

Floral ontogeny links *Dialypetalanthus* (Condamineae) with the floral developmental morphology of other Rubiaceae

Alexander Vrijdaghs^{1,2}, Petra De Block², Karen L.G. De Toni³, Erik Smets^{1,4}, Elmar Robbrecht²

¹ Laboratory of Plant Conservation and Population Biology, KU Leuven, Leuven, Belgium

² Meise Botanic Garden, Meise, Belgium

³ Rio de Janeiro Botanical Garden, Rio de Janeiro, Brazil

⁴ Naturalis Biodiversity Center, Leiden, The Netherlands

Corresponding author: Alexander Vrijdaghs (alexander.vrijdaghs@kuleuven.be)

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Abstract

Background – Vegetative and fruit characters of the Amazonian genus *Dialypetalanthus* point to a position in Rubiaceae. However, its floral morphology is so deviant that the genus was often placed in a family of its own. Even relationships outside Gentianales were postulated. Current molecular phylogenetic studies firmly show that *Dialypetalanthus* belongs to Rubiaceae.

Aims – This study aims to understand the idiosyncratic floral morphology in *Dialypetalanthus* and to compare it with the floral development in two other Condamineae genera as well as in other Rubiaceae for which ontogenetic data are available.

Material and methods – SEM and LM based floral ontogeny in *Dialypetalanthus fuscescens*, *Mussaendopsis beccariana*, and *Pogonopus exsertus*.

Results and main conclusions – Flowers in *Dialypetalanthus* develop a stamen-corolla-calyx tube, which can be considered as a floral morphological link between the genus and the other Rubiaceae. The polyandrous androecium originates from an annular intercalary meristem at the adaxial side of the stamen-corolla-calyx tube.

Keywords

Dialypetalanthus, floral cup, floral development, *Mussaendopsis*, *Pogonopus*, SEM, stamen-corolla tube, stamen-corolla-calyx tube

INTRODUCTION

Upon its recognition by Kuhlmann (1925), the monospecific Amazonian angiosperm genus *Dialypetalanthus* Kuhlmann was assigned to the family Rubiaceae. However, unlike its ovaries and fruits that fit well with Rubiaceae, the androecium and corolla are so deviant that many later authors postulated a monogeneric family Dialypetalanthaceae with an exceptional wide range of relationships in the orders Myrtales, Cornales, Rosales, or Gentianales. For references and a detailed overview see Piesschaert et al. (1997).

Flowers of Rubiaceae usually are sympetalous, actinomorphic and generally consist of a tetra- or pentamerous calyx and corolla, both often consisting of a tubular base with distal lobes, an androecium with a number of (often epipetalous) stamens usually equal to the number of calyx and corolla lobes. The gynoecium consists of an inferior, bilocular ovary, each locule with one to many unitegmic ovules, and a single style with two stigmas; on the top of the ovary, a gynoeceal annular nectary usually surrounds the base of the style (Robbrecht 1988). In contrast, the flowers of *Dialypetalanthus fuscescens* Kuhlmann are described as consisting of four free sepals, four free petals, and many stamens in two staminal

Table 1. Available floral developmental studies in Rubiaceae. Arranged according to the classification in two subfamilies (Robbrecht and Manen 2006; Antonelli et al. 2021).

CINCHONOIDEAE		
Coffeae	<i>Coffea</i> L.	Von Faber (1912); Van der Meulen (1939)
Octotropideae	<i>Canephora</i> Juss.	De Block and Vrijdaghs (2013)
RUBIOIDEAE		
Paederieae	<i>Paederia</i> L.	Vrijdaghs et al. (2015)
Knoxieae	<i>Pentas</i> Benth.	Vrijdaghs et al. (2015)
	<i>Sacosperma</i> G.Taylor	Vrijdaghs et al. (2015)
Spermacoceae	<i>Mitrasacmopsis</i> Jovet	Groeninckx et al. (2007)
	<i>Pentodon</i> Hochst.	Vrijdaghs et al. (2015)
	<i>Spermacoce</i> L.	Vrijdaghs et al. (2015)
Anthospermeae-Anthosperminae	<i>Galopina</i> Thunb.	Ronse Decraene and Smets (2000)
Rubieae-Theligoninae	<i>Theligonum</i> L.	Rutishauser et al. (1998)
Rubieae-Galiinae	<i>Asperula</i> L.	Payer (1857)
	<i>Asperula tinctoria</i> L.	Erbar and Leins (1996)
	<i>Crucianella</i> Boiss.	Vrijdaghs et al. (2020)
	<i>Cruciata</i> Opiz	Vrijdaghs et al. (2020)
	<i>Cruciata glabra</i> (L.) Ehrend.	Naghiloo and Classen-Bockhoff (2016)
	<i>Cruciata laevipes</i> Opiz.	Erbar and Leins (1996); Naghiloo and Classen-Bockhoff (2016)
	<i>Galium</i> L.	Payer (1857); Vrijdaghs et al. (2020)
	<i>Galium verum</i> L.	Erbar and Leins (1996)
	<i>Phuopsis stylosa</i> (Trin.) G.Nicholson	Erbar and Leins (1996); Vrijdaghs et al. (2020)
	<i>Sherardia</i> L.	Vrijdaghs et al. (2020)
Rubieae-Rubiinae	<i>Rubia</i> L.	Payer (1857); Vrijdaghs et al. (2020)
	<i>Rubia tinctorum</i> L.	Naghiloo and Classen-Bockhoff (2016)

whorls of which the filaments are basally fused. They have a bicarpellate, bilocular inferior ovary, each locule with a U-shaped placenta with many unitegmic ovules and a single style with two stigmas. There is no nectary. The ovary develops into a septicidal capsule with numerous winged seeds (e.g. Piesschaert et al. 1997; Figueiredo et al. 2017), features that are reminiscent of several rubiaceous genera, such as *Mitragyna* Korth. and other Naucleaeae.

In contrast to the confusing flower morphology, which was inconclusive as to the placement of the genus, all molecular phylogenetic studies showed that *Dialypetalanthus* belongs to Rubiaceae, Cinchonoideae sensu lato (Robbrecht and Manen 2006). Following Bremer and Eriksson (2009), many authors have considered three subfamilies in Rubiaceae, namely Rubioideae, Ixoroideae, and Cinchonoideae. However, recently, more support was found (Antonelli et al. 2021) for the approach of Robbrecht and Manen (2006), who recognised two subfamilies: Rubioideae, which concur with the Rubioideae sensu Bremer and Eriksson (2009), and Cinchonoideae, which include the Ixoroideae sensu Bremer and Eriksson (2009). Fay et al. (2000) found *Dialypetalanthus* in a clade with the New World taxa *Calycophyllum* DC., *Capirona* Spruce, *Condaminea* DC., *Hippotis* Ruiz & Pav., *Pentagonia* Benth., and *Pogonopus* Klotzsch, but

with a bootstrap support of less than 50%. Bremer and Eriksson (2009) presented a new and wide delimitation of the tribe Condamineae, comprising some 35 genera including the six mentioned above and *Dialypetalanthus*. Within this predominantly neotropical tribe, Kainulainen et al. (2010) suggested that *Dialypetalanthus* forms a well-supported clade together with the sister pair of the monospecific genera *Bothriospora* Hook.f. and *Wittmackanthus* Kuntze. However, Kainulainen et al. (2010: 1969) stated that the (floral) morphology within the *Dialypetalanthus-Bothriospora-Wittmackanthus* clade “is somewhat disparate, and no synapomorphies are known”. From literature, it can be concluded that a conflict remains between the floral morphological characteristics of *Dialypetalanthus* on the one hand and the firmly established phylogenetic position of *Dialypetalanthus* in Rubiaceae, tribe Condamineae on the other hand, a conflict that has not yet been resolved.

