogenetic tree (Endress, 2003; Endress and Matthews, 2006; Ronse De Craene, 2010). Our idea is supported by the mixture of petal-like and filamentous structures in some female flowers from various species of *Croton* (Fig. 4F). The reduction of petals in female flowers may also be explained by a shift in BC gene expression. In strongly heteromorphic species, female flowers

often have reduced petals or lack petals altogether, as in *Croton* (e.g. *Gunnera* in Gunneraceae: (Ronse De Craene and Wanntorp, 2006); *Grevea* in Montiniaceae: (Ronse De Craene, 2016); *Myriophyllum* in Haloragaceae: (Kubitzki, 2007). This suggests that petal reduction is linked to a shifting balance of B-genes responsible for petal identity. A reduced B-genes expression may be responsible for the reduction of petals to small appendages in most *Croton* species. However, in the case of *Croton schiedeanus* and *C. alabamensis* a regain of B-genes expression in the second whorl of female flowers might be the cause of regaining fully developed petals.

Mechanical pressure is another important factor that shapes the morphology of floral organs (Ronse De Craene, 2016). We found that the developmental sequence and mature morphology of petals in *Croton* is highly affected by the pressure exercised on the flower bud during development. In later developmental stages, some filamentous structures start to elongate. At this point, the centre of the flower is occupied by a massive ovary. Due to greater spatial pressure from the ovary and enclosing calyx, the filamentous structures become long and narrowly shaped (Fig. 4F; 6J-K) differing greatly from fully developed petals. The morphology of fully developed petals in female flowers is also shaped by mechanical pressure within the flower. It is found that petals are arranged alternate with nectary glands in both male and female flowers (Fig. 4A, F; 6L). However, nectary glands in female flowers are much larger and sometimes fuse together forming a ring. Pressure from adjacent nectary glands induce the petals in female flowers to have a very narrow base resembling clawed petals of staminodial origin (Fig. 11H-I). However, with ontogenetic data, we could confirm that the petals of female flowers represent bracteopetals and the claw-like morphology is a result of spatial pressure from nectary glands in the later stages.

We could not find any concrete evidence of ecological factors that lead to the development of fully developed petals in female flowers (Table 2). Evolutionary pressure from flower-insect interaction may be an important factor. However, very little attention has been paid to pollination studies in *Croton*. There are a small number of studies revealing different pollination syndromes in few species of *Croton*, e.g.,

wind pollination (Domínguez et al., 1989) or insect pollination (Freitas et al., 2001; Pires et al., 2004; Narbona and Dirzo, 2010) or mixed pollination (Novo et al., 2010). *C. alabamensis* was initially reported to have wind pollination but later observation suggested insect pollination supported by the presence of nectaries in both flower sexes (van Ee et al., 2006). It is known that all female flowers of *Croton* have nectar producing glands surrounding the ovary to attract insects. The presence of petals in female flowers may help increase flower visibility. However, petals in *Croton* are generally green or white in colour similar to sepals and may not contribute to insect attraction. We also observed that in *Croton* with fully develop petals, there are fewer female flowers in the inflorescence. So, perhaps the presence of petals may increase the chance of pollination. However, many *Croton* possess a smaller number of female flowers in inflorescences without having fully developed petals. All Neotropical *Croton* and African species with petals in female flowers also have lepidote trichomes. However, we think that this association is just a coincidence because other *Croton* with lepidote trichomes do not have fully developed petals in female flowers (Chapter 5). Further interdisciplinary studies, e.g., genetic, ecology, pollination and morphology, are needed to clarify the presence of fully developed petals in female flowers of some *Croton*.

3.5.2 The interpretation of nectaries in *Croton*

Five bilobed nectaries are present in both male and female flowers of all three species (except in male flower of *C. alabamensis*: Fig. 7G). They are arranged in antesepalous position within the corolla. We found that in female flowers, nectaries are initiated as two primordia adjacent to each petal (total of ten) which later expand and fuse together (Fig. 6E-I; De-Paula et al., 2011). We could not find the initiation stage of the nectary primordia in male flowers. However, results from a previous study suggest the emergence of a single primordium (De-Paula et al., 2011). In male flowers of *C. alabamensis*, however, there are ten nectary glands present in five pairs alternating with petals and stamens (Fig. 7G). Each pair of nectaries expands and fuses together forming a horse-shoe shaped nectary clasping the base of the outermost stamens (Fig. 7G). We hypothesize that during development, male flowers nectary

glands initiate alternating with the petals similar to other species of *Croton* (Fig. 2D-F). However, the space is already occupied by stamens in *C. alabamensis.* Therefore, two nectary glands initiate on the available space (adjacent to stamens/petals) and later expand surrounding the base of a filament in that position (Fig. 7G).

Throughout the genus, nectaries of female flowers are variously fused, often into a ring at the base of the gynoecium (Freitas et al., 2001; Caruzo and Cordeiro, 2007; De-Paula et al., 2011) while nectaries of male flowers are generally free. Different authors interpreted the origin of nectaries either as receptacular outgrowths (Caruzo and Cordeiro, 2007) or as transformed staminodes (De-Paula et al., 2011; Gagliardi et al., 2017). De-paula and colleagues (2011) interpreted nectaries as secretory staminodes based on their antesepalous position in the flower, despite their late initiation. They also reported vascularization of the nectary in *Croton.* However, nectaries from most species are supplied by traces derived from sepal bundles (De-Paula et al., 2011). Ronse De Craene and Smets (2001) suggested that staminodes can be transformed into nectaries with a retention of the original vasculature (e.g. *Harungana*, Hypericaceae; *Averrhoea*, Oxalidaceae). But in *Croton*, The nectaries are generally not supplied by a clear whorl of bundles as would be the case with stamen-derived nectaries (as in *Harungana* of Hypericaceae: Ronse De Craene and Smets, 1991). Moreover, nectaries in some species of *Jatropha* were found to be supplied by vascular bundle from both sepals and petals or petals alone (Venkata-Rao and Ramalakshmi, 1968).Venkata-Rao and Ramalakshmi (1968) interpreted the nectary of *Jatropha* as of receptacular origin because they are not supply by strands from the floral stele. Therefore, we interpret the vascular connections in *Croton* as opportunistic bundles supporting a receptacular origin of nectaries, since non-vascularized nectaries have also been reported in *C. bonplandianus* (Venkata-Rao and Ramalakshmi, 1968), *C. sarcopetalus* (Freitas et al., 2001), and in the related genus *Astraea* (De-Paula et al., 2011). Considering the nectary as of receptacular origin, links the floral morphology of Euphorbiaceae with other families in the order Malpighiales. A systematic survey by Bernardello (2007) suggests that most nectaries of Malpighiales are of receptacular (and hypanthial) origin, with the occasional presence of staminodial nectaries. Interpreting the nectaries as staminodial in *Croton* because of their position in

alternation with the other stamens is a static approach that does not consider the spatial constraints during development, where most space is available between the petals. The outer stamen whorl in most *Croton* is antepetalous and the greatest space for the late development of nectaries is in antesepalous position. In male flowers of *C. alabamensis*, the outermost stamen whorl is alternipetalous with the nectary appearing as five pairs of two nectaries clasping stamen bases (Fig. 7G). The plasticity of nectary number and position in this species support the interpretation of the nectary in *Croton* being of receptacular origin.

3.5.3 Floral diversity in male flowers

Our observations found that diversity of structure and development in male flowers of *Croton* is unexpectedly high. At maturity, the receptacle of male flowers of *C. chilensis* and *C. schiedeanus* is convex, as reported in many species from previous studies (Michaelis, 1924; De-Paula et al., 2011; Gagliardi et al., 2017) while *C. alabamensis* has a concave receptacle in the form of a hypanthium (Fig. 2E; 7G-H; 9). Cup-shaped flowers of *C. alabamensis* were reported before (Ginzbarg, 1992) but it was never considered to be unique among the genus *Croton* before. A similar condition has been found in male flowers from other Crotoneae genera, viz., *Sagotia racemosa* (slightly concave) and *Brasiliocroton muricatus* (flat receptacle), but in those species, stamens fully occupy the floral apex area contrary to *C. albamensis* with a central empty space (Chapter 2).

Moreover, our observation found that the second whorl of stamens from all three species develops centrifugally opposite to petals (Fig. 5; 9; 12). In *C. schiedeanus*, this whorl appears to be the outermost whorl throughout the whole ontogeny (Fig. 2F; 10G; 12) as was previously reported for other *Croton* species (De-Paula et al., 2011). In early stages of development of *C. chilensis*, the alternipetalous whorl is the outermost whorl (Fig. 5G-N) while at anthesis, it is the antepetalous whorl (Fig. 2D; 3A). This shift in stamen position in *C. chilensis* could be explained by the fact that in an early stage, growth of the outermost antepetalous stamens is restricted by the expanding petals. Therefore, the alternipetalous whorl appears to be outermost. In later stages,

nectary glands which develop on the empty space opposite the sepals push the alternipetalous stamen whorl inward. Finally, at anthesis petals start to spread out, making the antepetalous stamens to appear as the outermost whorl. *Croton alabamensis* also has a centrifugal stamen development but with alternipetalous stamens as the second and outermost whorl, while antepetalous stamens are the first and inner whorl (Fig. 7H; 9D-I). One possible explanation is that the vertical expansion of the hypanthium makes petals appear in a higher position than the stamen developing zone (Fig. 9D), eliminating pressure from petals on the stamen primordia; therefore, antepetalous stamens have the possibility to emerge first.

The centrifugal stamen development is unusual among angiosperms. This phenomenon is normally linked with a high stamen number (polyandry) (Corner, 1946; Ronse De Craene and Smets, 1992; Rudall, 2010). However, male flowers of *Croton* generally have about 11-16 stamens which fall in the range of oligandry (flowers with the number of stamen two times the number of petals) to lower scale polyandry (flowers with the number of stamens much higher than petals). Some cases of centrifugal stamen development in oligandric and lower-polyandric flower were reported before (Table 2). The centrifugal stamen development has often been linked with a reduction from ancestral flowers with polyandric centrifugal stamen development (Corner, 1946; Leins and Erbar, 1994). Alternatively, a centrifugal stamen development in oligandric flowers is the result of a shift linked with obdiplostemony (Ronse De Craene and Bull-Hereñu, 2016). However, as discussed in Ronse De Craene and Bull-Hereñu (2016), a shift in position is linked with differential growth processes associated with pressures in the flower. The different shifts in position of outer alternipetalous stamens in the investigated *Croton* are caused by differential pressures during the development of the flower. For *Croton*, it is possible that polyandry is a derived character since it is mostly found in subgenus *Adenophylli* (van Ee et al., 2011). Moreover, previously described cases of oligandric centrifugal stamen development occur in bisexual flowers, while centrifugal stamen development in unisexual (male) flowers is rare. There are few reports of this phenomenon in male flowers of Phytelephantoid palms (Uhl and Moore, 1977), *Populus* in Salicaceae (Kaul, 1995), *Medusagyne oppositifolia* in Ochnaceae (Ronse De Craene, 2017) and

Table 2. Examples of centrifugal stamen development in oligandric and lower-polyandric flowers.

Species/ Family (APG IV)	Stamen arrangement*	References
Arabidopsis thaliana (L.) Heynh./ Brassicaceae	A $\overleftarrow{2}$ + **4**	(Smyth et al., 1990)
Asarum caudatum Lindl./ Aristolociaceae	A $\overleftarrow{3}$ + $\overleftarrow{3}$ + **6**	(Leins and Erbar, 1985)
Bruguiera parviflora (Roxb.) W. A. ex Griff./ Rhizophoraceae	A $\overleftarrow{7}$-$\overleftarrow{8}$+ **7-8**	(Juncosa and Tomlinson, 1987)
Callisia Loefl. & *Tradescantia* L./ Commelinaceae	A $\overleftarrow{3}$ + **3**	(Payer, 1857; Hardy and Stevenson, 2000)
Combretum indicum (L.) DeFillps/ Combretaceae	A $\overleftarrow{5}$ + **5**	(Payer, 1857)
Harungana madagascariensis Poir./ Hypericaceae	A 4° + $^{\overleftarrow{3}}$**4**** (total 16 stamens and 4 staminodes)	(Ronse de Craene and Smets, 1991)
Hibbertia (=*Adrastaea*) *salicifolia* (DC.) F.Muell./ Dilleniaceae	A $\overleftarrow{5}$ + **5**	(Tucker and Bernhardt, 2000)
Monococcus echinophorus F.Muell./ Petiveriaceae	A $^{\overleftarrow{2}}$**4**** (total 12 stamens)	(Vanvinckenroye et al., 1997)
Nitraria L. *& Peganum* L./ Nitrariaceae	A $^{\overleftarrow{2}}$**5**** (total 15 stamens)	(Ronse De Craene and Smets, 1991a; Ronse De Craene et al., 1996; Bachelier et al., 2011)
Suriana maritima L./ Surianaceae	A $\overleftarrow{3°}$ + **5**	(Bello et al., 2008)
Visnea mocanera L.f./ Pentaphyllaceae	A $\overleftarrow{10}$ + **5**	(Payer, 1857)
Astraea & Croton/ Euphorbiaceae	A $\overleftarrow{5}$ + **5** + n*** A $\overleftarrow{5}$ + **5** + 5 +n*** A $\overleftarrow{5}$ + **5** + 5 + 5 + n***	(De-Paula et al., 2011 & the present study)

* left-to-right arrangement represent outermost to innermost stamen whorls. Number in bold indicates the first whorl to develop. Centrifugal whorls were indicated with an uppercase arrow. ** indicate centrifugally secondary increase stamens from a common fascicle. *** variable number ranging from 1 to 5.

Monococcus echinophorus in Petiveriaceae (Vanvinckenroye et al., 1997), but those development patterns are different from *Croton* (Table 2). Therefore, centrifugal stamen development in unisexual oligandric to lower-polyandric flowers such as

Croton and *Astraea,* may be a unique feature. So far there is no floral ontogenetic study in other genera in the tribe Crotoneae apart from *Astraea* and *Croton*. Since the presence of centrifugal stamen development appears in all previous studied *Croton*, and also *Astraea* (De-Paula et al., 2011), we hypothesize that this developmental pattern may be present in the ancestor of *Croton* and perhaps in the common ancestor of *Croton* and *Astraea* too. Further ontogenetic studies in other genera in the tribe Crotoneae are needed to confirm our hypothesis.

In the present study, the arrangement of stamens with the alternipetalous stamens as an outermost whorl is almost unique for *C. alabamensis* and has never been reported in *Croton* before. All previously investigated *Croton* (and also *Astraea*) have the outermost stamen whorl opposite to the petals (Baillon, 1858; Marchand, 1860; Michaelis, 1924; Nair and Abraham, 1962; Venkata-Rao and Ramalakshmi, 1968; Gandhi and Thomas, 1983; De-Paula et al., 2011; Gagliardi et al., 2017). The only other case of the alternipetalous stamen whorl as an outermost whorl is found in some species of *Croton* section *Moacroton* (Fig. 1F in van Ee et al., 2008 (*C. ekmanii* Urb.); Chapter 2, 3 and 4) which is a specialized group adapted to serpentine soils found in the Caribbean islands (Borhidi, 1991). Despite *C. alabamensis* and *Croton* section *Moacroton* belonging to the same subgenus *Quadrilobi*, they are not closely related (van Ee et al., 2008, 2011). Therefore, the presence of the alternipetalous outermost whorl should be considered as the result of parallel evolution (cf. Vasconcelos et al., 2017). *Croton alabamensis* and *Croton* section *Moacroton* (except *C. poecilanthus* and *C. maestrensis*) also share the presence of non-inflexed stamen which is unique among all *Croton* species (Berry et al., 2005b; van Ee et al., 2008, 2011). Nevertheless, stamen morphology from these two groups is different. Most of *Croton* section *Moacroton* have sessile anthers (Berry et al., 2005b; van Ee et al., 2008), while *C. alabamensis* has long filament that bend toward the centre of the flower in bud similar to other *Croton* species but are not inflexed (Fig. 7H, I; 9I). Therefore, the presence of non-inflexed anthers found in *C. alabamensis* and *Croton* section *Moacroton* is clearly a homoplasy.

Inner stamen whorls of *Croton chilensis* show great variation in number and arrangement in each flower. The variation may be influenced by spatial constraints between adjacent organs, which result in a different floral architecture in each flower. Developmental irregularity could occasionally happen as shown in a male flower of *C. fuscescens* from De-Paula et al. (2011). However, our observations found that irregular stamen development occurs more often in *Croton* subgenus *Adenophylli* in which many species have high stamen numbers (more than 20 stamens) leading to more than 100 stamen in some species from the *Medusae* group in section *Cyclostigma* (Chapter 5). Further studies on *Croton* with very high stamen numbers (section *Adenophylli*, section *Cyclostigma*), and very low numbers (section *Moacroton*, section *Crotonopsis*, section *Eremocarpus*, *C. monanthogynus* etc.) may reveal novel stamen development patterns waiting to be discovered.

3.6 Conclusion

Floral development in both male and female flowers of three species of *Croton* is highly diverse. Male flowers show variation of floral structures. The presence of a concave receptacle is reported for the first time in this study. The genus is also characterized by a high variation of stamen number and development that can be linked to spatial constraints during development. In female flowers, filamentous structures are interpreted as under-developed petals caused by a process of paedomorphosis. The presence of fully developed petals in *C. alabamensis* and *C. schiedeanus* suggest the independent reversal of this heterochronic process. However, from a combination of historical and ontogenetic perspectives, their occurrence should be considered as apomorphic tendencies rather than homoplasies. Being a mega-diverse genus of the family Euphorbiaceae, *Croton* represents a great model system for the study of floral evolution. However, there have been few detailed floral morphological investigations until now. We hope that this study will trigger further investigations on the floral ontogeny of *Croton* and other Euphorbiaceous genera to explore the evolutionarily dynamics of their floral morphology.