The floral development of only few species of Rubiaceae has been studied until now (see Table 1), and most of them belong to the subfamily Rubioideae starting with the historical work of Payer (1857) on *Asperula* L., *Galium* L., and *Rubia* L. Floral ontogenetic studies on Cinchonoideae are limited to *Coffea* L. (Von Faber 1912; Van der Meulen

1939) and a more recent publication on *Canephora* Juss. (De Block and Vrijdaghs 2013).

Piesschaert et al. (1997) provided a study, only based on herbarium material, of the floral ontogeny of *Dialypetalanthus*. Figueiredo et al. (2017) studied, using light microscopical observations, the development of the gynoecium in *Dialypetalanthus*. In this paper, based on light and scanning electronic microscopy, we present the results of a complete floral developmental study of *Dialypetalanthus*, including perianth and androecium. We compare the results on *Dialypetalanthus* with our floral ontogenetic observations of *Mussaendopsis* Baill. and *Pogonopus* Klotzsch, two genera of the Condamineae, a tribe in which Kainulainen et al. (2010) identified a large number of (sub)clades (Fig. 1). *Mussaendopsis* and *Pogonopus* were the only two genera of the tribe for which we could obtain sufficient material for a floral ontogenetic study. *Bothriospora* and *Wittmackanthus*, that form a clade with *Dialypetalanthus* as stated above, would have been better options, but we had no access to suitable flower material. *Pogonopus* (three species; POWO 2022) belongs to the “loculicidal clade”, one of the four clades of the crown group of Condamineae that form a polytomy. *Dialypetalanthus* (one species) belongs to the “septicidal clade”, and *Mussaendopsis* (three species; Puff and Igersheim 1994; Yamazaki 2001) belongs to the “Malesian-Pacific clade”, the only non-neotropical element of the Condamineae (Fig. 1).

According to Vrijdaghs et al. (2020), the variation in the commonly occurring tubular corolla and the presence (or not) of epipetalous stamens in all Rubiaceae species studied can be explained by four floral ontogenetic processes: 1) the development of a stamen-corolla tube from a common annular intercalary stamen-corolla meristem, 2) the development of a corolla tube sensu stricto from an annular intercalary corolla meristem, 3) fusion of petals, and 4) plastochron variation. In the present study, we aim to understand the idiosyncratic floral morphology in *Dialypetalanthus* and to compare it with the floral development in two related species of *Mussaendopsis* and *Pogonopus*.

MATERIAL AND METHODS

Collections were made in ethanol 70%. Partial inflorescences and floral buds of *Dialypetalanthus fuscescens* at all developmental stages were sampled by Karen De Toni from a tree in the Rio de Janeiro Botanic Garden, Brazil, and are preserved at Meise Botanic Garden, Belgium. Samples of *Mussaendopsis beccariana* Baill. came from the alcohol collection of Meise Botanic Garden and *Pogonopus exertus* (Oerst.) Oerst. was collected from the living collection of Meise Botanic Garden. For voucher data, see Table 2. Dissection was performed in ethanol 70%.

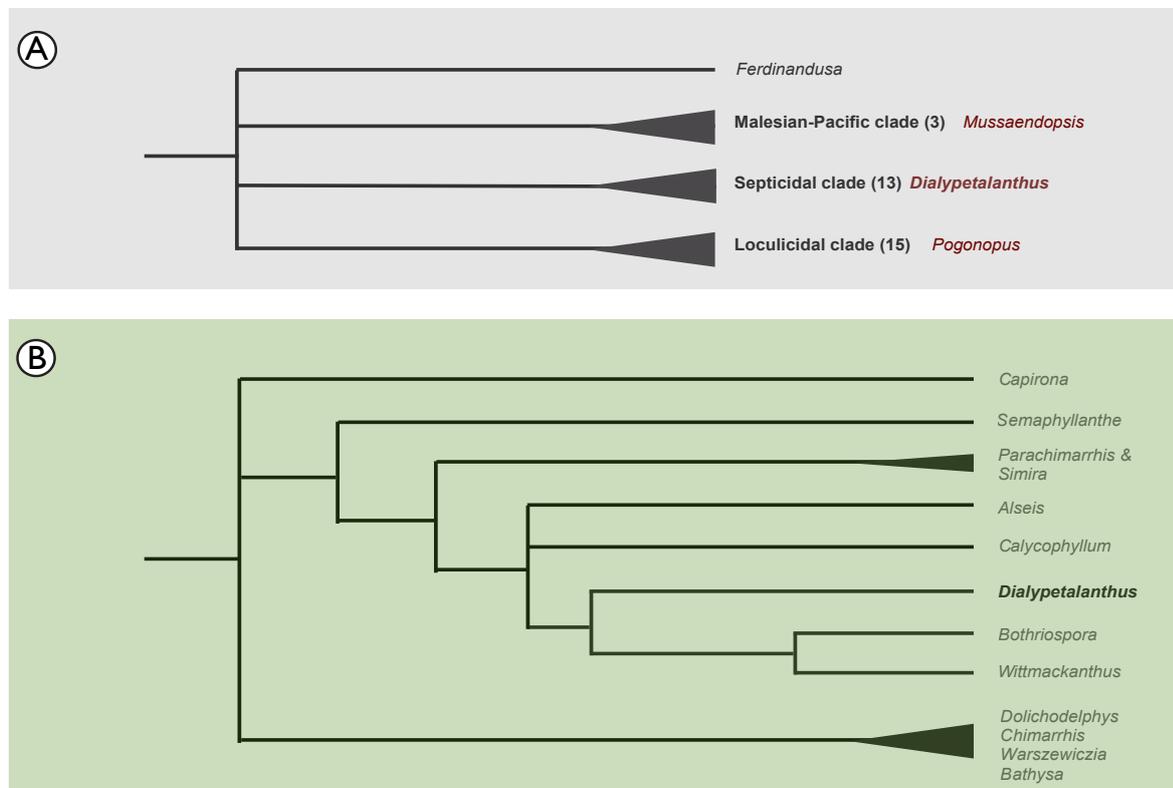
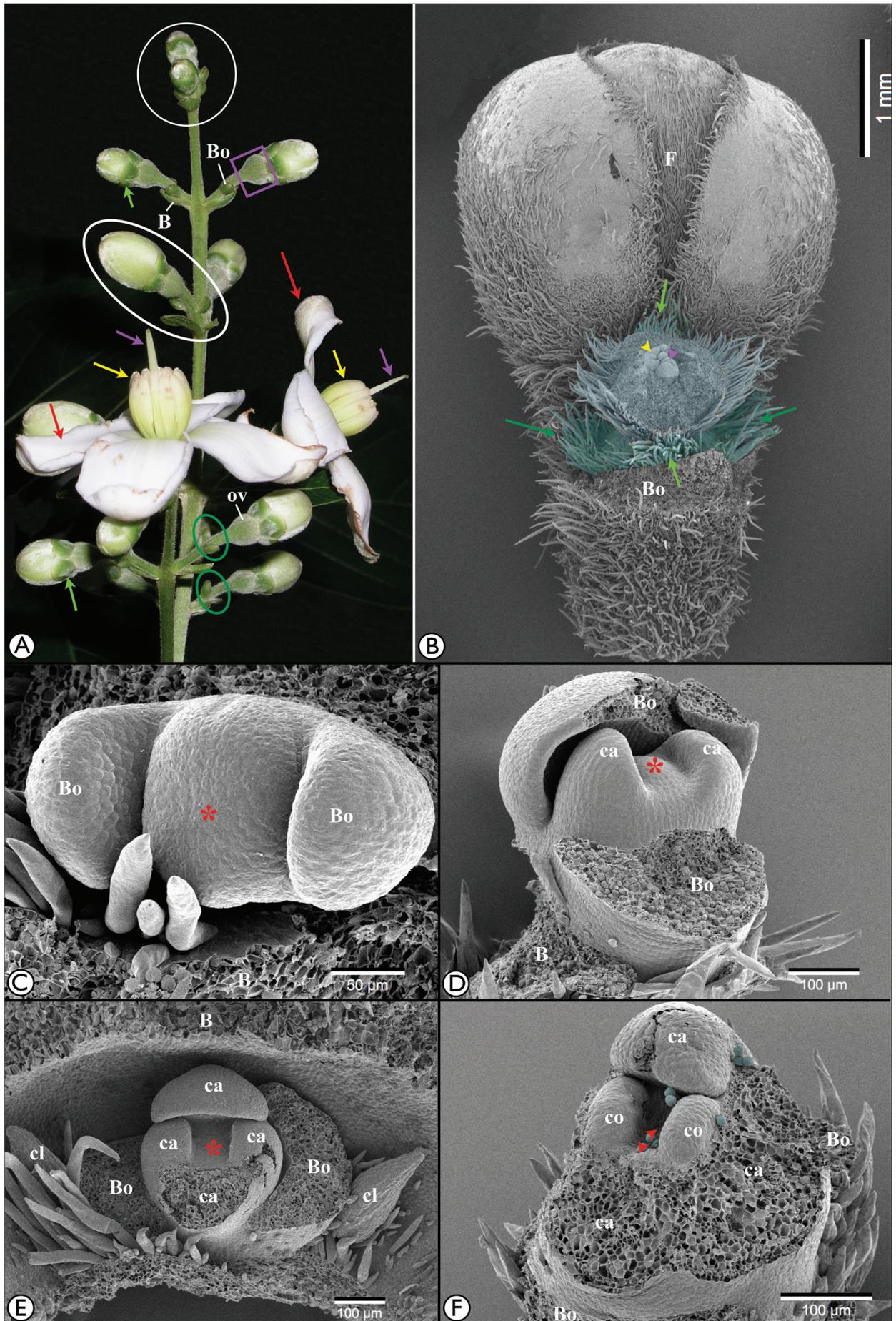


Figure 1. Simplified cladogram of the crown group of tribe Condamineae (Rubiaceae) after Kainulainen et al. (2010). **A.** Crown group (numbers between brackets = number of genera). **B.** Septicidal clade with *Dialypetalanthus*.



◀ **Figure 2.** Macrograph (A) and SEM images (B–F) of inflorescence (A–B) and successive stages of the early floral development (C–F) in *Dialypetalanthus fuscescens*. A and B: lateral view. C and E: apical view. D and F: lateral-apical view. **A.** Inflorescence axis with flowers in bud and at anthesis with calyx lobes, corolla lobes, androecium, and style indicated (resp. light green, red, yellow, and purple arrows). Pedicel of each flower with two opposite, possibly slightly displaced with respect to each other, bracteoles (encircled in dark green). Encircled in white the distal part of the main inflorescence axis and lower, an inflorescence unit. Framed in purple the inferior, bilocular ovary. **B.** Cymosely branched inflorescence unit with a terminal flower (F); scar of one of its bracteoles (Bo) subtending an inflorescence unit consisting of a developing lateral flower with two bracteoles (dark green arrows), a hairy calyx (light green arrows) and inner flower (blue) with centrally the appearing style (purple arrowhead) surrounded by stamens (yellow arrowhead). **C.** Flower primordium and two bracteoles. **D.** First two sepals appearing, opposite to each other and at 90° with respect to the pair of bracteoles. **E.** Four sepals, two by two, surrounding a flat central floral apex. The most recent pair of sepals opposite the bracteoles. **F.** Appearance of a first pair of petals, at 90° with respect of the latest developed pair of sepals. The floral axis (red arrow), situated in the centre of a cavity formed by the upwards growth of the bases of the surrounding floral parts. Colour code: blue, developing inner flower–pollen grains; green, calyx/bracteoles; purple, gynoecium; red, corolla; yellow, androecium. Symbols: B, bract; Bo, bracteole; ca, calyx (lobe); cl, colleter; co, corolla (lobe); F, flower.

Table 2. Voucher data of species studied.

Species	Collector and year	Origin	Preserved at
<i>Dialypetalanthus fuscescens</i> Kuhlm.	Karen De Toni 2017	Rio de Janeiro Botanical Garden, Brazil	RBv 7855, RB 462363
<i>Mussaendopsis beccariana</i> Baill.	Christian Puff, Anton Igersheim, and Gustavo Martinelli 1990	Sungai Liang Arboretum, Brunei	BR 900885/1/1
<i>Pogonopus exsertus</i> (Oerst.) Oerst.	Petra De Block 2020	Meise Botanic Garden, Belgium	BR 20180855-05

For SEM, in preparation to critical point drying, the dissected material was gradually brought from ethanol 70% to a 1:1 mixture of ethanol 70% and dimethoxymethane (DMM) and subsequently to pure DMM. Next, the material was critical point dried by gradually replacing the DMM by liquid CO₂ with the aid of a Balzers CPD 030 critical point dryer. The dried samples were mounted on aluminium stubs using carbon adhesive tape and sputter coated with gold with a Balzers SCD 020 sputter coater. SEM images were obtained with a JEOL JSM5800-LV scanning electron microscope at the laboratory of Plant Conservation and Population Biology, KU Leuven, Belgium.

For LM, the samples were gradually dehydrated through an ethanol series and subsequently embedded in KULZER's Technovit 7100 (based on HEMA, hydroxyethyl-meth-acrylate). Seven µm thick sections were obtained with the help of a rotary microtome Leica RM2135 with disposable blades (Leica DB80). Subsequently, the sections were stained in a 0.1% toluidine blue in aq. dest. solution. Observations were done using an Olympus BX51 microscope equipped with a Color View Soft Imaging System camera at Meise Botanic Garden.

RESULTS

Dialypetalanthus fuscescens

Inflorescence axes show decussate branching, the lateral axes occurring two-by-two, subtended by pairs of opposite

bracts. Each pair of bracts and lateral axes is at 90° with respect to the preceding pair. This pattern is continued by the inflorescence unit/flower subtending bracts, also occurring in pairs that are perpendicular to each other (Fig. 2A). The pedicel of each flower has two opposite bracteoles which may be slightly displaced with respect to each other (Fig. 2A). Bracteoles may each subtend (or not) a lateral flower (Fig. 2B), forming together with the first/terminal flower a cymosely branched inflorescence unit (Fig. 2B). Each flower originates from a flower meristem in the axil of a bract(eole) (Fig. 2B–F). Flower primordia are initially covered by a pair of bracteoles (Figs 2B, C, 5A). All perianth parts appear pairwise, continuing the decussate organisation of the inflorescence within the flower. First, two pairs of opposite sepals appear consecutively (Figs 2D, E, 5B), followed by two pairs of opposite petals (Figs 2F, 3A). Within the bract that subtends a flower/inflorescence unit, hairs and colleters develop (e.g. Fig. 2E). Simultaneously, in the flower a floral cup develops (Figs 3A, B, 5C), raising the calyx and corolla. Simultaneously, at the adaxial side of the central floral cup, one by one individual stamen primordia appear in centripetal order (Fig. 5D–F), eventually resulting in two ‘whorls’ of stamens, the distal stamens more developed relative to the proximal ones (Figs 3B, C, 5E). With the proximal stamens appearing, at the bottom of the central depression, two bulges appear surrounding a cavity (Figs 3C, 5E). The bulges develop into a single style with two stigmatic branches (Fig. 3D–H) and simultaneously two locules are formed (Figs 3E, 4A–E), each with a U-shaped placenta with multiple unitegmic ovules (Fig. 4A–E).