Chapter 4: Floral development and evolution in some enigmatic *Croton* L.

Data contribution: For the micro-computed tomography, sample preparations were done by the author. The scanning of samples and image stack construction were conducted by Dr Alexander Ziegler and Dr Julius Jeiter (Universität Bonn, Germany). Later visualisation and analyses were carried out by the author. Other examination and analyses were done by the author.

4.1 Chapter summary

This chapter aims to explore the developmental basis behind floral diversity in the genus *Croton*. There are several groups within *Croton* that have unusual floral morphology, e.g., low stamen number, very high stamen number, bilateral symmetry and low carpel number, but a comparative study with other typical *Croton* has never been done before. Both male and female flowers from unusual groups within *Croton* were examined with various techniques, e.g., light microscopy, scanning electron microscopy (SEM), resin sectioning and micro-computed tomography (μCT), to obtain information from different perspectives. In male flowers, great variations of stamen number and arrangement are observed. The ancestral androecium character is likely to have the outermost antepetalous stamen whorl developed centrifugally. Modification by reduction of the antepetalous whorl resulted in an outer alternipetalous stamen whorl in *Croton* section *Moacroton*. In *C. alabamensis*, the centrifugal development of the second whorl of stamens is retained, but there is a shift of the outermost stamens to an alternipetalous position caused by hypanthium formation. Several species in the subgenus *Geiseleria* show an independent reduction of stamen numbers leading to the same morphology, i.e., absence of a centrifugal development with the antepetalous whorl the first whorl to develop. Loss of petals is also observed in two distant groups but interestingly these phenomena do not affect androecial architecture. High stamen number with chaotic arrangement is found in *C.*

celtidifolius as a result of rapid development of stamens. In female flowers, the presence of reduced petals and a tricarpellate syncarpous ovary are the ancestral characters. Fully developed petals are reported in some species. Reduction of carpels number happened three times in the subgenus *Geiseleria*. Female flowers of *C. monanthogynus* have a pseudomonomerous bicarpellate ovary. Evidence supporting the true monomerous nature of the unilocular ovaries of *C. setiger* and *C. michauxii* is discussed. The basal placentation found in *C. mixhauxii* is unique among all *Croton* and possible the whole of Euphorbiaceae. *Croton setiger* has the most reduced female flower in the genus with a single unicarpellate carpel subtended by several colleters. Strong bilateral symmetry in sepals and nectary is observed in two species from section *Julocroton*. Therefore, the great floral diversity of the genus *Croton* could be explained by developmental modification of ancestral forms via reduction, increase, reversal and a shift in symmetry.

4.2 Introduction

Croton L. is the second largest genus in the family Euphorbiaceae with approximately 1,200 recognised species distributed in tropical and subtropical regions globally (Govaerts et al., 2000; Berry et al., 2005b; van Ee et al., 2011). Brazil is the centre of diversity while there is significant diversity in the Caribbean and Madagascar as well (Berry et al., 2005b). The genus is widely used in traditional medicine and also as a valuable source of various active compounds (Salatino et al., 2007; Xu et al., 2018). Flowers of *Croton* are unisexual and both flower sexes are usually borne on the same thyrsoid or racemose inflorescences with female flowers on the proximal part and male flowers on the distal part (Webster, 1993, 2014). In general, flowers of *Croton* are pentamerous with the perianth (bipartite in male flowers and sepals and with reduced or absent petals in female flowers) surrounding a nectary and 11-20 stamens in male flowers or a tricarpellate ovary with bifid styles in female flowers, reflecting a strong dimorphism between both flower sexes (Baillon, 1858; Michaelis, 1924; Webster, 1993, 2014; De-Paula et al., 2011; van Ee et al., 2011; Gagliardi et al., 2017). Male flowers are well known for their highly diverse stamen number ranging from one to more than 100 (Müller, 1866; Alain, 1960; Webster, 1993, 2014; van Ee et al., 2008; Riina et al., 2009). Female floral morphology is more stable than in male

flowers but there are reports of significant variation in style branching and occasionally in carpel number (Webster, 1993; Berry et al., 2005b; van Ee et al., 2011). Apart from the classical works of Baillon (1858) and Michaelis (1924), there are no other comparative floral morphological studies in the genus *Croton* until the 21st century. Two recent studies by De-Paula and colleagues (2011) and Gagliardi and colleagues (2017) examined the ontogeny of floral organs in both flower sexes of *Croton* using serial sections to determine the homology and origin of floral organs between male and female flowers. However, species included in those studies do not show variation in stamen nor carpel number (except for the strongly bilateral symmetry in female flowers of *C. triqueter* and *C. fuscescens* – section *Julocroton*). Our knowledge about the floral morphology of different groups in *Croton* is limited; therefore, a comparative floral morphological study is required.

Among all *Croton*, there are many species that have an unusual floral morphology which could be described by two mechanisms, i.e., reduction and increase. Reduced flowers were reported in several groups within *Croton,* e.g., section *Crotonopsis*, section *Eremocarpus* and section *Moacroton* (included *Cubacroton*). *Croton michauxii* from the monotypic section *Crotonopsis* was reported to have flowers with reduced morphology, e.g., low stamen number (five to seven) and an unicarpellate ovary (Baillon, 1858; Müller, 1866; Michaelis, 1924; Webster, 1993; Radcliffe-Smith, 2001; Berry et al., 2005b; van Ee and Berry, 2009a; van Ee et al., 2011). Reduced flowers were also observed in another monotypic section *Eremocarpus* (*Croton setiger*) with loss of petals and low stamen number (six to seven) in male flowers, and a reduced or absent corolla and unicarpellate ovary in female flowers (Müller, 1866; Webster, 1993; Radcliffe-Smith, 2001; van Ee et al., 2011). Some taxonomists treated both sections as separate genera up to the early 21th century (Webster, 1975; Radcliffe-Smith, 2001). However, Webster (1993) suggested these two species morphology resemble some *Croton* species, e.g., *C. monanthogynus* and *C. capitatus,* and merged them into *Croton.* Later phylogenetic studies supported their inclusion within *Croton* but found out that they are not closely related at all (Berry et al., 2005b; Ee and Berry, 2010; van Ee et al., 2011). *Croton* section *Moacroton* is another group with very low stamen number (one to six) (Leon and Alain, 1953; Alain,

1960; Radcliffe-Smith, 2001; van Ee et al., 2008; Webster, 2014). This section has non-inflexed stamens in bud contrary to the majority of *Croton* leading to the recognition of the section as a separate genus until later phylogenetic studies confirmed it to be a specialized group within *Croton* (Berry et al., 2005; Wurdack et al., 2005). Male flowers with low stamen number is common with the family Euphorbiaceae with an extreme case in the genus *Euphorbia* and related genera having a single stamen per flower (Prenner and Rudall, 2007; Prenner, Hopper, et al., 2008). Previous studies in *Croton* found that the outermost stamen whorls are generally arranged opposite petals (Baillon, 1858; Michaelis, 1924; De-Paula et al., 2011; Gagliardi et al., 2017). However, we do not know what the position of stamens would be when there are fewer stamens than perianth parts. At the opposite side of the floral diversity spectrum, stamen number increase is observed in the subgenus *Adenophylli*. Extreme cases of this phenomenon are found in species from the *Medusae* group of the section *Cyclostigma* where male flowers could have more than 100 stamens. Stamen increases are common among the Rosids with several observed mechanisms, e.g., fascicle formation, ring primordia or merism increase (Ronse De Craene and Smets, 1987, 1992, 1995; Ronse De Craene, 2010). Within the Euphorbiaceae, several mechanisms have been observed. In *Ricinus communis* L. (Acalyphoideae), fascicled stamen development result in highly branching stamens (Prenner, Box, et al., 2008). In *Vernicia*, stamen increase is the result of merism increase (McCann, 1942; Mao et al., 2017). In *Garcia nutans* (Crotonoideae), chaotic stamen development is the basis behind the stamen increase (Claßen-Bockhoff, 2016). In *Croton* species with oligandry and low polyandry, centrifugal development of an outermost stamen whorl is observed (Chapter 3; De-Paula et al., 2011). Stamen arrangement in *Croton* with a very high stamen number is chaotic, but we do not know which developmental mechanism generates this pattern. Floral ontogenetic studies in *Croton* with extremely high stamens will help us solve this question.

Up to now there are gaps in our knowledge about the ontogenetic basis behind the diversity of stamen number, stamen arrangement and carpel number in flowers of *Croton*. The present study aims to explore all spectra of floral diversity in the genus of *Croton* and explain floral morphology in a phylogenetic framework. Firstly, we will

explore the developmental mechanisms behind increase and reduction of flowers in *Croton*. Secondly, ancestral floral ontogenetic patterns in *Croton* will be drawn based on a combination of results of the present study and previous literature. Knowledge from the present study will contribute to the understanding floral diversity in the family Euphorbiaceae which is still under-explored.

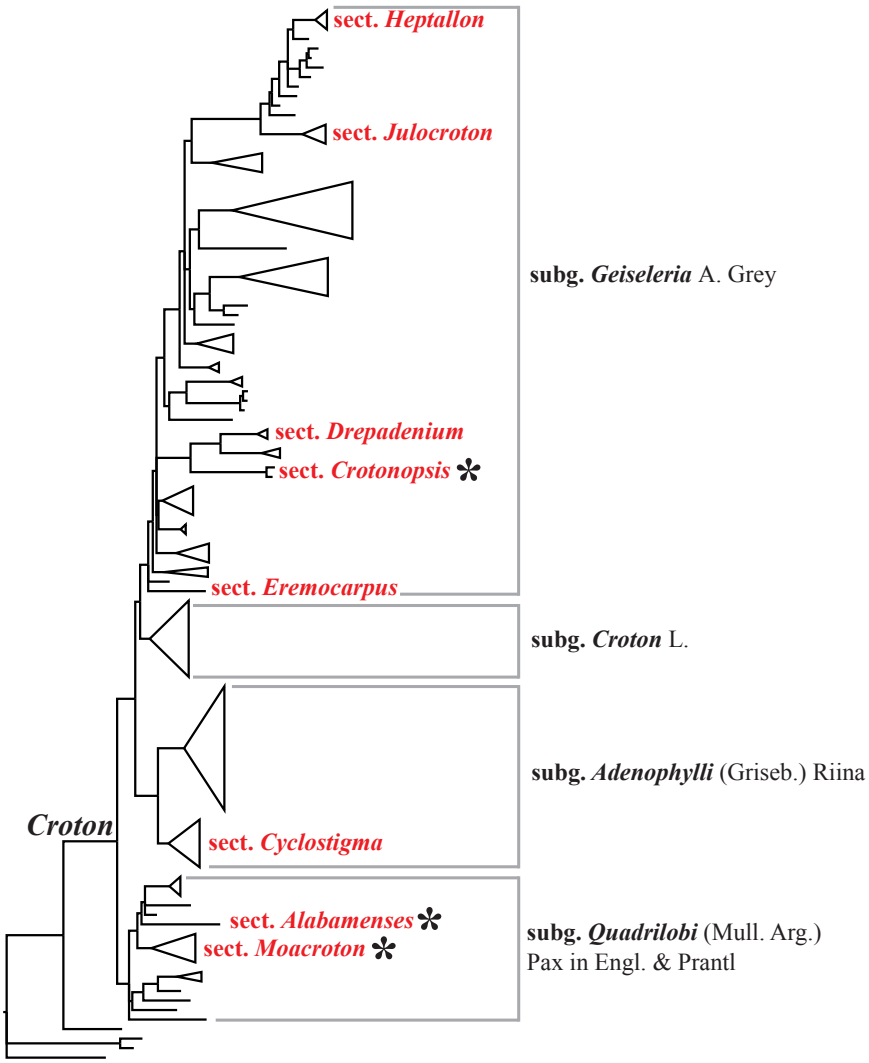

Figure 1. Simplified cladogram of *Croton* indicating four subgenera and their distribution. Sections which have representative species included in the present study are mapped on the tree; asterisk symbols indicate taxa that were used in the micro-computed tomography study. (modified from van Ee et al., 2011 provided by R. Riina).

4.3 Materials and methods

4.3.1 Sample collection

Samples from several *Croton* species with interesting morphology were included in this study (Fig. 1; Table 1). The majority of samples were collected in the field or from cultivation (Table 1), fixed in an FAA solution and then changed to 50% or 70% ethanol. Samples which were extracted from herbarium sheets (Table 1) were softened in a 6:1 10 % Aerosol-OT aqueous solution/acetone solution for 12-48 hours, washed with distilled water and stored in 70% alcohol (Peterson et al., 1978; Erbar, 1995).

Table 1. Source information of samples of *Croton* species included in this study

section	species	source	collection number
Alabamenses B.W. van Ee	*C. alabamensis* var. *alabamensis* E.A.Sm. ex Chapm.	wild collection	A. Wilkin
	C. alabamensis E.A.Sm. ex Chapm. var. *taxensis* Ginzbarg.	cultivation at RBGE from seed bank	LBJWC-1704
Crotonopsis (Michx.) G.L. Webster	*C. michauxii* G. L. Webster var. *elliptica* (Willd.) van Ee & P. E. Berry (syn. *Crotonopsis elliptica*)	wild collection	772 La
Cyclostigma Griseb.	*C. celtidifolius* Baill.	wild collection	M.B.R. Caruzo, R. Riina & A.P.N. Pereira 200 (SP)
		wild collection	A.P.N. Pereira, M.B.R. Caruzo & R. Riina 45 (SP)
Drepadenium (Raff.) Müll. Arg.	*C. dioicus* Cav.	cultivation at RBGE from seed bank	Serial no. 0348102 (millennium seed bank) 20160410 (RBGE code)

Eremocarpus (Benth.) G.L. Webster	*C. setiger* Hook. (syn. *Eremocarpus setigerus*)	wild collection/ California, USA	H. Forbes
		Royal botanic garden, Kew Herbarium	Leroy, A. 2966
Heptallon (Raf.) Müll. Arg.	*C. monanthogynus* Michx.	cultivation at RBGE from seed bank	LBJWC-48 (Lady Bird Johnsonwild flower centre, Texus USA)
Julocroton (Mart.) G.L. Webster	*C. argenteus* L. (syn. *Julocroton argenteus*)	Royal botanic garden, Kew Herbarium	Palmer, E. 103
	C. fuscescens Spreng. (syn. *Julocroton fuscescens*)	wild collection	Riina1951
Moacroton (Croizat) B.W. van Ee	*C. maestrensis* (Alain) B.W. van Ee & P.E. Berry (syn. *Cubacroton maestrense*)	The New York botanical garden Herbarium	Jorge Guiferrez, Paul E Berry, B van Ee, B Jestrow (HAJB 81958)
	C. trigonocarpus Wright ex. Griseb. (syn. *Moacroton trigonocarpus*)	University of Michigan Herbarium	HAJB #81962

4.3.2 Resin sectioning

Samples readily stored in 70% ethanol were dehydrated in ethanol series, alcohol/infiltration medium solution (Technovit® 7100 and hardener I) and immersed in 100% infiltration medium solution for at least three days. Later, samples were embedded in moulds filled with a 12:1 infiltration medium with hardener II solution. Moulds were put into an oven at 40ºC for at least one hour to complete the polymerisation of the resin. Sectioning was carried out in a Leica RM2235 rotary microtome with 5-10 µm thickness. Slides were stained with 0.05% Toluidine blue in aqueous solution, then de-stained in water, series of alcohol and Histoclear (Agar scientifict). DPX was used as mounting agent. Slides were observed under a light microscope (ZEISS Axioskop) and photographed by AxioCam MRc5.

4.3.3 Scanning electron microscope (SEM)

For morphological and ontogeneticl observations, samples were dissected under a light microscope (ZeissStemi SV6), dehydrated in an ethanol–acetone series, critical point dried in a K850 critical-point dryer (Quorum Technologies), coated with platinum in an Emitech K575Xv Sputter Coater, and examined with an LEO Supra 55VP scanning electron microscope.

Table 2. Micro-computed tomography scan settings.

Species/ voucher/ sample	Acceleration voltage (kV)	Source current (µA)	Exposure time (ms) (averaged frame)	Pictures per sample	Camera binning	Pixel size (µm)
C. alabamensis (A. Wilkins) male flower	45	165	755 (6)	633	2x2	3.5
(A. Wilkins) female flower	45	165	1166 (6)	627	2x2	2.5
C. maestrensis (Jorge Guiferrez, Paul E Berry, B van Ee, B Jestrow (HAJB 81958)) male flower	45	165	1166 (6)	627	2x2	2.5
C. michauxii (722La) inflorescence	45	165	1166(9)	627	2x2	2.5
(722La) male flower	45	165	1166(9)	627	2x2	1.5
(722La) Female flower 1	45	165	1166(9)	627	2x2	1.8

4.3.4 Micro-computed tomography (µCT)

Spirited samples (Table 2) were infiltrated with a contrasting solution of 1% phosphotungstic acid (PTA) dissolved in 70% ethanol for at least two weeks and the solution was changed every two days (Staedler et al., 2013). Delicate samples were scanned in ethanol solution while more tough sample were dried at the critical point of CO_2. Prepared samples were scanned in a SkyScan 1272 Micro-CT ultrahigh resolution desktop 3D scanner (Bruker Co., Billerica, MA, US) with Hamamatsu L11871 20 X-ray source (Hamamatsu Photonics, Japan) and a xiRAY16 camera (Ximea GmbH, Münster, Germany) with setting shown in the table 2. Image stacks were reconstructed using the InstaRecon Engine v.2 (InstaRecon, Champaign, IL, USA). Resolution, contrast, brightness and orientation of image sequences were edited with software Fiji (ImageJ 2.0.0-rc-69/1.52p, Schindelin et al., 2012). Image sequences were visualized as 3D volumes which were subsequently manipulated and captured in the AMIRA® 5.4.1 and AVIZO® 9 (FEI Visualization Sciences, France).