Simultaneously with the development of the gynoecium, the initial stamen primordia develop into short filaments and basifixed, tetrasporangiate, introrse anthers (Fig. 4A, B, D, F). The bases of the filaments of the stamens are fused (Fig. 3I). Later in the development, a dense whorl of hairs is formed at the base of the single style (Fig. 4F).

Pogonopus exertus

In a first stage of the floral development, a calyx with abaxially large trichomes and five petals develop, the latter united at the base (Fig. 6A, B). Simultaneously, five stamens develop (Fig. 6A, B, E, H, I), initially with short

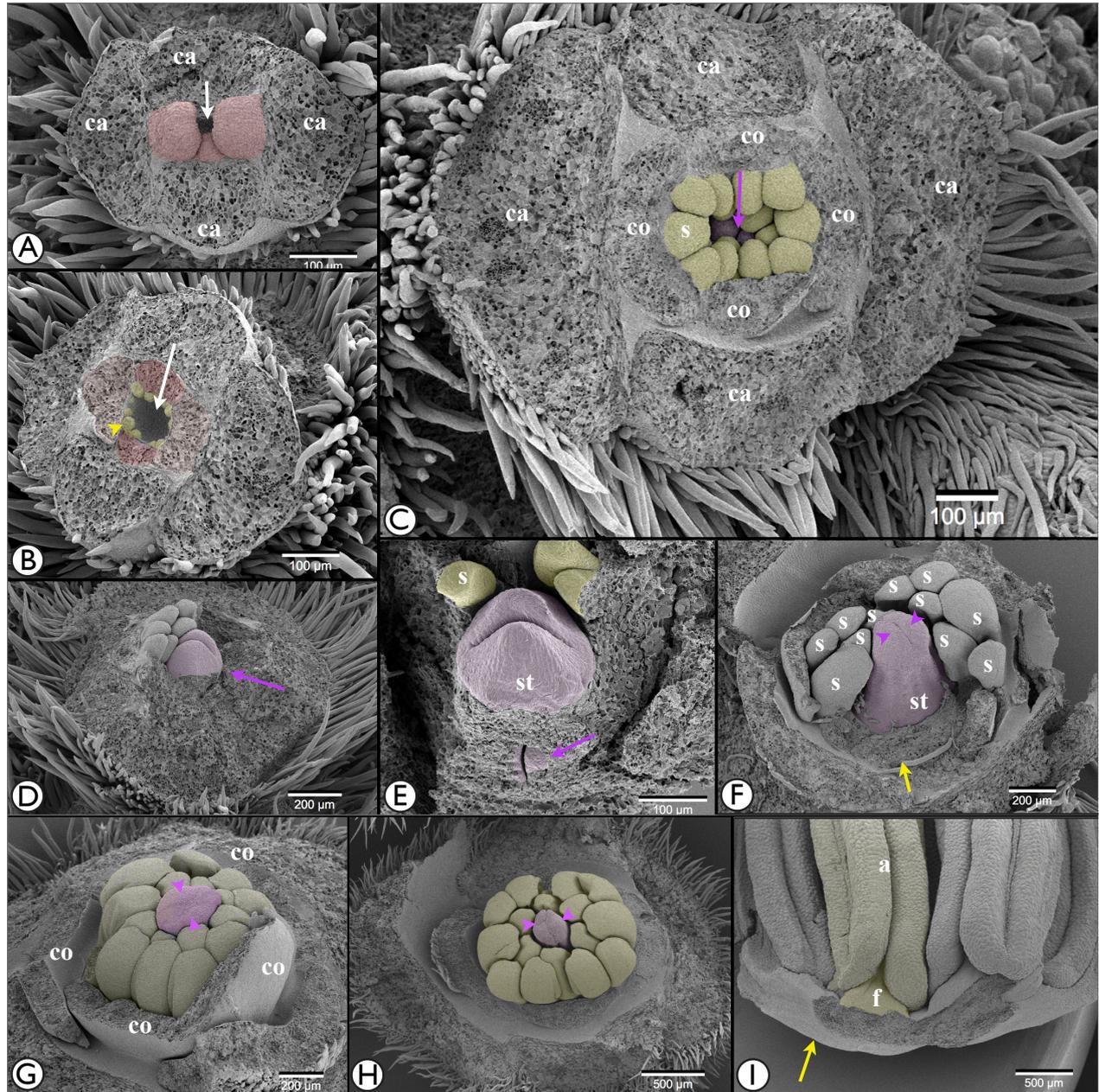


Figure 3. SEM images of successive stages of the floral development in *Dialypetalanthus fuscescens*. A–H: apical view, I: lateral view. A. Floral apex (white arrow) becomes a central cavity, surrounded by a second pair of opposite petals, now called corolla lobes at 90° with respect to the previous pair. Sepals are from now onwards called calyx lobes. B. Appearance of a row of stamen primordia (one indicated by yellow arrowhead) on the distal part of the apical central cavity (white arrow). C. Centripetally of the developing stamens, a second ‘whorl’ of stamen primordia appears. Floral apex differentiates into two bulges surrounding a central split (purple arrow). D. The central bulges are raised from a common base (purple arrow), consisting of a single style. E. Below the single style, one out of two locules filled up with a U-shaped placenta (purple arrow). F. Developing style with two stigmas (purple arrowheads). Surrounding the base of the style, a scar of removed stamens shows a common androecial base (yellow arrow). G–H. Variable number of stamens (16–17). Single style/stigmas (purple arrowheads) protruding above developing stamens. I. Stamens with a common base (yellow arrow). Colour code: purple, gynoecium; red, corolla; yellow, androecium. Symbols: a, anther; ca, calyx (lobe); f, filament; cl, colleter; co, corolla (lobe); s, stamen; st, style.

filaments. The dorsifixed anthers are tetrasporangiate and introrse and surround the equally developing single style with two stigmatic branches (Fig. 6H, I). With the anthers well developed, a stamen-corolla tube starts developing fastly (Fig. 6C–F) as well as a corolla tube sensu stricto (Fig. 6C–F), which raises the initial petals, now corolla lobes. A dense whorl of trichomes at the bases of the filaments gradually develops, separating the stamen-corolla tube and corolla tube sensu stricto (Fig. 6B–F). Until semi-maturity, the filaments stretch somewhat slower than the corolla tube sensu stricto (Fig. 6C–G). Meanwhile a four-lobed annular nectary surrounding the base of the single style develops (Fig. 6I–K), and below it two locules, each with a U-shaped placenta (Fig. 6I, K) with multiple unitegmic ovules (Fig. 6K, L).

Mussaendopsis beccariana

In (semi-)mature flowers, a calyx is present consisting of a short calyx tube and five calyx lobes (Fig. 7A, B). Alternating with the calyx lobes, five free petals are inserted on the hypanthium (Fig. 7A, B). Alternating with the petals, five stamens with dorsifixed, tetrasporangiate and introrse anthers are inserted on the hypanthium, ‘below’ the protruding rim of a conspicuous annular nectary that surrounds the base of a single style (Fig. 7B, C). Below the nectary, the inferior ovary consists of two locules, each with a U-shaped (not shown) placenta with multiple unitegmic ovules (Fig. 7C). At maturity, the stamens are stretched with a long filament (Fig. 7D). The anther is curved on top of the filament, releasing pollen through longitudinal slits (Fig. 7D, E). The style ends in two short stigmatic branches (Fig. 7F).

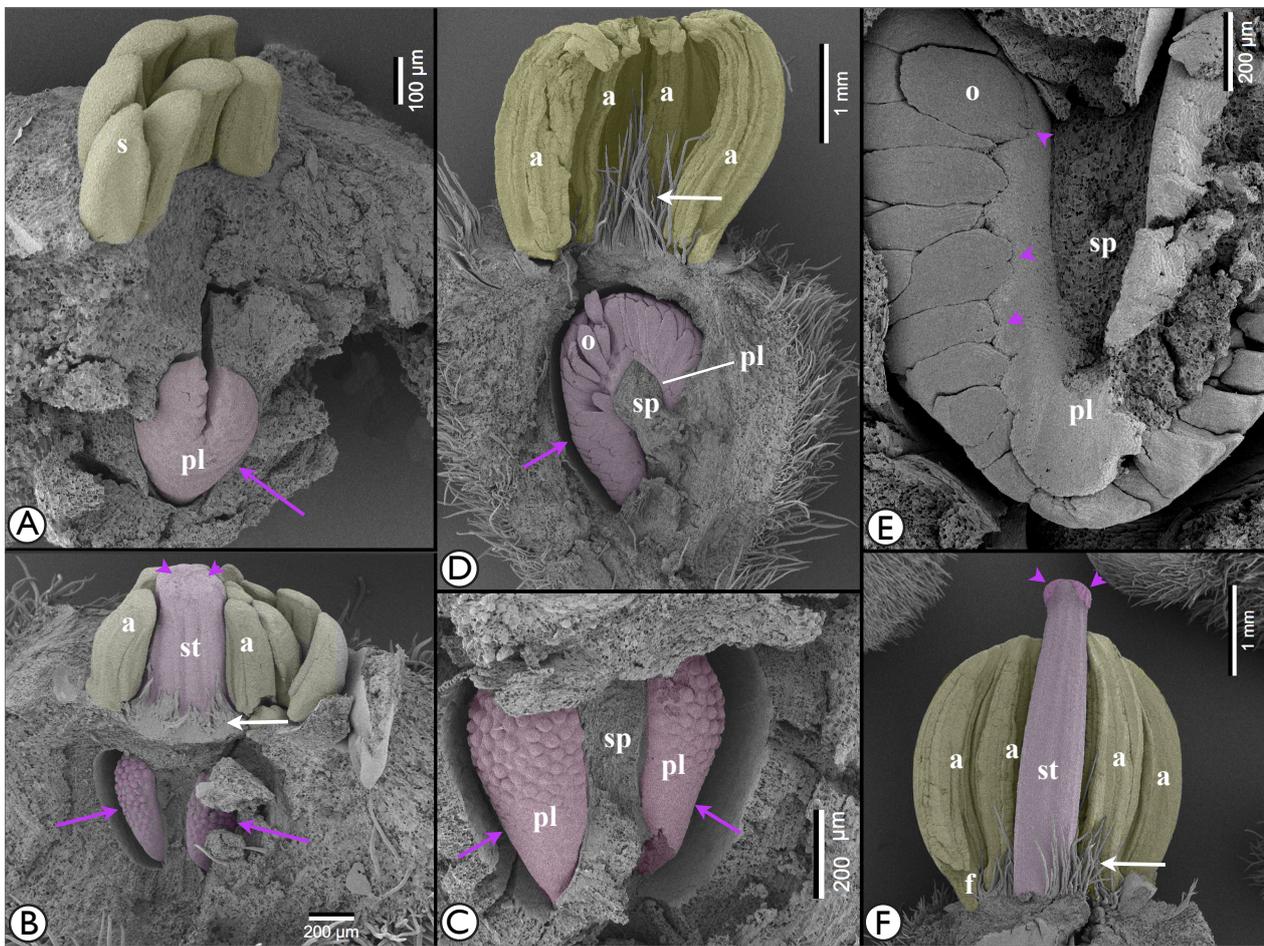


Figure 4. SEM images of successive developmental stages of the gynoecium in *Dialypetalanthus fuscescens*. All images are lateral views. **A.** Opened locule with U-shaped early placenta with multiple ovule primordia. **B–E.** Idem, ovules develop top to bottom. **B–C.** Side view on U-shaped placenta. **B.** Developing single style starting to protrude above the stamens, its base surrounded by a whorl of trichomes. **D.** Adaxial view on placenta with distally semi-mature ovules. **E.** Adaxial view on placenta with unitegmic mature ovules (purple arrowheads). **F.** Semi-mature single style and stigmas (purple arrowheads). Colour code: purple, gynoecium; yellow, androecium. Symbols: a, anther; f, filament; pl, placenta; s, stamen; sp, septum; st, style; purple arrow, placenta; white arrow, whorl of hairs at style base.