4.4 Results

4.4.1 *Croton alabamensis* (section *Alabamenses*)

Morphology and ontogeny of flowers

Male flowers of *C. alabamensis* are pentamerous with five outer sepals alternating with five petals (Fig. 2A). The flowers are cup-shaped with approximately 15-18 stamens arranged in three whorls of five on the concave receptacle (Fig. 2B). The outermost stamens alternate to petals (Fig. 2B). In buds, stamens are curved toward the central part of the flower but are not inflexed (Fig. 2B). Outside of the androecium, there are 10 nectary glands present (Fig. 2A, 2C). Nectary glands are sometimes fused together forming a horse-shoe shaped structure surrounding the filaments (Fig. 2A, 2C). Female flowers are also pentamerous with the presence of five sepals and five fully developed petals (Fig. 2D). A nectary ring is present inside

both perianth whorls (Fig. 2D), surrounding a tricarpellate syncarpous ovary (Fig. 2E). On top of the ovary, there are three simple or slightly bifid styles (Fig. 2F).

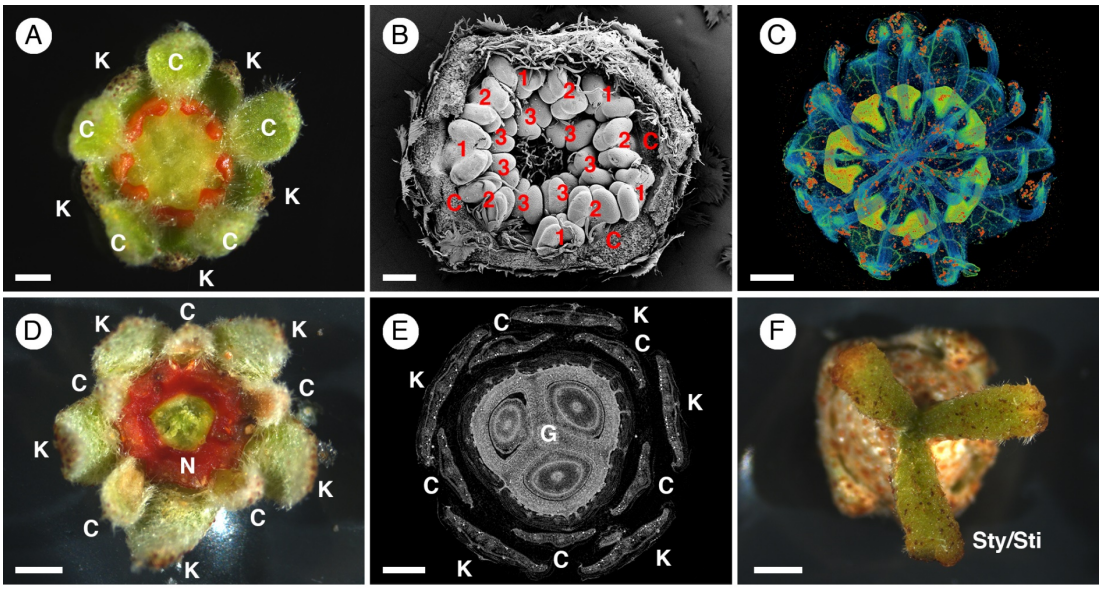

Figure 2. Morphology of male and female flowers of *C. alabamensis*. (A) A male flower with five sepals, five petals and ten orange nectary glands. Stamens were removed. (B) A male flower shows three whorls of stamens inserted on the hypanthium with an empty space in the middle. Perianths were removed. (C) An image obtained from micro-computed tomography shows ten nectary glands surrounding the androecium (yellow colour) Some of the glands fuse together forming horse-shoe shaped structures. (D) A female flower with five sepals, five fully developed petals and a red nectary ring. The ovary was removed. (E) A section from micro-computed tomography shows the presence of tricarpellate syncarpous ovary with axile placentation surrounded by petals and sepals. (F) A female flower shows a simple style with slight branching at the tip. K, sepal; C, petal; N, nectary, Sty, style; Sti, stigma. Scale bar: (A, C, D, F) = 1000 µm; (B) = 200 µm; (E) = 500 µm.

4.4.2 *Croton monanthogynus* (section *Heptallon*)

Morphology and ontogeny of inflorescence and male flowers

Inflorescences of *C. monanthogynus* are racemes with two to three female flowers on the proximal part and several male flowers on the distal part (Fig. 3A-B). The inflorescence is protogynous in all *Croton* (Fig. 3A-B). Flowers are arranged on the inflorescence in a spiral sequence without a terminal flower (Fig. 3C). Male flowers are initiated in the axil of a glandular bract adjacent to two glandular bracteoles

(Fig. 3D-E). Sepals are generally tetramerous with an abaxial sepal initiated first (Fig. 3E, 3F). Then four petals primordia emerge alternating with sepals (Fig. 3G). Next, four outer stamens are initiated opposite to petals following by one, two or three central stamens (Fig. 3H-J). Later, stamens become inflexed in bud (Fig. 3J). Four nectary glands are located alternate with petals and outermost stamens (Fig. 3K-L).

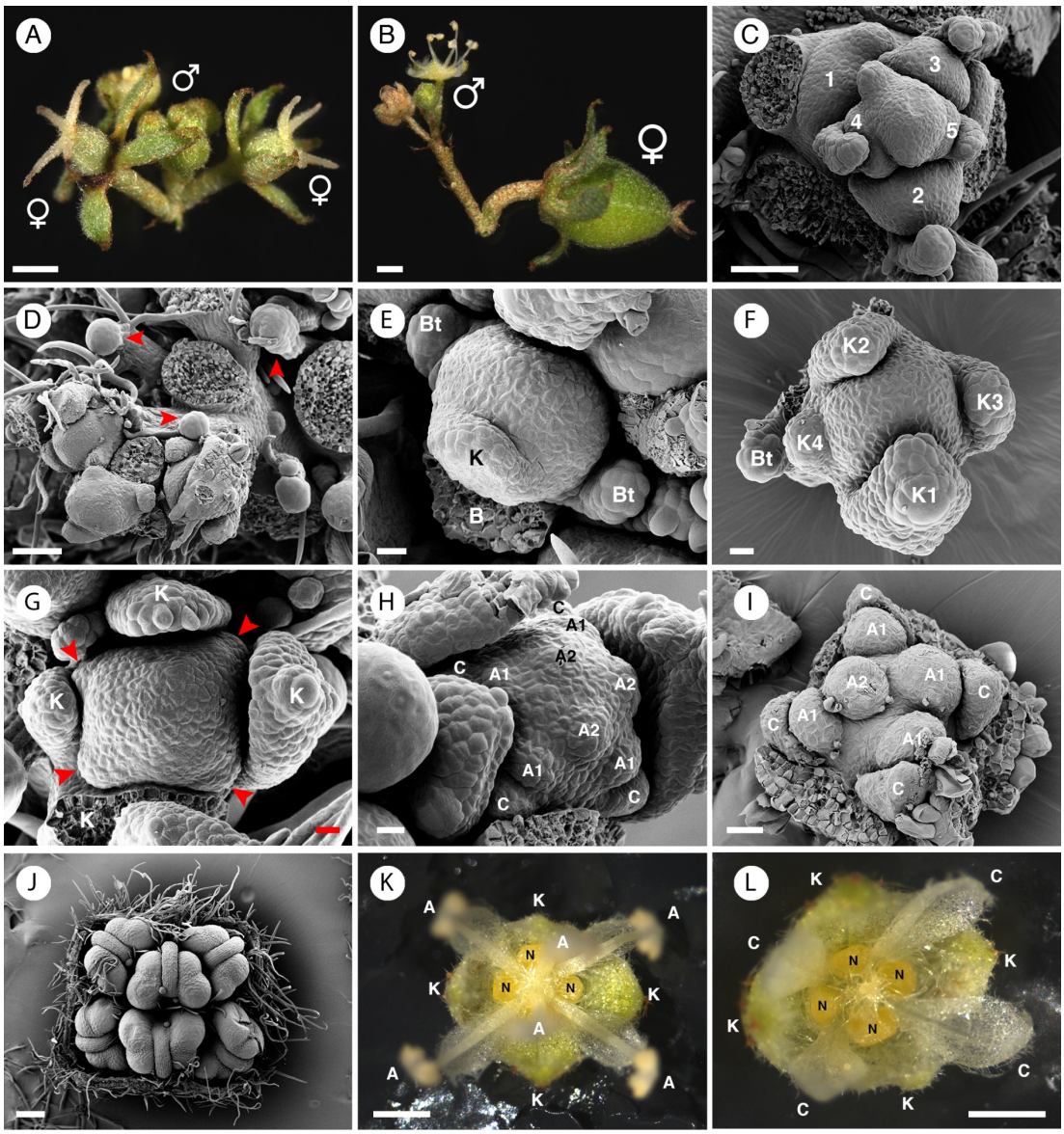

Figure 3. Morphology of male flowers of *C. monanthogynus*. (A) A female stage inflorescence with two female flowers blooming and a male flower starting to bloom. (B) A late male stage inflorescence with a young fruit at the lower part and three male flowers on the upper part. (C) A young inflorescence shows spirally arranged flowers without a terminal flower. (D) A young inflorescence with some flowers removed showing glandular bract and bracteoles (arrowhead). (E) A male flower in an early stage with a first sepals developing. The flower is subtended by a bract and two bracteoles.

Figure 3 continued. (F) A very young male flowers with four sepals developed. (G) A young male flower shows initiation of petal primordia alternating with sepals. (H) Early development of stamens with first four stamen primordia initiated opposite to petals followed by three central stamens. (I) A male flower with five stamens. (J) A male flower bud with perianth removed shows the presence of six stamens. Note stamens are inflexed in bud. (K) An anthetic male flower shows the presence of nectary glands arranged alternating with stamens and petals. (L) An anthetic male flower with stamens removed shows four nectary glands. B, bract; Bt, bracteole; K, sepal; C, petal; A, stamen; N, nectary. Scale bar: (A, B) = 1,000 μm; (C, D) = 50 μm; (E, F, G, H) = 10 μm; (I) = 20 μm; (J) = 100 μm; (K, L) = 500 μm.

Morphology and ontogeny of female flowers

Female flowers are located at the lower part of the inflorescence subtended by a glandular bract and two glandular bracteoles (Fig. 4A, B). Five sepals are the first organs to develop (Fig. 4C, 4D). After that, two carpels with axile placentation emerge on the central part of the flower (Fig. 4D). There is no trace of petals or filamentous structures in female flowers of this species (Fig. 4E-H). Later, two bifid styles are formed on top of the ovary (Fig. 4F-H). The ovary wall is covered with stellate trichomes (Fig. G-H). All sepals have the potential to become glandular with a gland on the apex (I). Five sepals are generally present (Fig. 4J) but in many flowers reduction of an adaxial sepal is observed (Fig. 4K) resulting in four sepals in some flowers (Fig. 4L). Four or five separate nectary glands are located opposite sepals (Fig. 4J, 4L).

Figure 4 (next page). Morphology of female flowers of *C. monanthogynus*. (A) A young inflorescence with flowers arranged in spiral pattern. Female flowers are at the lower part, while male flowers are on the upper part. (B) A female flower is subtended by a bract with colleter present on the apex and two colleter-bearing bracteoles. The flower is removed. (C) An early stage female flower with five sepals developed in al 2/5 pattern. (D) A young ovary with two fused carpels surrounded by five sepals.

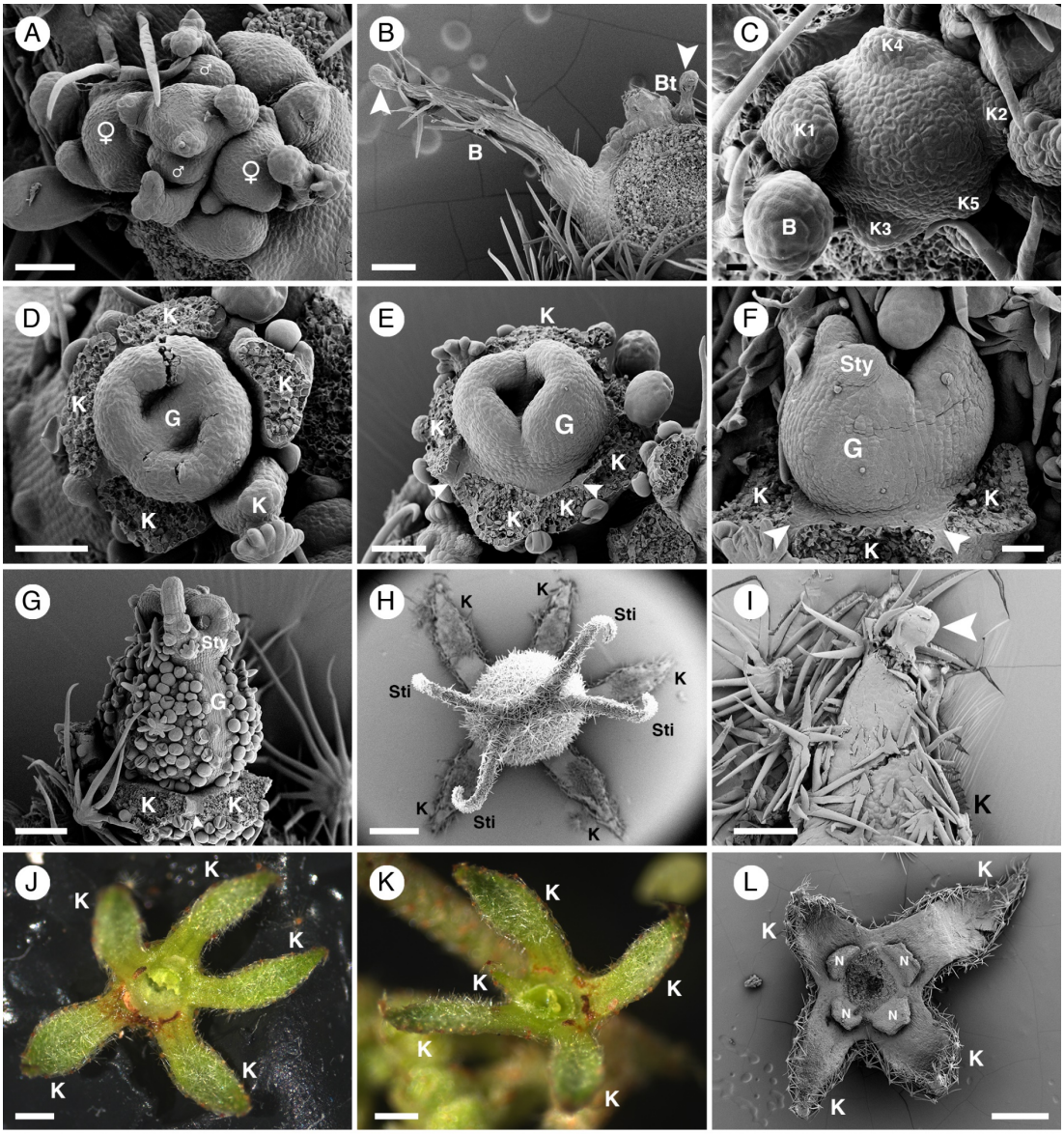

Figure 4 continued. (E) A young ovary with two carpels starting to close. Note there are no primordia of petals or filamentous structures visible (arrowhead). (F) A young ovary with closing carpels. A bilobed style develops on top of each carpel. Petals or filamentous structures are absent (arrowheads). (G) An ovary covered with young trichomes. There is no trace of petals in the empty space alternating with sepals (arrowhead). (H) An anthetic flower with five sepals and an ovary with two fused carpel and two bifid styles. (I) A sepal with a colleter present at the apex (arrowhead). (J) A female flower with five sepals. The ovary was removed. (K) A female flower with five sepals. One sepal is much smaller than other fours. The ovary was removed. (L) A female flower with the ovary removed. There are four sepals present superposed by four separate nectary glands. B, bract; Bt, bracteole; K, sepal; G, ovary; Sty, style; Sti, stigma; N, nectary. Scale bar: (A, D, E) = 50 µm; (B, G) = 100 µm; (C) = 10 µm; (F) = 300 µm; (H, J, K, L) = 500 µm; (I) = 90 µm.

4.4.3 *Croton dioicus* (section *Drepadenium*)

Morphology and ontogeny of flowers

Croton species in this section have their unisexual flowers on separate plants (dioecious). Flowers from both sex types are born on racemose inflorescences. In male flowers, after the development of five sepals, 11 stamens initiate in two whorls of five surrounding a central stamen (A 5+5+1) (Fig. 5A). There are five nectary glands arranged opposite to sepals (Fig. 5B). There is no trace of petals visible, but the outermost stamen whorl alternates with sepals (Fig. 5A, 5C). In female flowers, there is no trace of petals nor filamentous structures (Fig. 5D). Inside the calyx, there is a nectary ring present (Fig. 5E). The ovary is tricarpellate syncarpous superposed by three multifid styles (Fig. 5F).