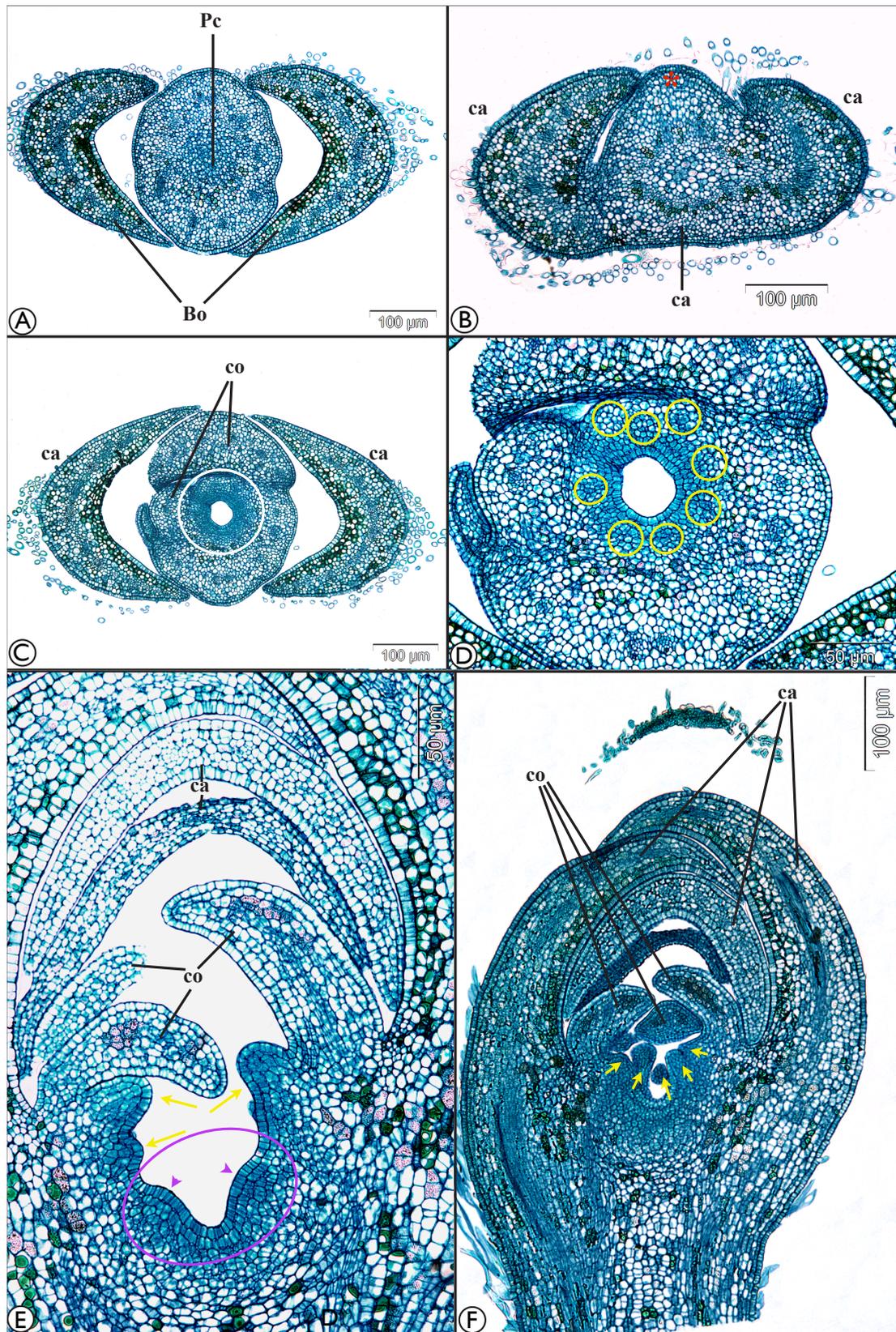


Figure 5. LM images of transverse (A, C, D) and longitudinal sections (B, E, F) through developing flowers of *Dialypetalanthus fuscescens*. A. Through pedicel at height of bracteoles. B. Through early floral developmental stage. C. Through successive floral developmental stage with central depression (encircled in white). D. Detail of central depression with early stamens (encircled in yellow). E–F. Through developing flower, with central depression and apical cavity (encircled in purple) with developing stamens (yellow arrows) surrounded by two originating carpillary bulges (purple arrowheads). Colour code: purple, gynoecium; yellow, androecium. Symbols: Bo, bracteole; ca, calyx (lobe); co, corolla (lobe); Pc, pedicel; red asterisk, floral apex.

DISCUSSION

Dialypetalanthus

The inflorescence is essentially decussate and dichasial, with successive pairs of opposite bracts, each subtending a lateral inflorescence axis (Figs 2A, 8A). According to Piesschaert et al. (1997: 205), each inflorescence axis ends in a terminal bud: "...that at first sight may be confused with a single terminal flower. Dissection... reveals a floral meristem where acropetal inceptions of lateral flowers occurs". In our opinion, this implies that each branch eventually must end in a terminal flower, though Piesschaert et al. (1997) also mentioned that the distal flowers are poorly developed. We conclude that each branch ends in an underdeveloped cymosely branched inflorescence unit. In a typical Rubiaceae inflorescence, usually two oppositely situated bracteoles occur on the pedicel of each flower. From a floral ontogenetic viewpoint, the term 'bract' has a relative meaning as it refers to any appendage that subtends a vegetative or generative axis. In this sense, in Rubiaceae, each bracteole on the pedicel of the first flower of an inflorescence unit may act (or not) as a bract by subtending a lateral flower (Weberling 1992; Robbrecht 1988). As a result, such an inflorescence unit ideally consists of a first or terminal flower with a younger, lateral flower in each of its bracteoles. In *D. fuscescens*, similar inflorescence units occur (Figs 2B, 8A).

Flowers in *D. fuscescens* at first view show a quite idiosyncratic development, compared to other Rubiaceae, as already mentioned by Piesschaert et al. (1997). In Rubiaceae, a flower is mostly 4- to 5-merous, and usually develops a calyx tube with lobes (the calyx may appear late in the development or not at all), a tubular corolla with distinct lobes, epipetalous stamens in the same number as the calyx and corolla lobes (Fig. 8B, left). In contrast, our present study as well as previous studies (e.g. Piesschaert et al. 1997) show that in *Dialypetalanthus*, during early development, it is difficult to distinguish between the bracteoles and the first perianth parts, all of which appear as similar pairs of opposite scales (Fig. 2). In total, five of such pairs are formed, which in the course of the floral development reveal their 'identity' as bracteoles (one pair), sepals (two pairs), and petals (two pairs), respectively. The bracteoles soon show a slight metatopic displacement with respect to each other (Fig. 2A, E–G). The four sepals and four petals eventually seem to constitute two whorls, respectively a greenish calyx of four small sepals and a corolla of four white petals (Fig. 2A, F), instead of the four whorls of two perianth parts each as described by Piesschaert et al. (1997). Whether the calyx and corolla each consist of two whorls of two members, or of one whorl of four members is rather semantic; at anthesis, the positions and identities of the four petals and sepals suggest two whorls, despite the pairwise development of the perianth members. In this context, we refer to the perianths in e.g. Amaranthaceae

but also in many other families, where the five perianth part primordia have a quincuncial aestivation (sequence of appearance of perianth parts and consequent arrangement). Nevertheless, they are considered to constitute one whorl (e.g. Flores Olvera et al. 2011). In that sense, *Dialypetalanthus* can be compared with the corolla in some Papaveraceae and the calyx in Brassicaceae with four sepals, where the apparent tetramery results from a rather dimerous or successive appearance of floral primordia (e.g. Payer 1857: 210, 218, plates 44, 48). On the other hand, the apparent dimery in *Dialypetalanthus* seems systemic in the vegetative as well as the generative parts of the plant, which suggests a deep regulatory origin which goes beyond so-called isomeric changes (Ronse De Craene 2016) within the flower. Without a doubt, in *Dialypetalanthus*, the opposite arrangement of petals and sepals contributes to the atypical floral morphology for a genus in Rubiaceae.

Moreover, during floral development, the initially slightly convex floral apex (Fig. 2B) becomes conspicuously concave. Actually, a floral cup sensu Leins and Erbar (2010) is formed by the development of a stamen-corolla-calyx tube (Figs 3A, 8B right; see also figure 99 in Leins and Erbar 2010: 102). Sepals and petals are lifted up by the development of this stamen-corolla-calyx tube. We consider the presence of a stamen-corolla-calyx tube to be the morphological link, hitherto missing, with the other Rubiaceae, since strictly speaking, the sepals should be seen as calyx lobes and the petals as corolla lobes. Hence, it can be argued that also in *Dialypetalanthus*, there are no free sepals nor petals since the sequence of appearance and subsequent arrangement of the calyx and corolla lobes is not related to the appearance of individual perianth part primordia. The pairwise sequence of appearance may be due to spatial constraints.

During the development of the perianth lobes, individual stamen primordia appear in a more or less centripetal order at the adaxial side of the central floral cup (Figs 3B–C, 8B right). Eventually, two 'whorls' of stamens develop and fill up the initial floral cup. There is no visible primary androecium primordium but individual stamen primordia seem to originate from an annular meristem surrounding the floral apex. Initially, we were inclined to interpret the androecium as consisting of four parts, each one positioned below the base of a corolla lobe (petal) upon the common stamen-corolla-calyx socle. However, our observations do not support this hypothesis (Fig. 3). Moreover, Piesschaert et al. (1997) already mentioned that the androecium falls off as a whole. Since indeed, all stamens appear to be connected to each other at the very base of the filaments, we conclude that they originate from an annular meristem at the inner (adaxial) side of a short stamen-corolla-calyx tube. The fact that at later developmental stages there are two 'whorls' of stamens is rather a consequence of the spatial constraints within the central cavity in the flower. Consequently, although the stamens seem free-standing at first glance, they should be considered as epipetalous on the stamen-corolla-calyx



◀ **Figure 6.** SEM images of stages of the floral development in *Pogonopus exsertus*. A–L: lateral views. **A.** Longitudinally opened developing flower. **B.** Tetrasporangiate introrse anther on short (invisible) filament. **C.** Adaxial view of part of tubular corolla; encircled in red, the haired rims of adherent corolla lobes. Proximally, a ‘whorl’ of hairs between stamen-corolla tube and corolla tube sensu stricto. **D–E.** Scar of filament at adhesion point of an epipetalous stamen at the tubular corolla. Below the scar, the stamen-corolla tube grows faster than the corolla tube sensu stricto above the scar. **E.** Longitudinal section of developing flower. An annular nectary (purple arrows) developing at the base of a single style with two stigma branches. **F–G.** Adaxial view of part of a semi-mature tubular corolla with two out of five epipetalous stamens. **F.** Proximal part. **G.** Distal part; the corolla lobes (one indicated by red triangle) with hairy margins. **H–L.** Development of the gynoecium. **H.** Developing single style with two style branches. **I.** Same stage, longitudinally opened flower with opened locule with U-shaped placenta. **J–K.** Successive stage with developing annular nectary surrounding the style base. Below the nectary, at right hand side, developing ovules. **K.** Idem from more apical view. Developing ovules encircled in purple. **L.** Developing ovules, detail of unitegmic ovule in frame. Obviously unitegmic ones indicated by purple arrowheads. Colour code: purple, gynoecium; red, corolla; yellow, androecium. Symbols: a, anther; ca, calyx (lobe); co, corolla (lobe); f, filament (scar of); ne, nectary; o, ovule; pl, placenta; s, stamen; sg, stigma; st, style; red double arrow, corolla tube sensu stricto; red-yellow double arrow, stamen-corolla tube.

tube. If we consider the usual epipetaly in Rubiaceae with stamens positioned on a stamen-corolla tube as epipetaly sensu stricto, the epipetaly in *Dialypetalanthus* can be considered as another character state (Ochoterena et al. 2019).