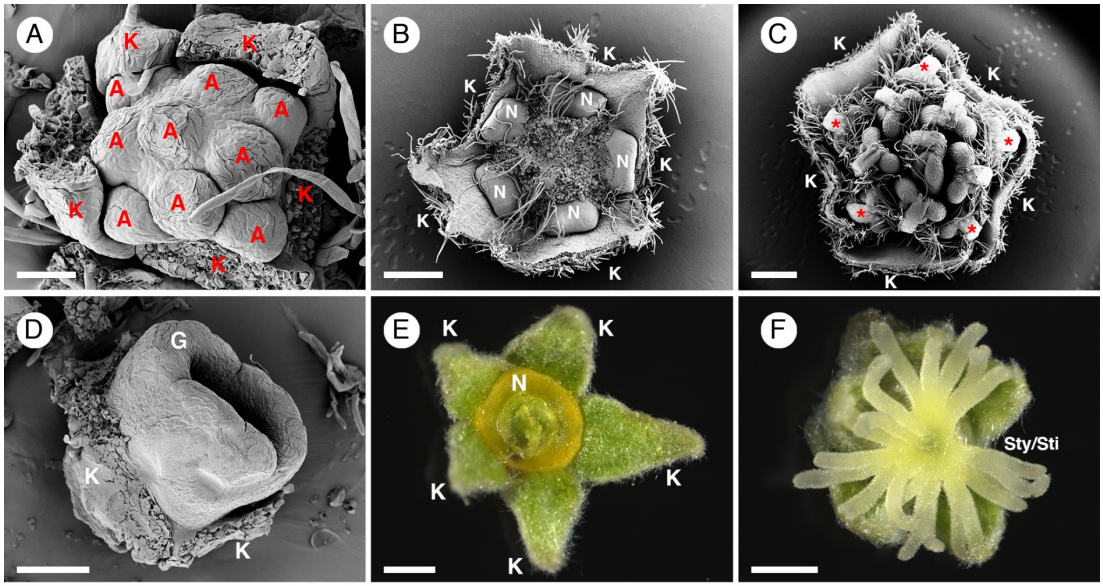

Figure 5. Morphology of male and female flowers of *C. dioicus*. (A) A male flower with stamen primordia. There are two whorls of four stamens surrounding a central stamen in an unusual tetramerous flower. The outermost stamens alternate with sepals. There are no petals in the flower. (B) A male flower with the androecium removed shows the presence of five nectary glands opposite to sepals. (C) A male flower bud with 11 stamens arranged in two whorls of five surrounding a central stamen. The outermost whorl is alternate with sepals (asterisk). (D) A young female flower with tricarpellate ovary. (E) An anthetic flower with an ovary removed shows a nectary ring surrounded by five sepals. Petals or filamentous structures are absent. (F) An anthetic flower with multifid styles. K, sepal; A, stamen; N, nectary; G, ovary; Sty, style; Sti, stigma. Scale bar: (A) = 50 µm; (B, C) = 500 µm; (D) = 100 µm; (E, F) = 1,000 µm.

4.4.4 *Croton setiger* (section *Eremocarpus*)

Morphology and ontogeny of inflorescence and male flowers

Flowers of *C. setiger* are spirally arranged on racemose inflorescences (Fig. 6A-C) with a terminal flower (Fig. 6C). There are about three female flowers on the proximal part and several male flowers on the distal part (Fig. 6A). Each male flower is subtended by a glandular bract and two glandular bracteoles (Fig. 6A; 6D-F). Pedicels show recaulescent growth uplifting bract and bracteoles (Fig. 6A; 6D-E). In male flowers, five sepals are initiated in spiral sequence (Fig. 6F-G). There is no trace of petal primordia. Next, five outer stamens develop alternate with sepals followed by a central stamen (Fig. 6H-J). At maturity, stamens become inflexed in bud (Fig. 6J). Nectary glands are present in male flowers (Fig. 6K).

Morphology and ontogeny of female flowers

Female flowers of *C. setiger* are present on the lower part of the inflorescence (Fig. 7A). Flowers are pedicellate and originate in the axil of a leaf-like bract flanked by two glandular bracteoles (could be interpreted as a leaf and two stipules?) (Fig. 7A-D). There is no perianth in female flowers but there are about six glandular trichomes present around the ovary (Fig. 7E-F). Thin sectioning reveals that those glandular structures are colleters (Fig. 7G). Young ovaries are urn-shaped with long slender tip (Fig. 7E-F). The ovary is unicarpellate and plicate with a visible groove along the adaxial side (Fig. 7F, H). There is an unbranched long slender style on top of the carpel (Fig. 7A, I). All parts are covered with long stellate trichomes (Fig. 7I). There is one ovule attached to the upper part of the locule (apical placentation) (Fig. 7J). Two integuments are present with the inner one thicker than the outer one (Fig. 7K). The nucellus is crassinucellate partly protruding through the micropyle (nucellar beak) (Fig. 7L-M). Above the ovule, an obturator tissue is present (Fig. 7O).

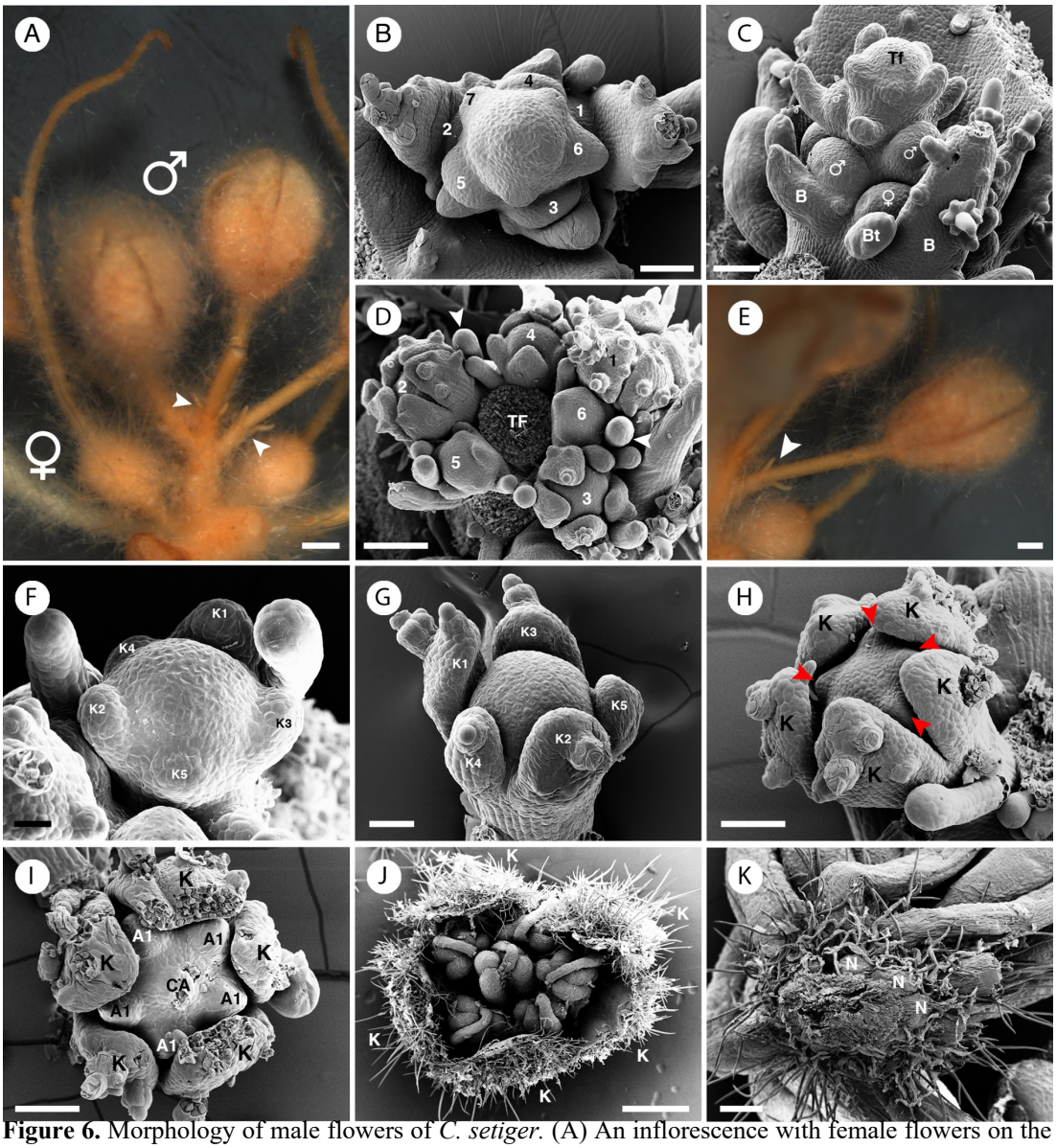

Figure 6. Morphology of male flowers of *C. setiger*. (A) An inflorescence with female flowers on the lower part and male flowers on the upper part. Note the recaulescent growth of the male flower pedicel with uplifting of bract and bracteoles (arrowhead). (B) A young inflorescence with flowers arranged in spiral pattern. (C) A young inflorescence with a terminal flower. Note sepal initiation in the terminal flower (D) An inflorescence with a terminal flower removed shows male flowers in different developing stages. (E) A male flower with uplifting bract and bracteoles (arrowhead). (F) A young male flower with five sepals developing in 2/5 pattern. (G) A young male flower with the presence of five sepals. There is no trace of petal primordia. (H) The first stamen whorl develops alternate with sepals (arrowhead). Young developing trichomes are visible on the abaxial surface of sepals. (I) A male flower shows five outer alternisepalous stamen primordia surrounding a central stamen. (J) A flower bud near anthesis with the upper part of sepals removed shows the presence of six stamens. Petals are absent. (K) The lower part of a flower shows the presence of a nectary. B, bract; Bt, bracteole; TF, terminal flower; K, sepal; A, stamen; N, nectary. Scale bar: (A, J) = 500 µm; (B, C, H, I) = 50 µm; (D, K) = 100 µm; (E) = 200 µm; (F) = 20 µm; (G) = 30 µm.

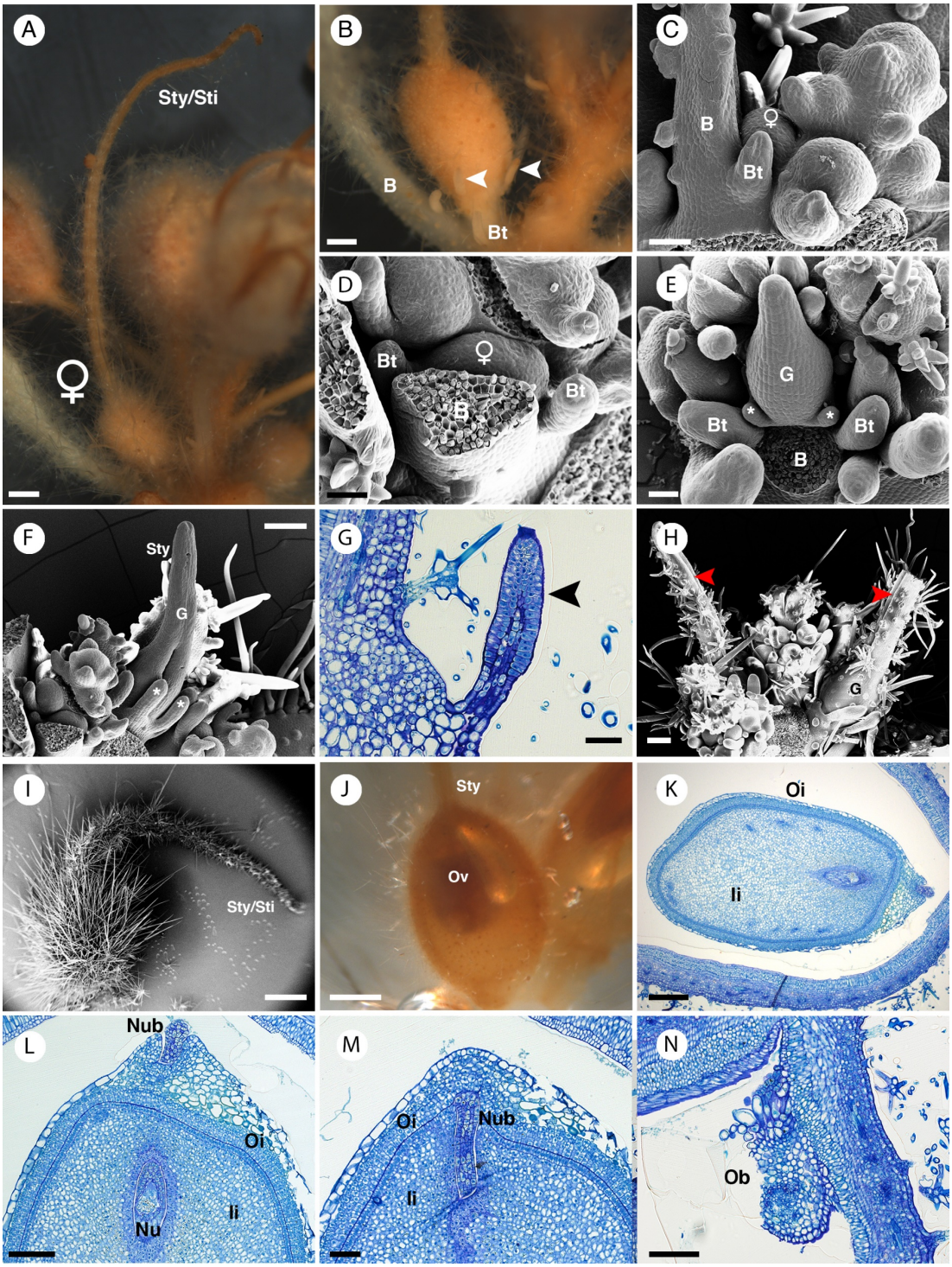

Figure 7. Morphology of female flowers of *C. setiger*. (A) A female flower with long unbranched style. (B) A female flower is subtended by a leaf-like bract and two colleter-like bracteoles. Note, there are colleters present surrounding the ovary (arrowhead). (C) A young inflorescence shows a female flower subtended by a leaf-like bract and bracteoles. (D) The top view of a female flower primordium subtended by a bract and two adjacent bracteoles. (E) A cone-shaped young ovary with two primordia develop on the abaxial side (asterisk).

Figure 7 continued. (F) An adaxial view of a young flower. The ovary is unicarpellate with a groove on the adaxial side. Two filamentous structures (later becoming colleters) are visible (asterisk). (G) An image from resin thin sectioning reveals the glandular structures surrounding the ovary are colleters. (H) Older flowers with a groove visible on the adaxial side of each ovary (astersisk). The upper part of the ovary is elongate forming a style. (I) A mature flower is covered with rosulate and stellate trichomes. (J) A female flower embedded in resin shows an ovule attached to the upper part (apical placentation). (K) An ovule with bitegmic integument. The inner integument is much thicker than the outer layer. (L) The nucellus is crassinucellate with a part protruding through the micropyle (nucellar beak). (M) The nucellar beak protrudes through the micropyle. (N) There is an obturator presentabove the ovule. B, bract; Bt, bracteole; G, ovary; Sty, style; Sti, stigma; Ov, ovule; Oi, outer integument; Ii, inner integument; Nu, nucellus; Nub, nucellar beak; Ob, obturator. Scale bar: (A) = 400 μm; (B, K) = 200 μm; (C, E) = 40 μm; (D) = 30 μm; (F, H, L, N) = 100 μm; (G, M) = 50 μm; (I) = 900 μm; (J) = 500 μm.

4.4.5 *Croton michauxii* (section *Crotonopsis*)

Morphology and ontogeny of inflorescence and male flowers

Flowers of *C. michauxii* are spirally arranged on raceme inflorescences with about two female flowers on the proximal part and several male flowers on the upper part (Fig. 8A-B). Each flower from both sex types is subtended by a bract and two glandular bracteoles (Fig. 8C). Male flowers generally have four sepals with unidirectional growth toward the abaxial side (Fig. 8D). Petal primordia initiate alternate with sepals (Fig. 8E). Next, four stamens develop opposite to petals followed by a single central stamen (Fig. 8F-G). Trimerous flowers with four stamens are occasionally found (Fig. 8H). Some flowers show a reduction of petals on the adaxial side (Fig. 8G). In mature buds, filaments of stamens are inflexed (Fig. 8I). Later, four nectary glands will develop alternate with petals and stamens (Fig. 8J).