The polyandry exhibited by *D. fuscescens* can theoretically find its origin in either ‘dédoulement’ of initially individual stamen primordia or polygenesis

(Ronse De Craene 1988; Ronse Decraene and Smets 1993), or by the development of an annular primary androecium primordium, from which secondarily stamen primordia originate. In the first case, one might expect that the stamens are grouped in four ‘groups’ of stamens, the position of each corresponding to the position of the initial stamen. Our results show that this is not the case. In contrast, our observations suggest that the

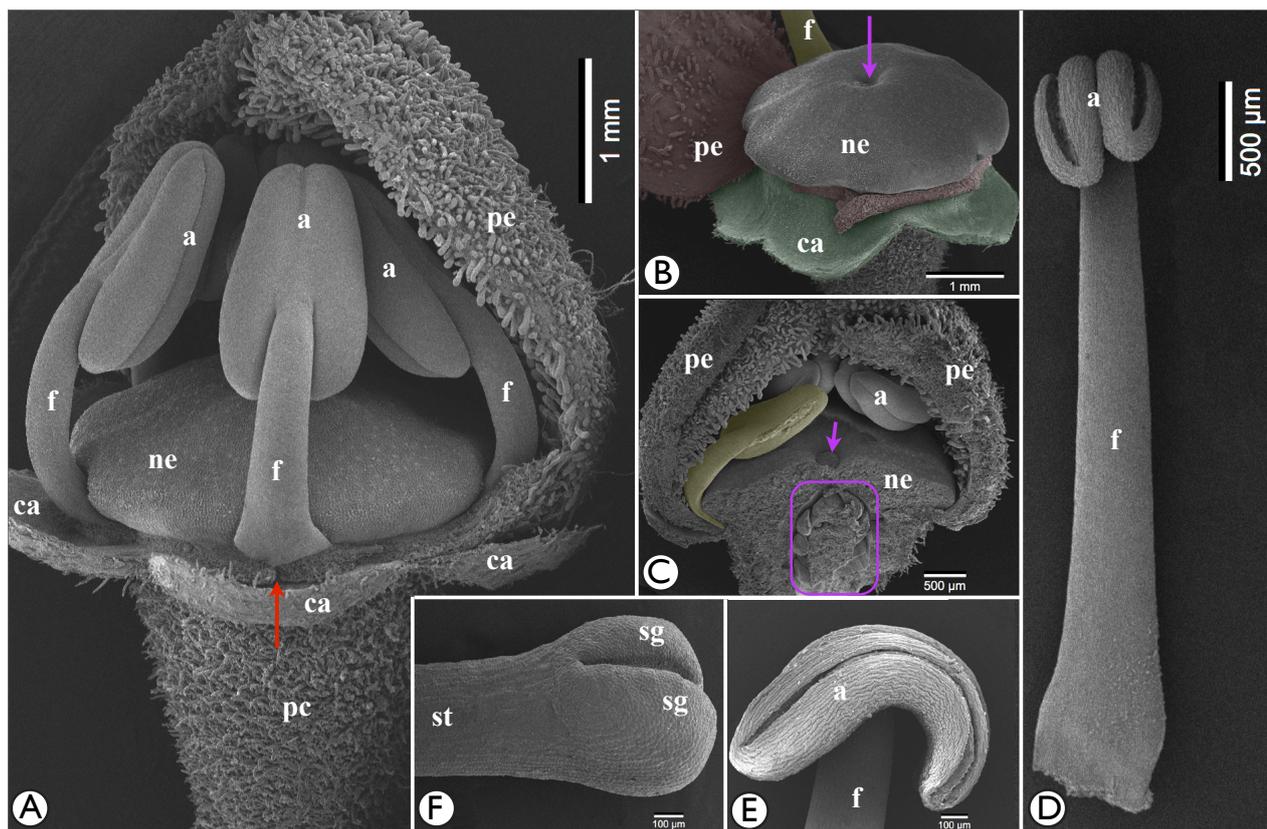


Figure 7. SEM images of stages of the floral development in *Mussaendopsis beccariana*. A–F: lateral views. **A–B.** Semi-mature flower with part of corolla removed. Calyx consisting of a calyx tube and calyx lobes. Corolla consisting of free petals embedded in the hypanthium (red arrow). Five stamens with tetrasporangiate dorsifixed and introrse anthers. Conspicuous annular nectary surrounding the style base (scar of removed style indicated by purple arrow). **C.** Longitudinally opened semi-mature flower with encircled in purple a placenta with multiple ovules filling up one out of two locules. Centrally in the flower, scar of removed style indicated by purple arrow. **D–E.** Mature stamen with stretched filament, longitudinally opened tetrasporangiate dorsifixed and curved anther. **E.** Detail of anther. **F.** Detail of style and two stigmas. Colour code: green, calyx; purple, gynoecium; red, corolla; yellow, androecium. Symbols: a, anther; ca, calyx (lobe); f, filament; ne, nectary; pc, pedicel; pe, petal; sg, stigma; st, style.

many stamens in *D. fuscescens* originate from an annular meristem on the adaxial flanks of the central floral cup (which can also be considered as a primary androecium primordium since a particular structure, the central floral cup, is formed preceding the appearance of the stamens). The androecial development in *D. fuscescens* resembles the development of the numerous perianth parts, often called silky hairs, in flowers of the Cyperaceae genus *Eriophorum* (Vrijdaghs et al. 2005), which also originate one by one and centripetally, from an annular perianth meristem. Apparently, within angiosperms, there is a potential to switch from a limited default number of individual floral part primordia within a whorl to a common annular meristem/primary primordium from which a large number of floral parts secondarily develops.

Only after the appearance of most stamens, the development of the gynoecium starts, similar as in other Rubiaceae (De Block and Vrijdaghs 2013; Vrijdaghs et al. 2020), though without gynoeical nectary (Fig. 3C; Figueiredo et al. 2017). Perhaps, investing in polyandry

instead of a nectary gives an ecological advantage to *Dialypetalanthus*, but this is a subject for another study.

Mussaendopsis beccariana and *Pogonopus exsertus*

The floral morphology in *M. beccariana* differs from that in most Rubiaceae studied by the absence of a tubular corolla and epipetaly. However, in contrast to the flowers of *Dialypetalanthus*, the flowers of *M. beccariana* have floral features that are considered to be common in Rubiaceae, such as a calyx tube, the same number of stamens as corolla lobes (no polyandry) and the presence of an annular nectary surrounding the base of the single style.