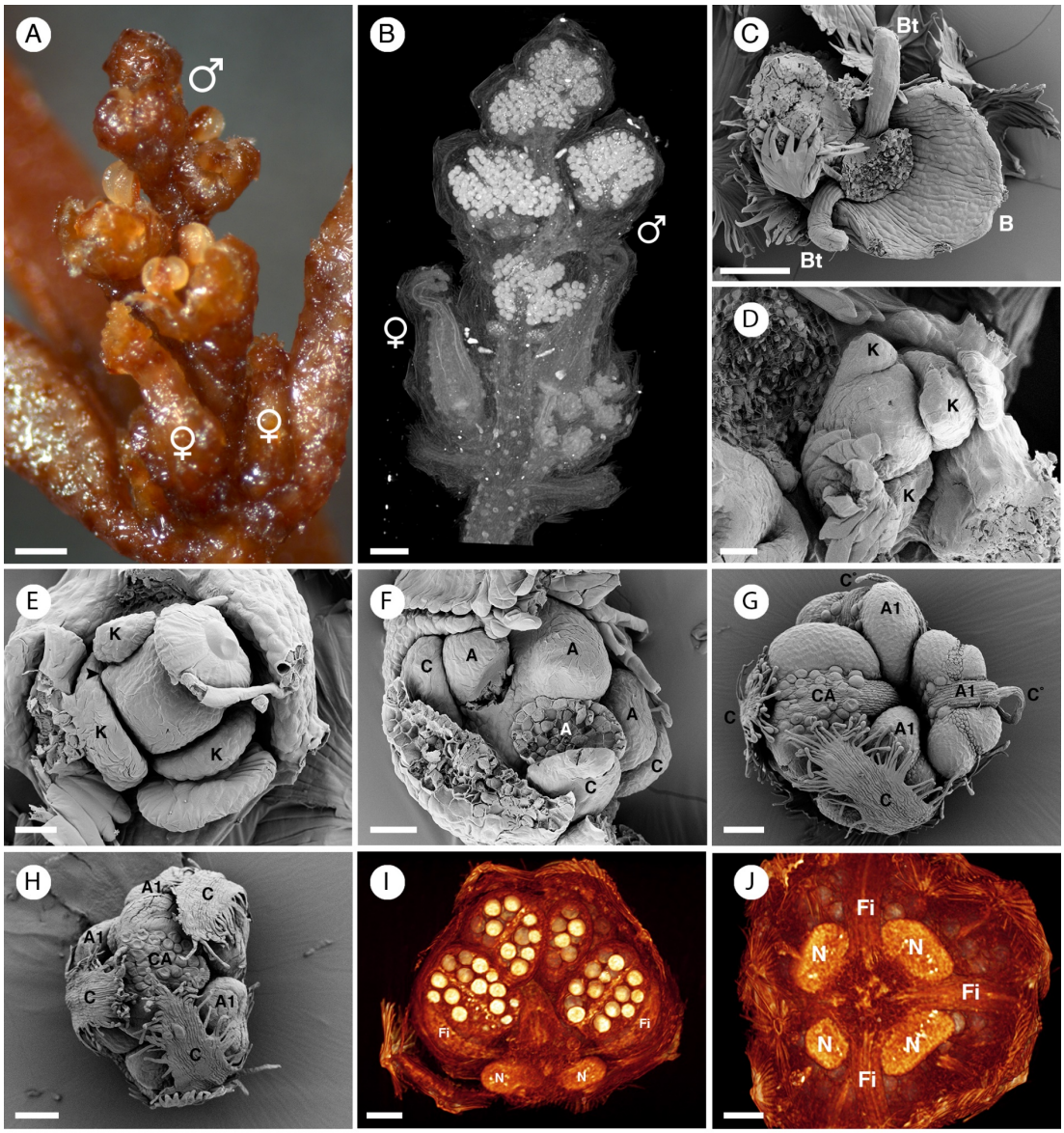

Figure 8. Morphology of male flowers of *C. michauxii*. (A) An inflorescence with female flowers on the lower part and male flowers on the upper part. (B) An image from micro-computed tomography reveals floral structures which are covered with lepidote trichomes. (C) Each flower is subtended by a flattened bract and two glandular bracteoles. (D) A very young male flower with four sepals developed (three visible). (E) Initiation of petal primordia alternating with sepals. (F) The outermost whorl of stamen initiates opposite to petals surrounding a central stamen. (G) A flower with five stamens. There are four antepetalous stamens surrounding a central stamen. Note, two filmentous-like reduced petals. Sepals were removed. (H) A trimerous flower with four stamens. (I) Stamens are inflexed in bud. There are nectaries present at the lower part of androecium. (J) In a tetramerous flower, there are four nectary glands present alternating with petals and stamens. B, bract; Bt, bracteole; k, sepal; C, petal; A, stamen; N, nectary. Scale bar: (A) = 400 µm; (B) = 200 µm; (C, I, J) = 100 µm; (D, E, F) = 20 µm; (G, H) = 90 µm.

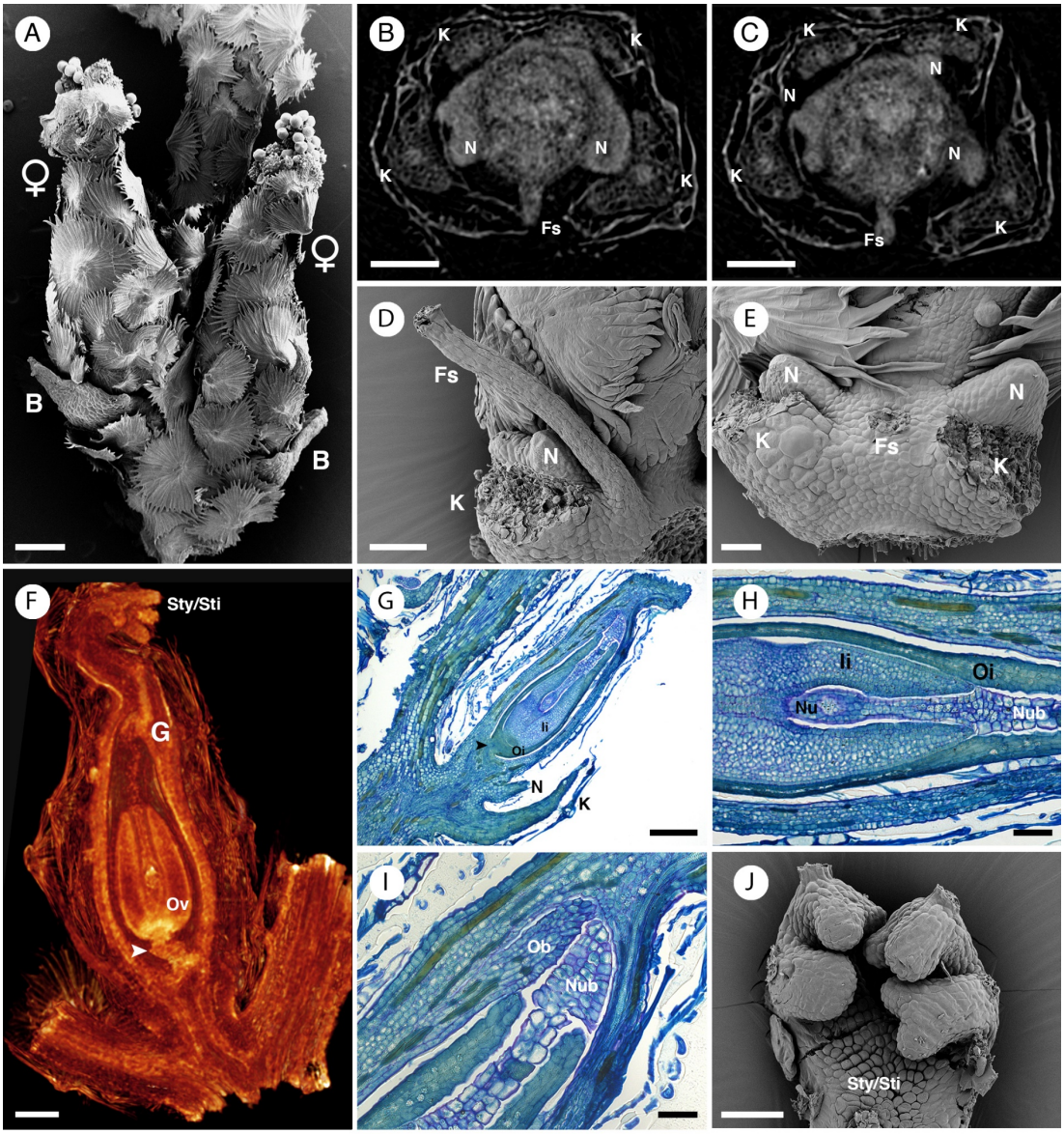

Figure 9. Morphology of female flowers of *C. michauxii*. (A) Two female flowers which are covered with lepidote trichomes. (B-C) A sectioning shows the presence of four sepals, a filamentous structure and four nectary glands surrounding an ovary. Nectary glands could fuse together forming a partial ring. (D) A filamentous structure on the abaxial side of a flower alternating with sepals and nectary. (E) Two abaxial sepals superposed by nectary. There is a scar of fallen-off filamentous structure. (F) Sectioning reveals that the ovary is unicarpellate with a single style. The ovule is attached to the lower part inside the locule (basal placentation) (arrowhead). (G) Longitudinal section of a flower shows the basal placentation of the ovule. (H) The ovule is bitegmic with the outer integument much thinner that the inner intergument. The nucellus is crassinucellate partly protruding through the micropyle (nucellar beak).

Figure 9 continued. (I) There is an obturator present above the ovule. (J) The stigma is generally tetrafid, sometimes with slightly further branching. B, bract; K, sepal; Fs, filamentous structure; N, nectary; G, ovary; Ov, ovule; Sty, style; Sti, stigma; Oi, outer integument; Ii, inner integument; Nu, nucellus; Nub, nucellar beak. Scale bar: (A, B, C, G) = 200 μm; (D, H, J) = 50 μm; (E, I) = 40 μm; (F) = 100 μm.

Morphology and ontogeny of female flowers

Female flowers of *C. michauxii* are covered with dentate-lepidote trichomes (Fig. 9A). Four unequal sepals are present with two outer sepals slightly bigger than two inners (Fig. 9B). A single filamentous structure is present on the abaxial side alternate with sepals (Fig. 9B-E). Four nectary glands are present opposite sepals and are sometimes fused together (Fig. 9A-E). In the middle of the flower, there is a unicarpellate ovary (Fig. 9F-G). There is a single basal ovule (Fig. 9F-G). Two integuments are present with the outer layer much thinner than the inner one (Fig. 9G-H). The apex of the nucellus protrudes beyond the micropyle (nucellar beak) (Fig. 9G-H). Above the ovule, there is an outgrowth of the carpel wall cover the ovule (obturator) (Fig. 9I). A tetrafid style exists on the abaxial side of the carpel but bends toward the adaxial side (Fig. 9J).

4.4.6 *Croton trigonocarpus* and *Croton maestrensis* (section *Moacroton*)

Morphology and ontogeny of flowers

Male flowers are covered with stellate-lepidote trichomes (Fig. 10A). In *C. trigonocarpus*, the perianth consists of five sepals alternate with five petals (Fig. 10B-C). Inside, six stamens are present with five outer stamens alternate with petals and surrounding a single central stamen (Fig. 10B-C). Filaments of stamens are short, making stamens erect in bud (Fig. 10D). In *C. maestrensis*, four sepals are present alternating with four petals (Fig. 10E). Four nectary glands are present opposite sepals (Fig. 10E). Two stamens are located in the central part of flowers alternating with petals (Fig. 10F). Contrary to *C. trigonocarpus*, stamens of *C. maestrensis* are inflexed in buds (Fig. 10G). Only female flowers of *C. trigonocarpus* are examined. There are

five sepals surrounding a tricarpellate syncarpous ovary with three short bifid styles (Fig. 10H). A flower with removed ovary reveals a nectary ring and reduced petals alternating with the sepals (Fig. 10I).

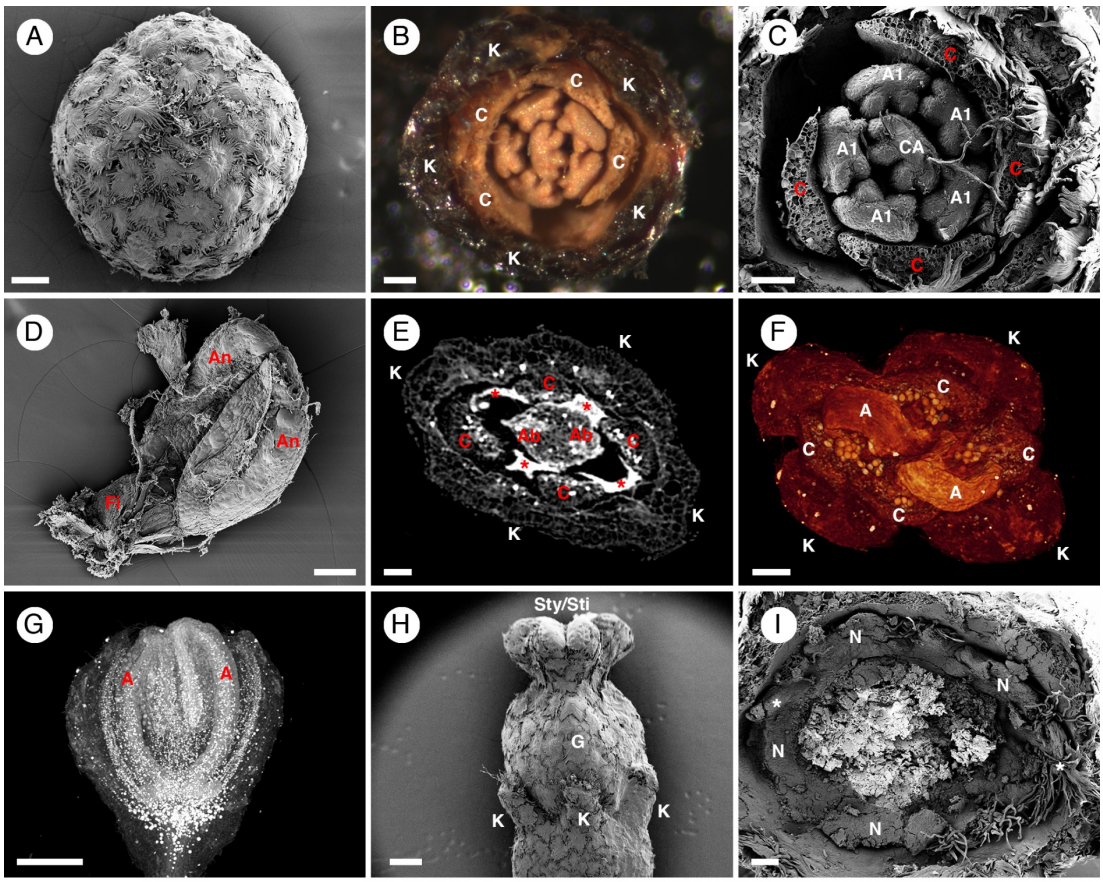

Figure 10. Morphology of male and female flowers of *Croton* section *Moacroton*. (A-D, H-I) *C. trigonocarpus*; (E-G) *C. maestrensis*. (A) A male flower bud covered with lepidote trichomes. (B) A male flower with upper part of perianth was removed. The flower is pentamerous with five sepals, five petals and six stamens visible. Stamens are erect in bud. (C) Five outer stamens are alternate with petals surrounding a central stamen. Anthers are tetrasporangiate dithecal. (D) A stamen shows a short filament and anthers. (E) A transverse section of a flower shows a tetramerous flower with four sepals, four petals, four nectaries and two stamen bundles. (F) A male flower of *C. maestrensis* with two stamens alternating with petals. (G) Stamens of *C. maestensis* are inflexed in bud. (H) A female flower with small sepals and short styles. (I) A female flower with sepals and an ovary removed shows the presence of filamentous structure (asterisk) and nectary. K, sepal; C, petal; A, stamen; CA, central stamen, Sty, style; Sti, stigma; N, nectary. Scale bar: (A, F) = 200 µm; (B, E, I) = 100 µm; (C, D) = 90 µm; (G) = 500 µm; (H) = 400 µm.

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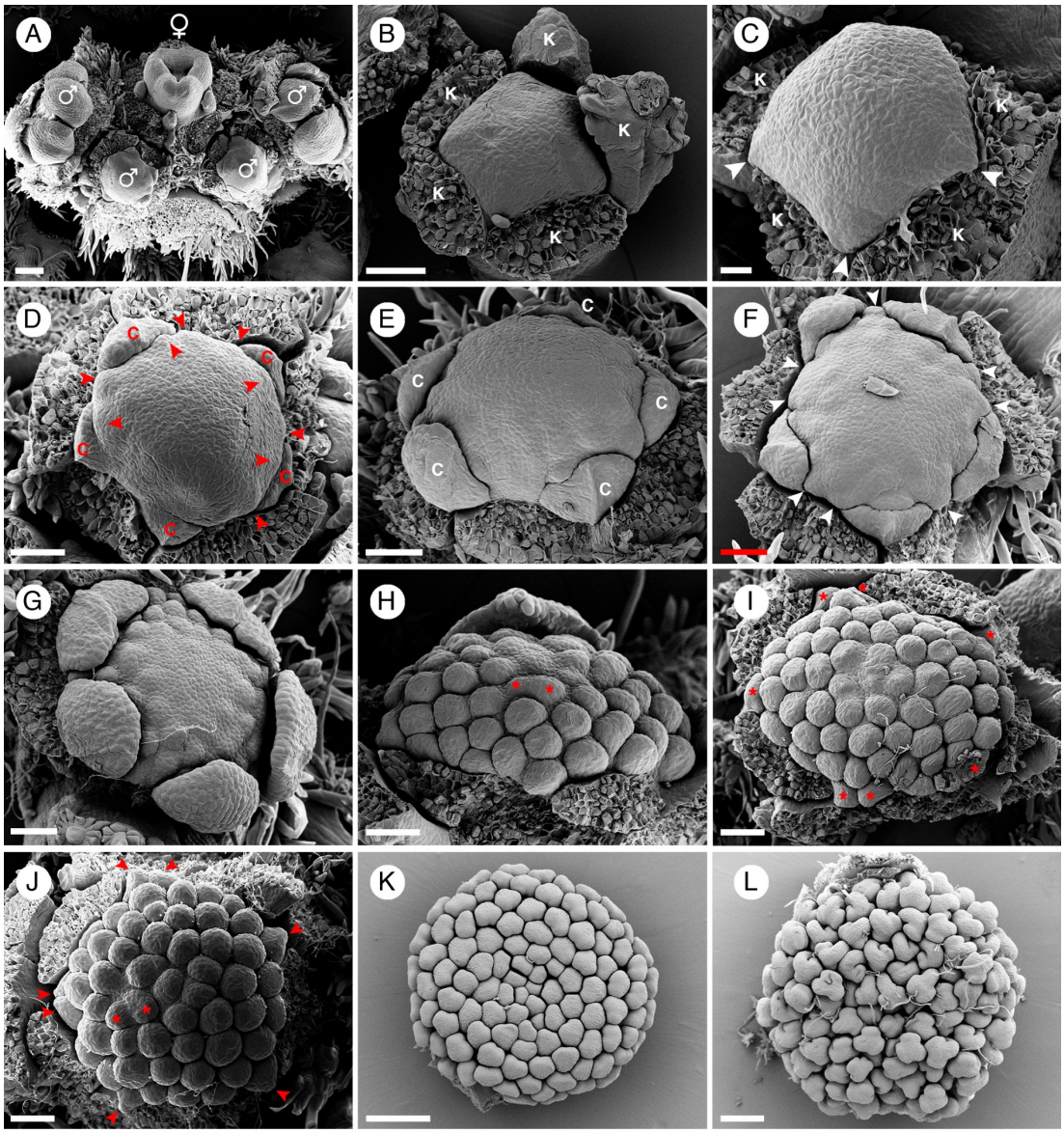

Figure 11. Morphology of male flowers of *C. celtidifolius*. (A) A bisexual dichasial cyme with a female flower surrounded by male flowers. (B) A young male flower with five sepals. (C) Initiation of petal primordia alternating with sepals. (D) An early stage of stamen development. Some stamen primordia were highlighted (arrowheads). (E) Stamen primordia proliferate on the receptacle. (F) Two stamens emerge in an outer alternipetalous position reflecting a labile stamen number in a whorl. (G) Stamens develop rapidly without clear boundary between stamen whorls. (H) A side view of a flower shows a splitting of a primordium into two. (I) A flower with irregular number of stamens per whorl. The outermost stamen whorl is highlighted (asterisks). (J) Top view of a young flower with perianth removed shows labile stamen number and splitting of stamen primordium. (K) A young flower bud with very high stamen number arranged chaotically. (L) An older flower bud with perianth removed show inflexed stamens in bud. The arrangement is chaotic. The orientation of the inflexed stamen is also chaotic. K, sepal; C, petal. Scale bar: (A) = 100 µm; (B, D, E, F, G, H, I, J) = 50 µm; ;(C) = 20 µm; (K, L) = 200 µm.

4.4.7 *Croton celtidifolius* (section *Cyclostigma*)

Morphology and ontogeny of male flowers

Flowers of *C. celtidifolius* are borne on thyrses with bisexual cymules on the proximal part and male cymules on the distal part. Cymules are dichasial (Fig. 11A). Perianths of male flowers of *C. celtidifolius* are pentamerous. Five sepals are initiated first (Fig. 11B) alternating with later developed petals (Fig. 11C). Stamens develop firstly on the brim of the dome shape receptacle (Fig. 11D-G). Stamens then proliferate rapidly through the remaining receptacle (Fig. 11E-L). Some stamens are smaller which may be the result of primordium cleavage (Fig. 11H-J). Outermost stamens are located in an alternipetalous position with one or two primordia visible (Fig. 11F, 11I-J). The arrangement of stamens is chaotic (Fig. 11K, 11L). At maturity, stamens are inflexed in bud (Fig. 11L).

4.4.8 *Croton fuscescens* and *Croton argenteus* (section *Julocroton*)

Morphology and ontogeny of inflorescence and male flowers

Inflorescences of *Croton* section *Julocroton* are compact racemes in which the internode of each flower does not elongate as in other groups of *Croton* (Fig. 12A). In male flowers, the calyx consists of five sepals with unequal size (Fig. 12B). Two sepals on the abaxial side are slightly larger and covered with denser stellate trichomes than three adaxial sepals (Fig. 12B-C). However, unequal growth is only observed in the calyx. Five petals and nectary glands are present in alternation and do not show unequal growth (Fig. 12C-D). In the central part of the flower, there are two whorls of five stamens surrounding a central stamen (Fig. 12E). The outermost stamens are arranged opposite petals (Fig. 12C).

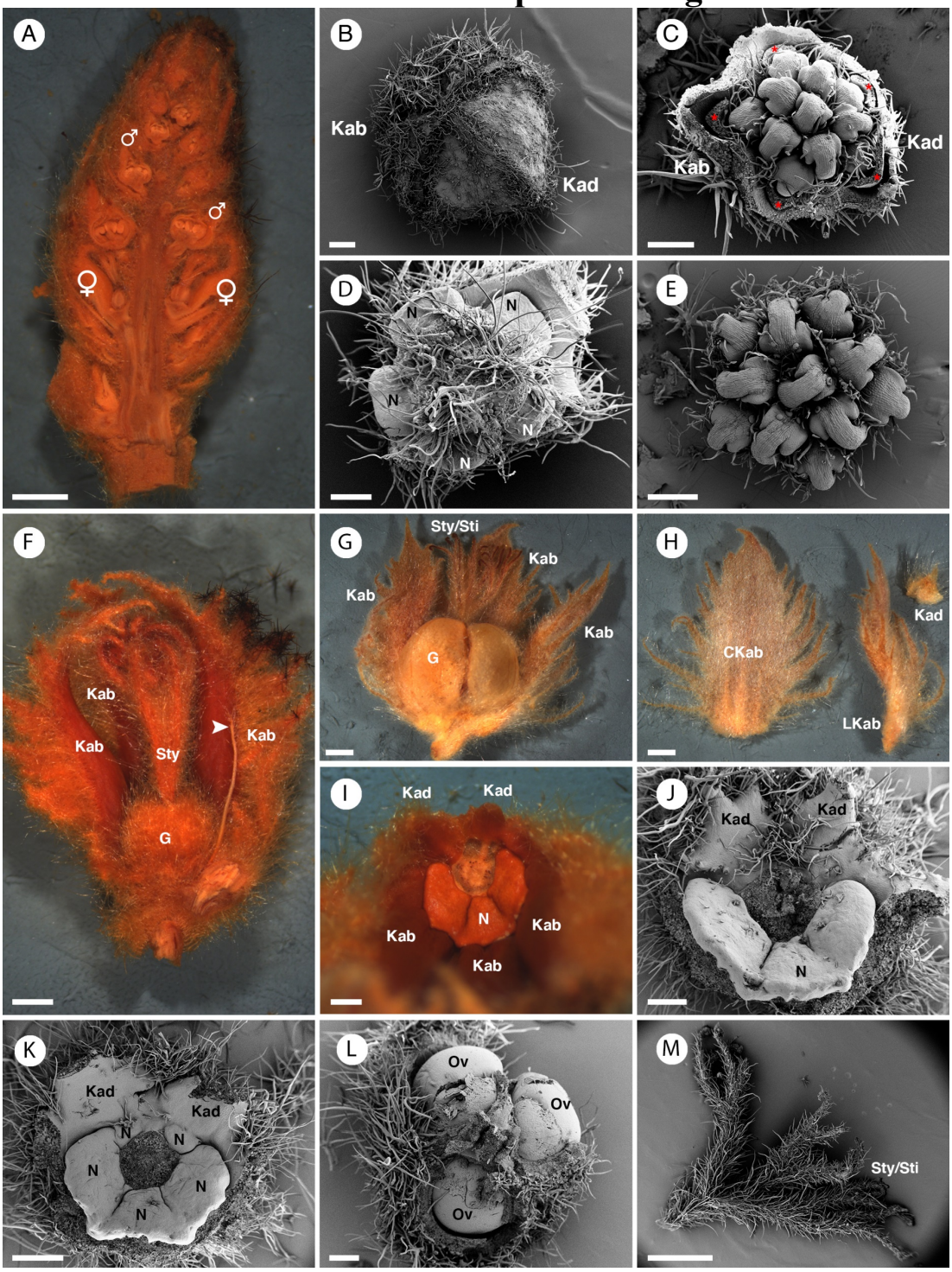

Figure 12. Morphology of male and female flowers of *Croton* section *Julocroton*. (A, C-F, I-M) *C. fuscescens*; (B, G-H) *C. argenteus*. (A) A condensed inflorescence of the raceme type with solitary flowers spirally inserted. (B) A male flower bud shows the difference between sepals on the abaxial side and sepals on the adaxial side. (C) A flower bud with upper part of perianth removed shows bilateral symmetry affecting the calyx whorl. The outermost stamens are opposite to petals (asterisks). (D) Five nectary glands are present surrounding the androecium.

Figure 12 continued. (E) There are 11 stamen presence in a male flower arranged in two whorls of five surrounding a central stamen. (F-G) A view from the adaxial side of a female flowers shows unequal sepals (only the abaxial sepals are clearly visible). Note, a filamentous structure is occasionally present (arrowhead). (H) A comparison of three sepals from different angless. The central abaxial sepal is wider with laciniate margin. The lateral abaxial sepal also has a laciniate margin but is narrower than the central one. The sepal on the adaxial side is the smallest. (I) A top view of a flower shows unequal sepals and a horse-shoe shaped nectary. The ovary was removed. (J) A female flower generally has a horse-shoe shaped nectary developed from three nectary lobes. Two adaxial sepals are visible in the background. (K) Occasionally, two adaxial nectary glands are present completing the whorl of five nectary glands. (L) An ovary comprises three fused carpels with axile placentation. There is one ovule present in each locule. (M) Three tetrafid styles (12 stigmatic tips) covered with stellate trichomes. Kab, sepal on the abaxial side; Kad, sepal on the adaxial side; N, nectary; Sty, style; G, ovary; Sti; stigma; CKab, central sepal on the abaxial side; LKab, lateral sepal on the abaxial side; Ov, ovule. Scale bar: (A, F, G, H, M) = 1,000 μm; (B, C, D, E, L) = 200 μm; (I, K) = 500 μm; (J) = 300 μm.

Morphology and ontogeny of female flowers

Female flowers of *Croton* section *Julocroton* have strongly bilateral symmetry (Fig. 12F-G). Five sepals are present. Three abaxial sepals with laciniate margin are much bigger than two adaxial sepals (Fig. 12F-I). Within the three abaxial sepals, the central sepal is larger than two adjacent petals (Fig. 12G-H). Inside the calyx, horse-shoe shaped nectary glands are present (Fig. 12I-J). Nectary glands are generally three-lobed opposite the three abaxial sepals but a fourth and fifth lobe are sometimes visible (Fig. 12I-K). Filamentous structures are occasionally present (Fig. 12F). In the middle part of the flower, there is a tricarpellate syncarpous ovary with axile placentation (Fig. 12L). Ovules on the adaxial side are slightly bigger than the ovule on the abaxial side (Fig. 12L). On top of the ovary, there are three tetrafid styles present (Fig. 12F-G, 12M).

4.5 Discussion

4.5.1 Diversity and evolution of stamen number and arrangement

Stamen number is highly variable within *Croton* ranging from one to hundreds (Webster, 1993, 2014; Radcliffe-Smith, 2001). Previous studies found that stamens of *Croton* are arranged in several whorls of five with the outermost whorl located opposite to petals (Baillon, 1858; Marchand, 1860; Michaelis, 1924; Nair and Abraham, 1962; Venkata-Rao and Ramalakshmi, 1968; Gandhi and Thomas, 1983; De-Paula et al., 2011; Gagliardi et al., 2017). Previous ontogenetic studies found that the outermost antepetalous stamen whorl of some *Croton* develops centrifugally (Chapter 3; De-Paula et al., 2011). This developmental process is similar to a condition described as secondary obdiplostemony (or centrifugal obdiplostemony) which is a rare developmental pattern among angiosperms (Ronse De Craene and Smets, 1995; Ronse De Craene and Bull-Hereñu, 2016). This pattern is observed in several species of *Croton* from different lineages and also in the genus *Astraea* from the tribe Crotoneae (Chapter 3; De-Paula et al., 2011; Gagliardi et al., 2017), so it is likely that this pattern is the ancestral state of the genus *Croton*. However, variations of this pattern were observed in *Croton*. In male flowers of *C. alabamensis*, the outermost stamen whorl develops centrifugally in alternation with petals as under the influence of the hypanthium formation (Chapter 3). Ten nectary glands (instead of normally five as in other species) later develop alternate with the outermost stamens (Chapter 3). Moreover, the outermost stamen whorl of *Croton* section *Moacroton* is arranged alternate with petals (Fig. 10B-C, E-F). Stamen number of this clade is generally low (one to six) which prevents the formation of more than one whorl of stamens (except in *C. trigonocarpus* with 5+1 stamens, Fig. 10B-C). There is no centrifugal antepetalous stamen whorl development in this group which explains why the outermost stamen whorl is arranged alternate with petals. This stamen developmental process is a shift from secondary obdiplostemony type II directly to haplostemony without intermediate with staminodes which has not been described before (Ronse De Craene and Bull-Hereñu, 2016). Interestingly, stamens in most species from this group are non-inflexed in bud due to their short filaments (Fig. 10B-C)(Radcliffe-Smith,

2001; Berry et al., 2005b; van Ee et al., 2008), except in *C. maestrensis*, a separate clade from the rest of the section, which has inflexed filaments in bud (Fig. 10G). This case implies that the reduction of stamen number (also rearrangement of stamens) occurred before the change of filament length. In subgenus *Geiseleria*, a decrease of stamen numbers also affects the stamen arrangement in flowers. In *Croton* flowers with less than 10 stamens, e.g., *C. trinitatis*, *C. argyranthemus*, *C. michauxii*, *C. monanthogynus*, and *C. setiger*, five antepetalous or alternisepalous stamens are the first whorl to develop followed by central stamens with a variable number ranging from one to four (Fig. 3A; 6H; 8F; 13). Therefore, there is no centrifugal stamen development in these species. This phenomenon could be described by as a shift from secondary obdiplostemony type II to obhaplostemony without petal loss (except in *C. setiger*) which has never been described before. The erroneous floral diagram of male flowers of *C. michauxii* (previously named *Crotonopsis elliptica*) with five stamens alternating with petals (haplostemony) was published by Baillon (1858). Later, more accurate diagrams of tetramerous and pentamerous male flowers with outermost stamens opposite to petals were published by *Michaelis* (1924). Interestingly, the decrease of stamen number and loss of the centrifugally initiated whorl coexist in different clades independently, which reflects the nature of this character as an apomorphic tendency specifically found in *Croton* subgenus *Geiseleria*. Merism change of the whorled flower strongly affects the stamen number. *Croton maestrensis* with a tetramerous perianth was found to be the *Croton* species with lowest stamen number ranging from one to three (Alain, 1960; van Ee et al., 2008). Tetramerous male flowers are also found in *C. sapiifolius* and *C. monanthogynus* with 10-15 and six to seven stamens respectively (present study; Riina et al., 2010b). Merism in male flowers of *C. michauxii* is highly variable ranging from three to five (present study; Michaelis, 1924) but the majority of flowers from our sample is tetramerous with five stamens (A 4+1) (Fig. 8G). The presence of trimerous flowers with four stamens (A 3+1) (Fig. 8H) is the lowest stamen number in the subgenus *Geiseleria*. Interestingly, the outermost stamens of *C. dioicus* and *C. setiger* alternate to sepals despite the fact that there are no petals present in their flowers (Fig. 5A, C; 6H-J). Therefore, it could be assumed that the loss of petals does not affect stamen arrangement in flowers of *Croton*.

Increase of stamen number is observed in *Croton* subgenus *Adenophylli*. The ontogenetic study in *C. chilensis* with approximately 20 stamens demonstrated that the alternipetalous whorl is the first whorl to develop followed by the centrifugal antepetalous stamen whorl (Chapter 3). The third and fourth whorls develop centripetally but their numbers are labile, resulting in different stamen numbers in each flower (Chapter 3). These cases of variation in stamen number in a whorl are further stressed in male flowers with higher stamen number. Our ontogenetic observation in male flowers of *C. celtidifolius* (sect. *Cyclostigma*) with more than 100 stamens found that stamens develop rapidly with no clear boundary between each whorl (Fig. 11G-L). Moreover, its stamen number is highly variable as observed in the alternipetalous stamens which could be one or two (Fig. 11F, I-J). A combination of stamen number increase, rapidly developed stamens and labile stamen number in a whorl give rise to a chaotic stamen arrangement of *C. celtidifolius* (Fig. 11K-L; 13) which is shared among other species from the *Medusae* clade of sect. *Cyclostigma* (Riina et al., 2009).

Diversity of stamen number and arrangement is widespread at the family level. There are only few cases of stamen diversity in the same genus. *Clusia* L. (Clusiaceae) is a genus with great diversity in its synandrium structure ranging from low stamen number (eight to twelve) to more than one hundred stamens resulting from ring primordia with centrifugal development (Bittrich and Amaral, 1996; Gustafsson, 2000; Hochwallner and Weber, 2006; Sá-Haiad et al., 2015). The androecial structure in the genus *Hibbertia* Andrews (Dilleniaceae) is highly diverse. Different modes of stamen proliferation, e.g., free stamens, ring primordia and stamen fascicles, are found in the genus (Payer, 1857; Tucker and Bernhardt, 2000). The stamens of *Hibbertia* are generally developed centrifugally but Tucker and Bernhardt (2000) also mentioned acropetal stamen development in species with low stamen number. Moreover, bilateral symmetry of the androecium in some *Hibbertia* is derived from a partial reduction of stamen primordia (Tucker and Bernhardt, 2000). Diversity in stamen arrangement is reported in the genus *Mittela* Tourn. ex L. (Saxifragaceae) with the presence of obdiplostemony (e.g., *M. nuda* L. and *M. diphylla* L.), obhaplostemony (e.g., *M. pauciflora* Rosend.) and haplostemony (e.g., *M. ovalis* Greene and *M. trifida* Graham) in the same genus (Rosendahl, 1914; Katsuhara et al., 2017). However, a

later phylogenetic study found that the genus *Mitella* is polyphyletic (Okuyama et al., 2008). Further comparative floral morphology is required to confirm if there is stamen arrangement diversity in this genus or not. In *Croton*, the diversity of stamen number and arrangement is caused by the plasticity in the stamen development. The presence of the second outermost stamen whorl with centrifugal development seems to be the ancestral character. Stamen architecture diversity in the genus is derived from a modification of the ancestral form via reduction or multiplication.

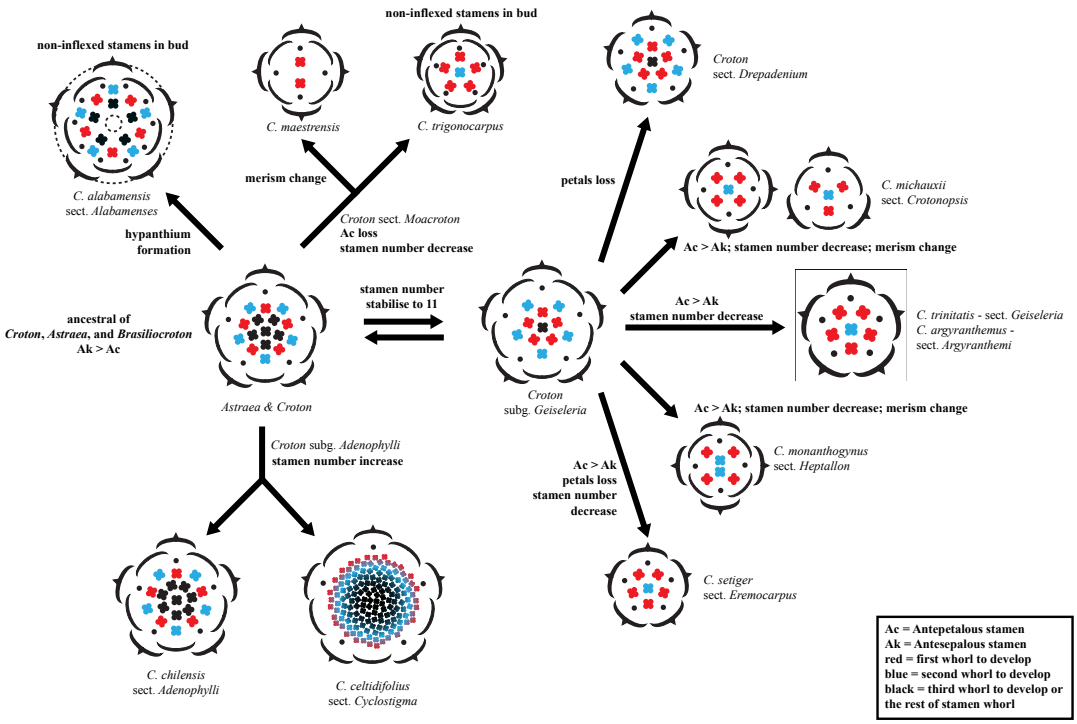

Figure 13. A diagram shows the hypothetical evolutionary pathway of stamen arrangement in male flowers from different groups within *Croton*. Data of floral development in *C. alabamensis* and *C. chilensis* were obtained from chapter 3. The position of floral diagrams of representative species follows their phylogenetic relationship (Fig. 44 from chapter 2; Fig. 1 from this chapter). The possible ancestral stamen number of *Croton* is found to be 15-19 with shift to 10-14 in the subgenus *Geiseleria* (Fig. 44 from chapter 2). Species with reduced stamen number are found to be not closely related (Fig. 44 from chapter 2; Fig. 1 from this chapter) suggesting an independent origin.

4.5.2 Diversity and evolution in female flowers

The general morphology of female flowers of *Croton* includes the presence of sepals, filamentous structures, nectary and tricarpellate ovary (Radcliffe-Smith, 2001; Webster, 2014). It is found that the architecture of female flowers of *Croton* is much more stable than male flowers. However, some groups of *Croton* have female flowers with unusual morphology, e.g., strong bilateral symmetry, fully developed petals and reduction of carpel number (Fig. 14). Fully developed petals are found in *Croton* section *Alabamenses* (subg. *Quadrilobi*), many species from section *Eluteria* (subg. *Geiseleria*), and some African species (subg. *Croton*) (Webster, 1993; Berry et al., 2005b; Friis and Gilbert, 2008; van Ee and Berry, 2009b; van Ee et al., 2011; Berry et al., 2016). Results from the floral ontogenetic study suggested that filamentous structures are reduced petals derived via paedomorphosis, while fully developed petals in female flowers are derived via reversal (Chapter 3). *Croton* species from section *Julocroton* are well known for their highly unequal sepals and horse-shoe shaped nectary in female flowers (Radcliffe-Smith, 2001; De-Paula et al., 2011; van Ee et al., 2011). A previous ontogenetic study found that the unequal sepals are the result of unidirectional growth of sepal primordia (De-Paula et al., 2011; Gagliardi et al., 2017). Unequal sepals are common among *Croton* species but are highly abundant in some groups, e.g., sect. *Geiseleria* (van Ee et al., 2011). In *Croton* sect. *Geiseleria*, the smaller sepals are on the adaxial side (Sodré and Silva, 2017; Sodré et al., 2019) suggesting that this may be the result of mechanical pressure from the inflorescence axis during floral development. Strong bilateral symmetry in sepals and nectary of *Croton* sect. *Julocroton* may be the result from condensed inflorescences in the section. This also helps explain why male flowers of *Croton* sect. *Julocroton* exhibit slightly unequal growth only in the calyx whorl (Fig. 12B, C). Previous examination in *C. triqueter* and *C. fuscescens* found that a horseshoe-shape nectary is found on the abaxial side of female flowers. However, our examination found that two nectary lobes could be present on the adaxial side (Fig. 12K). Filamentous structures could be occasionally present on the adaxial side as well as in lateral position (Fig. 12F; 14) (De-Paula et al., 2011).

Reduction of the perianth also occurs in three non-closely related species with reduced carpel number, i.e., *C. setiger*, *C. michauxii* and *C. monanthogynus* (Fig. 14). Sepals in female flowers of *C. monanthogynus* have a tendency to change from pentamery to tetramery with intermediate forms (Fig. 4J-L) conforming with the tetramerous male flowers (Fig. 3J, K). Interestingly, intermediate form sepals are highly similar to some filamentous structures supporting the hypothesis of their origin as reduced perianth (petals). In *C. michauxii*, four sepals are present in female flowers which is also the same norm as in most of male flowers. The abaxial sepals are slightly bigger than those on the adaxial side. On the abaxial side, there is one long filamentous structure present which we thought to be homologous to filamentous structures (reduced petals) in other *Croton* species (Fig. 9B-E). The occurrence of unequal sepals, a single abaxial filamentous structure and an unicarpellate ovary suggest that female flowers of *C. michauxii* express a strong bilateral symmetry. Female flowers of *C. setiger* are the most reduced flowers of the whole genus with an unicarpellate ovary, and absence of both perianth and nectary. However, we found several colleters present around the ovary (Fig. 7B, E, F-G). We think that these colleters are derived via reduction of colleter bearing sepals similar to reduction of a sepal in *C. monanthogynus* or the colleter occupy the empty space left by the lost sepals.

Female flowers of *C. setiger*, *C. michauxii* and *C. monanthogynus* also present a reduction of carpel numbers. The ovary of *C. monanthogynus* clearly comprises two carpels from observation of the young stages (Fig. 4D-E). The unilocular ovary of *C. setiger* is found to be unicarpellate as a carpel slit is observed on the adaxial side of young ovaries (Fig. 7F). We could not observe a young ovary of *C. michauxii*. However, we found that there is only one style present on top on the abaxial side of the ovary. Evidence from floral ontogeny in other species of *Croton* shows that each style develops specifically on top of each carpel (the present study) (Chapter 3; De-Paula et al., 2011; Gagliardi et al., 2017). Therefore, the unilocular ovary of *C. michauxii* is constructed from a single carpel.

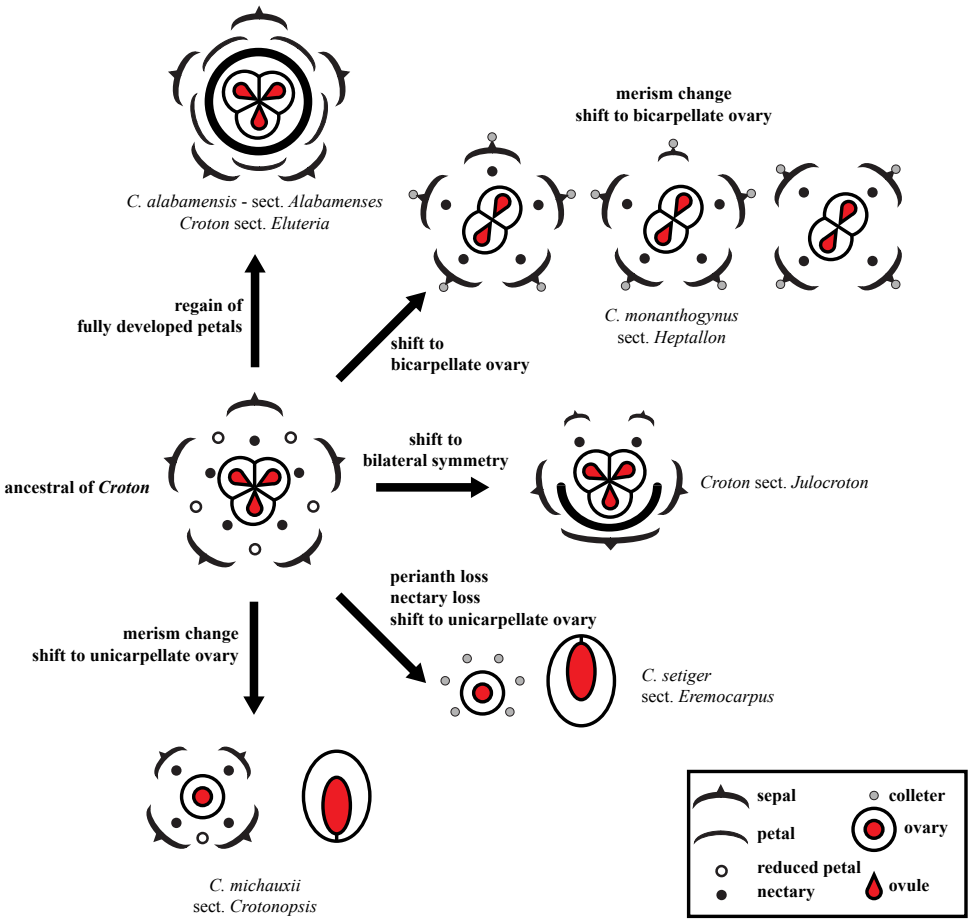

Figure 14. A diagram shows the hypothetical evolutionary pathway of female flowers from different groups within *Croton*. The position of floral diagrams of representative species follows their phylogenetic relationship (Fig. 1). Species with unusual gynoecial characters are not closely related on the phylogenetic tree (Fig. 1) suggesting an independent origin.

4.5.3 Independent reduction of carpel number in different lineages within *Croton*

The combination of tricarpellate syncarpous gynoecia with axile placentation (three locules) and an anatropous ovule in each locule is a characteristic of female flowers of the family Euphorbiaceae and also *Croton* (Marchand, 1860; Sutter and Endress, 1995; De-Paula and Sajo, 2011; Webster, 2014). Reduction of carpel number from three to two carpels is common among the Euphorbiaceae especially in the subfamily Acalyphoideae, e.g, several *Mallotus* (Kulju et al., 2007), *Alchornea* (Secco, 2004; Secco and Giulietti, 2004; Webster, 2014; Gama et al., 2019), *Mercurialis* and related genera (Webster 2014), and subfamily Crotonoideae, e.g.,

Joannesia princeps, *Hylandia*, *Pantadenia* (*Parapantadenia?*), *Borneodendron*, *Annesijoa* (Radcliffe-Smith, 2001; Webster, 2014). However, further reduction of the carpel number to one is much rarer as was reported in a few species, e.g., *Mallotus cumingii* Müll.Arg. (syn. *Neotrewia*) (Acalyphoideae) (Kulju et al., 2007), *Plagiostyles africana* (Euphorbioideae) (Radcliffe-Smith, 2001; Webster, 2014), *Radcliffea* Petra Hoffm. & K. Wurdack (Crotonoideae) (Hoffmann and Wurdack, 2006; Webster, 2014), *Jatropha* section *Mozinna* (Dehgan and Webster, 1979; Dehgan and Schutzman, 1994; Dehgan, 2012), *C. setiger* and *C. michauxii* (present study; Webster 1993; Radcliffe-Smith 2001; Berry *et al.* 2005; van Ee *et al.* 2011). The unicarpellate ovaries of *M. cumingii* and *Plagiostyles* are likely to be derived via bicarpellate intermediates since bicarpellate ovaries are common among *Mallotus*, and related genera of *Plagiostyles* (Kulju et al., 2007; Webster, 2014). Webster (Webster, 1967, 1993) suggested that the unicarpellate ovary of *C. michauxii* may be derived via ancestors with bicarpellate ovaries resembling *C. monanthogynus* based on their morphological similarity, and *Croton setiger* might be related to the annual herbaceous species which are actually placed in section *Heptallon*. Later phylogenetic studies reveal that all these groups are in the same subgenus *Geiseleria* but far apart from each other, with *C. setiger* as one of basal clades, *C. michauxii* associated with sect. *Drepadenium* and sect. *Argyranthemi*, and *C. monanthogynus* nested within other species in section *Heptallon* with tricarpellate ovary (Fig. 1) (Berry et al., 2005b; van Ee and Berry, 2010b; van Ee et al., 2011). Bicarpellate ovaries also occur in other lineages within *Croton*, e.g., *C. sapiifolius* (sect. *Quadrilobi*, subg. *Quadrilobi*) and *C. miarensis* Leandri. (subg. *Croton*) (Riina et al., 2010b; van Ee and Berry, 2010b; van Ee et al., 2011). Therefore, reduction of carpel number occurred separately within *Croton*. Moreover, data from a morphological examination and previous studies infer different origins of these ovary reductions. In *C. monanthogynus*, fruits are dehiscent with usually one of two ovules aborted suggesting that the bicarpellate ovary of this species is pseudomonomerous (Webster, 1967; Sokoloff et al., 2017). A similar reduction by abortion is found in the bicarpellate ovary of *C. sapiifolius* which has three styles, but in this case the abortion affects only one of the carpel (Riina et al., 2010). There is no trace of a second carpel in the unicarpellate ovaries of *C. setiger* and *C. michauxii* which demonstrate their true monomerous nature (Sokoloff et al.,

2017). The gynoecium of *C. michauxii* was erroneously described as having three styles (Baillon, 1858; van Ee and Berry, 2009a; van Ee et al., 2011) making some authors interpret its unilocular ovary as pseudomonomerous (Gama et al., 2019). However, our examination reveals there is only one style on the ovary supporting its true monomery (Fig. 9F-G). It is clearly that these true monomerous ovaries in both species are derived from ancestors with tricarpellate ovary, because both taxa are nested among other *Croton* with typical ovary (Berry et al., 2005b; van Ee et al., 2011). However, ovaries of these two species are derived via different pathways. Ovaries of *C. setiger* have an anatropous ovule attached to the apical part of the carpel indicating that they evolved from ancestors with axile placentation which is found in the rest of *Croton* (Fig. 7J-K; 14). Fruits of *C. setiger* are also dehiscent which is a typical fruit character in *Croton* (Radcliffe-Smith, 2001; Berry et al., 2005b). On the other hand, ovaries of *C. michauxii* have an orthotropous ovule attached to the base of the carpel (basal placentation) different from all other *Croton* and perhaps all other Euphorbiaceae (Fig. 9F-G; 14). Within Rosids, unicarpellate ovaries with basal placentation similar to *C. michauxii* are found in Nyctaginaceae and Petiveriaceae which is likely to be derived from polycarpellate ancestor (Sattler and Perlin, 1982; Volgin, 1988; Ronse De Craene and Smets, 1991b; Bittrich and Kühn, 1993; Rohwer, 1993; Vanvinckenroye et al., 1993; Ronse De Craene and Stuppy, 2010). However, ovules in both families are curved (hemianatropous, anacampylotropous campylo-anatropous to campylotropous) which are different from the upright ovule (orthotropous) in *C. michauxii* (Ronse De Craene and Smets, 1991b; Bittrich and Kühn, 1993; Vanvinckenroye et al., 1993). Unicarpellate ovaries of *C. michauxii* produce indehiscent fruits which are unique within *Croton* (Pax, 1896; Webster, 1967, 1993; Radcliffe-Smith, 2001; Berry et al., 2005b; van Ee and Berry, 2009a; van Ee et al., 2011). Explosive capsular fruit types are common in the family Euphorbiaceae, but shifts to indehiscent fruits happened several times independently which may be linked to animal dispersal (Haegens, 2000; Esser, 2003; Wurdack et al., 2004, 2005). There is a report that ripe fruits of *C. michauxii* will fall to the soil (Ware, 2010). However, there is no mention of a seed disperser.

4.6 Conclusion

The androecium of *Croton* exhibits great variation in number and arrangement. The development of the outermost centrifugal antepetalous stamen whorl is likely to be an ancestral character of the genus. We found that a modification of floral structures gives rise to new patterns of stamen arrangement. An outermost whorl of alternipetalous stamens is observed in male flowers of *C. alabamensis* with hypanthium formation. In section *Moacroton*, the antepetalous centrifugal whorl is lost linked with a decrease of stamen number. In contrast, in *Croton* subg. *Geiseleria* with low stamen number the anterpetalous stamens are the first to develop. Increase of stamen number is linked with lower stability of stamen number within a whorl. An extreme case is found in *C. celtidifolius* with chaotic stamen arrangement and highly variable stamen number. Contrary to male flowers, the morphology female flowers of *Croton* is more stable. However, some variation was observed, e.g., the presence of fully developed petals, strong bilateral symmetry and reduced floral structures. In *Croton* subgenus *Geiseleria*, the reduction of carpel number happened three times independently. *Croton monanthogynus* has a pseudomonomerous bicarpellate ovary. Female flowers of *C. setiger* have a unicarpellate ovary surrounded by colleters. *Croton michauxii* has bilateral symmetrical female flowers with minute sepals and a nectary surrounding a unicarpellate ovary. Unicarpellate ovaries of *C. setiger* and *C. michauxii* are highly different with the presence of apical placentation and a simple style in the former and the presence of basal placentation and a twice-bifid style in the latter. Therefore, flowers of *Croton* exhibit great morphological diversity. However, only flowers from a small number of species were examined. Further study may reveal greater floral morphological diversity waiting to be discovered in the genus.

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Chapter 5: General conclusion

5.1 Evolution of the inflorescence and floral diversity in the Crotoneae and what causes the difference between them

Inflorescence and floral morphology in the tribe Crotoneae are highly diverse. In chapter 2, we used the technique of micro-computed tomography to conduct non-damaged examination of delicate flowers. Relationships between the Crotoneae and the Jatropheae have been discussed. Several synapomorphies of the tribe were identified, i.e., the presence of inflorescences with male terminal parts, reduced petals in female flowers, and more than 10 free stamens. Morphological characters to support internal relationships are also described. Botryoid inflorescences, recaulescent petioles, high stamen number, with the outermost whorl alternate to petals, are unique characters of the SS clade comprising *Sagotia* and *Sandwithia*. For the AABC clade, condensation and reduction of the cymule are present in *Astraea*, *Croton* and *Acidocroton*. *Brasilicroton* has unique paniculate inflorescences which could be derived from the elongation of the cymule primordia similar to the process that occurs in *C. rusbyi*. Moreover, the synapomorphies of *Astraea-Acidocroton* are also identified, i.e., the presence of colleters on female sepal margin and style with tetrafid or more branching pattern. We also discussed three hypotheses for the origin of petals in the core crotonoideae, i.e., staminodial origin, secondary sepal origin and retention of petals. Trichomes are exceptionally diverse in the genus *Croton,* ranging from stellate to lepidote to dendritic. We identify a possible link between simple trichomes and stellate trichomes via a pair of trichomes in the genus *Sandwithia*. The nectary of *Acidocroton* is unique among all genera in showing multiple lobes.

Croton is the most well-known genus in the tribe Crotoneae. It also has the highest species number contrary to other genera. Previously, apart from *Croton*, the floral morphology of other genera in the tribe was poorly known. Results from chapter 2 reveal that female flowers of all genera have highly conserved floral structures with the presence of five sepals, reduced petals, nectary lobes or ring, and tricarpellate

ovary with bifid styles. One major diversity is the branching pattern of style which occurs in *Acidocroton*, *Astraea* and *Croton*. The female flowers of *Sagotia* show the reduction of the nectary into a narrow ring which requires field observation and chemical tests to confirm its function as nectar secreting structure. Female flowers of *Acidocroton* have a unique system of multiple nectary lobes differing from female flowers of other genera which have generally five lobes. Contrary to female flowers, the androecium in male flower is highly diverse with variable stamen number, arrangement and form. *Sagotia* and *Sandwithia* have a high stamen number with the outermost stamen whorl alternate with petals. In the AABC clade, *Astraea* and *Croton* share the presence of inflexed stamens in bud with the outermost whorl opposite to petals. *Acidocroton* has curved stamen with the outermost whorl alternate with the petals. *Brasiliocroton* has erect stamens in bud with the outermost stamen whorl opposite to petals, but the outermost stamens could appear in pairs or be single. The analysis of results revealed that the ancestral androecial character of the tribe is likely to have more than 10 stamens with outermost stamens opposite to petals and stamen whorls developing simultaneously. The arrangement of outermost stamens alternates to petals in *Sagotia* and *Sandwithia* possibly links with an increase of stamen number in this clade, contrary to genera in the AABC clade with generally lower stamen number that have the outermost stamen opposite to petals (except *Acidocroton*). For the diverse forms of stamens occurring in the tribe, we thought that they are the result of an independent adaptation in each genus to pack a maximum stamen number in the limited space of the flower bud. The petal is another floral organ with variable morphology among all genera. Petals of *Sagotia* and *Sandwithia* are bigger than sepals at anthesis supporting their close relationship. Petals of *Astraea*, *Brasiliocroton* and *Croton* from the AABC clade have petals smaller than sepals at anthesis and share the presence of long trichomes on the margin. However, petals of *Acidocroton* are more similar to petals in the SS clade with bigger size than sepals at anthesis and lack of long trichomes on margin. So, it could be speculated that petals of *Acidocroton* may have been selected by similar ecological factors as in the petals from the SS clade. Moreover, Petals of *Astraea* possess unique moniliform trichomes on the lower part different from all other genera. *Croton* and *Brasiliocroton* share the presence of dense trichomes on the petal margin. The nectary in male flowers is generally conserved with

the presence of five lobes that could be separate or fused together. Male flowers of *Sagotia* are found to have lost the nectary, and this may be linked with its possible shift to a pollen feeding strategy accompanied with colour changing petals as possible signal for bees. Male flowers of *Acidocroton* have multiple nectary lobes as in female flowers but there is an expansion of the nectary into the inner receptacle area among the filament bases resulting in a honeycomb-like nectary which is highly uncommon among Euphorbiaceae and all angiosperms. The expansion of the nectary as well as the variation in lobe number support the interpretation that thenectary in both male and female flowers of all genera in this tribe is of receptacular origin. However, the function or benefit ofa nectary lobe increase could not be inferred from the present study. Further field observations of pollinator behaviour and chemical components of the nectar may reveal the ecological role of this character which may explain how it has evolved.

Therefore, the difference in inflorescence and floral structures in all genera in the tribe Crotoneae may be the result of diverse developmental process. Throughout the tribe, female flowers are much more conserved in structure as happens in the whorl family. In contrast, male flowers of this tribe show great diversity in androecium structure and organisation. Our findings suggested that the variable stamen arrangement in the tribe may be derived via modification of an ancestral opdiplostemonous arrangement. For the diversity of stamen form, we hypothesise that it may be caused by independent strategies of each genus to pack a high stamen number in a limited space. Moreover, the interpretation of nectary as of receptacular origin helps to explain the variability in nectary structure. However, the present study only provides information from a morphological and anatomical point of view. Further examination of floral ontogeny in all genera of the tribe together with field observatiosn of pollination biology may give us a more complete knowledge behind floral diversity of this group. Our investigation is the first step to understand and explain the complex diversity in this group which we hope will contribute to better knowledge of other Euphorbiaceous flowers.

5.2 The mechanism behind floral dimorphism in *Croton*

Male and female flowers of *Croton* show strong dimorphism especially in the presence of petals. In the third chapter we compare floral ontogeny between male and female flowers and also between female flowers with filamentous structures and female flowers with fully developed petals. Our results reveal that petals in male flowers and filamentous structures in female flowers have the same origin as bracteopetals. However, petals in each flower type undergo different developmental pathways. The difference in morphology between filamentous structures and fully developed petals is found to be caused by heterochrony. Petals primordia in female flowers are considered to be a paedomorphic form of petals in male flowers. The presence of fully developed petals in female flowers of *C. alabamensis* and *C. schiedeanus* is considered to be an independent reversal of this developmental process. However, the breakdown of developmental constraints should be treated as apomorphic tendencies instead of a homoplasy. Moreover, the nectary from both flower types also shares the late development on the same position suggesting the same origin. Previous studies suggested a staminodial origin of the nectary based on its position. However, our results reveal that the nectary in male flowers could have lability in number and position supporting a receptacular origin. Interestingly, male flowers included in the study also show unexpected diversity in stamen number, stamen arrangement and receptacle shape. We found that the character of the presence of an outermost stamen whorl opposite to petals, which is a characteristic of *Croton,* is derived via centrifugal development of the second stamen whorl. This phenomenon is more common in species with bisexual flowers and a high stamen number. The occurrence of this developmental process in the unisexual flowers of *Croton* with moderate stamen number is really unexpected. A concave receptacle is uniquely found in *C. alabamensis* and linked with the presence of the outermost alternipetalous stamens, contrary to other species. Therefore, the results from this chapter tell us that flowers of *Croton* are full of wonder in plain sight. Further examination in the groups of *Croton* with unusual flowers in the fourth chapter tells us more about the great floral morphological diversity in this genus.

5.3 Comparative floral morphology of the whole genus and the developmental basis behind some unusual *Croton*.

In the fourth chapter, we examine floral morphology of several unusual groups within *Croton*. In male flowers, diversity of stamen development is exceptionally high. In the third chapter we identified the centrifugal development of stamens in flowers with moderate stamen number. The presence of a centrifugally developing outermost antepetalous stamen whorl is likely to be an ancestral character of the genus since it is found in several species from different subgroups within *Croton* (chapter 3). In chapter 4, we examined flowers with unusual characters, e.g., low stamen number (< 10) and very high stamen number (> 50). When we examined species with low stamen number from the subgenus *Geiseleria*, it was found that stamens of these flowers only develop centripetally with the outermost stamens opposite to petals. Lower stamen numbers occur independently in several species from the subgenus *Geiseleria* suggesting parallel loss of centrifugal development. Low stamen number is also found in the section *Moacroton*, subgenus *Quadrilobi* (generally 2-6 stamens except in *C. poecilanthus*). Male flowers in this group could produce one full whorl of stamens at the maximum (six stamen). So, the centrifugally developing whorl is lost in this group since it requires at least two whorls of stamens to occur. Interestingly, the outermost stamen position is different between these two groups, alternipetalous in the section *Moacroton* vs. antepetalous in the section *Geiseleria*, suggesting different pathways of development. Additionally, we examined flowers with a high stamen number (more than 20). Stamens of this type of flowers develop rapidly causing conflict between stamens number and space availability, resulting in a chaotic arrangement of stamens in the flower bud.

Contrary to high androecial diversity in male flowers, gynoecial structures of female flowers are highly stable with the presence of three carpels. Independent reduction of carpel number is found in few species with different outcomes. In chapter 4, we examined three cases of carpel number reduction. *Croton monanthogynus* has a bicarpellate ovary but during the fruiting only on seed develops, suggesting a pseudomonomerous nature. For *C. setiger*, young stages of the flowers were observed helping to confirm its true monomerous nature. Moreover, the female flower of this

species is the simplest flower in the whole genus with on carpel and a simple style surrounded by several colleters. The last case is in *C. michauxii*, with previous literature recording the ovary of this plant to be pseudomonomerous. However, our examination is in contrast to previous observations in confirming the true monomerous nature of the flower. Moreover, the ovule of this plant is attached to the lower part of the locule (basal placentation), which is a unique character not just in *Croton* but in the whole family Euphorbiaceae. Other variations of female flowers are also found, e.g., regain of female fully developed petals in section *Alabamenses* and *Eluteria* (Chapter 3; 4) and strong bilateral symmetry in section *Julocroton* (Chapter 4).

Therefore, in the fourth chapter, most aspects of floral diversity in the genus *Croton* were explored. Hypothetical diagrams were constructed to explain the evolution behind the floral diversity in the genus. In male flowers, the presence of a centrifugal development of the second whorl of stamens is considered to be an ancestral character. We found that extreme change of stamen number is linkedd with new developmental pathways. Loss of centrifugal stamen development evolved independently in male flowers of different groups that have low stamen number (< 9), while rapid stamen development causing chaotic stamen arrangement is found in flowers with very high stamen number (>50). In female flowers, different independent developmental processes (pseudomonomery and true monomery) give rise to different forms of a reduced gynoecium in different species of *Croton*. Our findings emphasise the high floral diversity in the genus *Croton*. However, only a fraction of species has been studied. Further examination in the old-world clade of *Croton* may reveal other hidden floral diversity. We hope that our work is a step towards a better understanding of the great diversity in the family Euphorbiaceae.

5.4 Future prospects

5.4.1 Exploration of the floral diversity and evolution in the tribe Crotoneae with an eco-evo-devo approach

The present study explores floral diversity and evolution in the tribe Crotoneae from an anatomical, morphological, ontogenetic and phylogenetic perspective.

However, there is one aspect that is still mostly underexplored, i.e., ecology. Apart from *Croton*, there is no study dealing with the ecology of flowers in other genera in the tribe Crotoneae. In the present study, we could not explain the ecological function related to pollination of many floral structures, e.g., inaperturate pollen, colleters in sepals of *Sagotia*, *Astraea*, *Acidocroton* and many *Croton*, loss of a nectary in male flowers of *Sagotia*, colour change in flowers of *Sagotia* and *Astraea*, moniliform trichomes in *Astraea*, a nectary ring in *Sagotia* and *Acidocroton*, a honeycomb-like nectary in male flowers of *Acidocroton*, shift in stamen position in *Croton* and connective appendages in stamens of *Acidocroton*. Moreover, the present study examined herbarium- and alcohol-preserved sampled, so many data are missing, e.g., colour of floral structure, and chemical components of nectar, which may contribute to pollination process. Another interesting aspect is a developmental genetic study which could identify genetic factors that contribute to floral diversity in the tribe Crotoneae. Investigations of gene expression profiles of ABC genes which control the identity of floral organ may contribute to the understanding of the origin of some floral structures, e.g., filamentous structures and nectary. However, this approach requires fresh samples from living plants which could be challenging to obtain for most of genera. Field observation together with genetic studies of flowers will provide us with valuable data that could be combined with morphological evolution and development data from the present study (eco-evo-devo approach) to explain the evolution that creates great floral morphological diversity in the tribe Crotoneae.

5.4.2 Towards an understanding of the floral diversity in the Crotonoideae

The present study explores inflorescence and floral diversity and evolution within the tribe Crotoneae which belong to the subfamily Crotonoideae. This subfamily comprises four groups, e.g., Gelonieae, articulated crotonoid, clade C1 (Crotoneae and Jatropheae) and clade C2 (Wurdack et al., 2005; van Welzen et al., 2020). Clade C1 and clade C2 together form the inaperturate crotonoid clade with the presence of petals and inaperturate pollen as synapomorphies contrary to two basal clades with apetalous flowers (taxa from articulated crotonoid have petaloid sepals) (Wurdack et al., 2005). The present study and some previous studies examine

inflorescence and flowers of the tribe Jatropheae and Crotoneae (clade C1), but there is no comparative floral morphological study conducted in other groups of the Crotonoideae. Three possible hypothetical origins of petals in the core-crotonoideae were discussed in chapter two based on data from some genera in clade C1 and C2 but data from other groups are lacking. To test our hypothesis, it is important to perform comparative ontogenetic studies on a bigger scale. In the near future, we plan to investigate floral ontogeny in apetalous Crotonoideae, i.e., Gelonieae and articulated crotonoid, for better understanding of petal evolution in the core-crotonoideae. Reduction of petals is common among inaperturate crotonoid genera. The present study reveals that the reduced petals of *Croton* are derived via paedomorphosis compared to fully developed petals in male flowers. However, it is still unknown if the same mechanism occurs in other genera in the clade C2 or not (or in Gelonieae and articulated crotonoid if their flowers were derived from flowers with bipartite perianth). The origin of the floral nectary is another controversial subject with polarized opinion whether it has staminodial origin or receptacular origin (De-Paula et al., 2011). The present study found that the nectary in the Crotoneae shows lability in number and position supporting the receptacular origin (Chapter 2, 3). However, there are no other developmental data from other Crotonoideae genera available. So, we intend to fill the knowledge gap and find more evidence to clarify the nectary origin by examining the floral nectary morphology in different groups of Crotonoideae. Androecial morphology is another subject that is included in our future plan since it has great diversity in stamen form, stamen arrangement and development in the tribe Crotoneae. High androecial diversity is also observed in other groups within the Crotonoideae, e.g., merism change, increase or decrease in number and stamen column formation (Radcliffe-smith, 2001; Webster, 2014), but there are no ontogenetic data available. So, there is big gap of data needed to be filled which we plan to conduct in the near future. However, better understanding of character evolution requires well resolved relationships among all clades. Thanks to the improvement of the phylogenetic topology in recent years the mapping of characters on the phylogenetic trees is easier. However, there are still some inconsistencies in the position of some taxa in the phylogeny (Wurdack et al., 2005). We think that further phylogenetic reconstructions using the new technology of next generation sequencing would greatly

improve and resolve the question about relationship between different clade in the core-Crotonoideae.

5.4.3 Towards a comparative study of the whole Euphorbiaceae

In recent years, there are several floral ontogenetic studies conducted in Euphorbiaceae (Prenner and Rudall, 2007; Prenner, Box, et al., 2008; Prenner, Hopper, et al., 2008; De-Paula and Sajo, 2011; Gagliardi, 2018; Gagliardi et al., 2018; Gama et al., 2019; Tobias et al., 2019). However, these works only represent a fraction of the whole diversity in the Euphorbiaceae. Apart from the classic works of Baillon (1858) and Michaelis (1924), there are no wide ranging comparative floral morphological studies conducted in the family Euphorbiaceae. There are many cases of unusual floral morphology in the family that are still underexplored, e.g., branched stamens in *Homonoia*, allomorphic female flowers in *Acalypha*, obscure male structures in the tribe *Hippomaneae* and origin of petals in some genera in subfamily *Acalyphoideae*. New techniques of microcomputed tomography used in the present study is a good step to explore the floral diversity in the family. This technique allows us to examine samples without damaging them, so that rare or type specimens could be examined safely with this technique. We hope that in the near future we will be able to use this new technique combined with traditional methods to explore the great diversity in the family Euphorbiaceae.

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