The development of the flower in *P. exsertus* concurs with the floral development as described by Vrijdaghs et al. (2020) for *Crucianella* Boiss. and *Phuopsis* (Griseb.) Hook.f., both genera belonging to the Rubioideae-Rubieae. The floral ontogeny in *P. exsertus* is characterised by the development of a stamen-corolla tube as well as a corolla tube *sensu stricto* and corresponds with figure

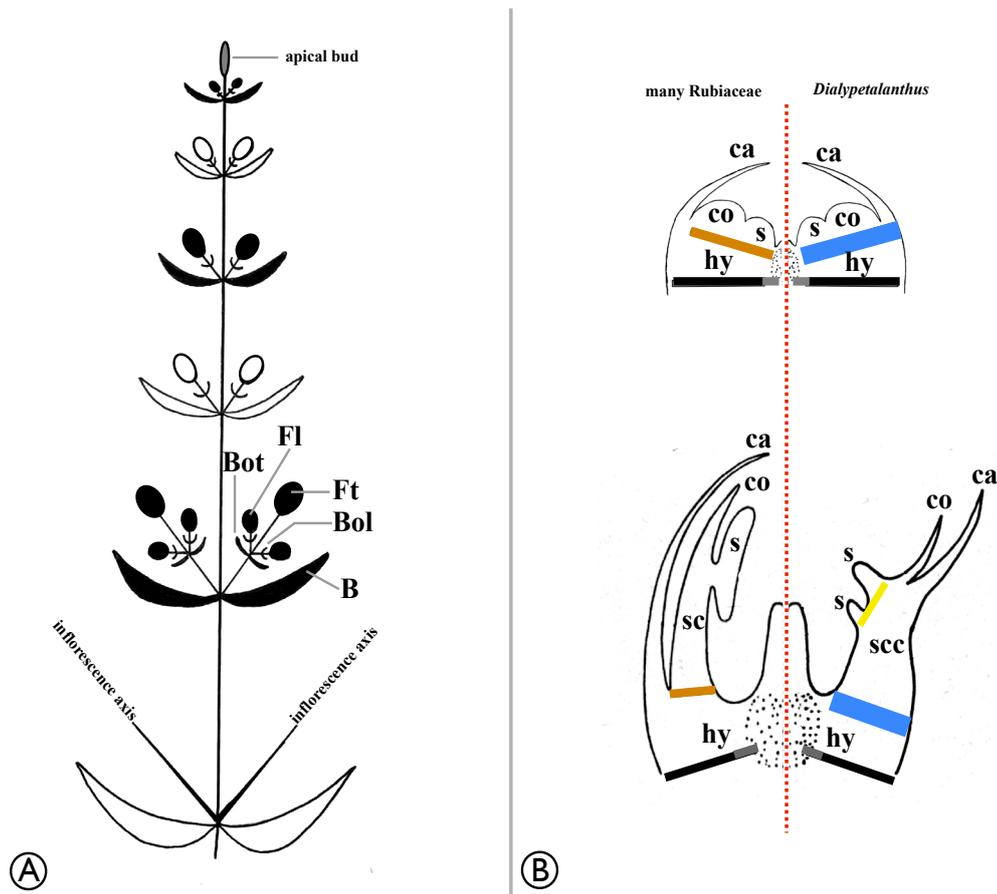


Figure 8. Theoretical outline of the inflorescence (A) and floral development (B, after Leins and Erbar (2010: 102) and Vrijdaghs et al. (2015: 251)) in *Dialypetalanthus*. **A.** White and black pairwise structures perpendicular to each other. Apical bud consisting of two opposite bracts surrounding an underdeveloped, terminal, cymosely branched inflorescence unit. **B.** Left, floral development in many Rubiaceae, right in *Dialypetalanthus*. Bars represent circular intercalary meristems respectively developing a hypanthium (black-grey), a stamen-corolla tube (amber), a stamen-corolla-calyx tube (blue), stamens (yellow). Dotted area, gynoeical locular zone. Symbols: B, bract; Bol, bracteole of lateral flower; Bot, bracteole of terminal flower; ca, calyx (lobes); co, corolla (lobes); Fl, lateral flower; Ft, terminal flower; hy, hypanthium; s, stamen; sc, stamen-corolla tube; scc, stamen-corolla-calyx tube.

Table 3. Floral developmental characters in the three species studied based on the developmental hypothesis of Vrijdaghs et al. (2020).

Characters	<i>Dialypetalanthus fuscescens</i>	<i>Mussaendopsis beccariana</i>	<i>Pogonopus exsertus</i>
ca free sepals (1) or floral cup* + lobes (0)	0	0	0
stamen-corolla tube	0	0	1
co corolla tube sensu stricto	0	0	1
stamen-corolla-calyx tube	1	0	0
petal fusion	0	0	0
free stamens (1) or epipetaly (0)**	1	1	0
an stamens on: stamen-corolla tube (1) or stamen-corolla-calyx tube (0)	0	1	–
4–5 stamens (1) or polyandry (0)	0	1	1
inferior (1) or (half)-superior (0)	1	1	1
gy bilocular (1) or other (0)	1	1	1
single style/ 2 stigmatic branches (1) or two styles (0)	1	1	1
ne gynoecial annular nectary	0	1	1

* The floral cup usually is a calyx tube. It can also be any combination with the inner whorls, such as a corolla-calyx tube or a stamen-corolla-calyx tube.

** Two character states are defined for ‘epipetaly’: stamens attached on a stamen-corolla tube or on a stamen-corolla-calyx tube. A third state, which is not relevant here, is stamens attached on free petals.

12C in Vrijdaghs et al. (2020: 484). However, in *P. exsertus*, the development of the stamen-corolla tube is initially somewhat delayed compared to the flowers in *Crucianella* and *Phuopsis*. Adaxially and at the base of the stamens, a thick whorl of hairs develops. Also the development of the androecium and inferior and bilocular gynoecium occurs as observed in all other Rubiaceae studied.

According to Puff and Igersheim (1994), the corolla of the two then known *Mussaendopsis* species consists of a tube and lobes. This is in contrast to our observations in semi-mature flowers. We cannot exclude that very late in the floral development, shortly before anthesis, a stamen-corolla tube is still formed, which would explain the observations of Puff and Igersheim (1994). However, this seems improbable, since no indications of a common (meristemetic) base of petals and stamens were found as for example is the case in the genera *Pentodon* (Spermacoceae) and *Sacosperma* (Knoxieae) (Vrijdaghs et al. 2015). Of the two taxa compared here with *Dialypetalanthus*, *P. exsertus* is also neotropical. Yet it is the floral morphology of the Asiatic *M. beccariana* that shares two unusual features with that of *D. fuscescens*, namely the free petals and free stamens, while the floral morphology of *P. exsertus* is similar to what may be expected from a Rubiaceae flower.

Following the hypothesis of Vrijdaghs et al. (2020), Table 3 compares the floral development and morphology in *D. fuscescens* with that in *M. beccariana* and *P. exsertus*. It follows that: 1) the typical and quite common floral Bauplan in Rubiaceae is maintained, albeit by means of a stamen-corolla-calyx tube instead of a stamen-corolla tube, 2) the development of floral whorls (in casu, the

androecium) from a fixed number of individual primordia can be reorganised into an annular meristem from which multiple whorl members secondarily originate. This was also observed in other families than Rubiaceae (e.g. Ronse De Craene 1988; Vrijdaghs et al. 2005; Ronse Decraene and Smets 1993).

CONCLUSIONS

The early development of a stamen-corolla-calyx tube sensu Leins and Erbar (2010) can be considered as a floral morphological link between *Dialypetalanthus* and the other Rubiaceae. In this interpretation, the perianth in flowers of *Dialypetalanthus* matches that of most other Rubiaceae, albeit through a stamen-corolla-calyx tube instead of a stamen-corolla tube. Moreover, as a consequence, the apparent sepals and petals actually are calyx and corolla lobes respectively, developing from a stamen-corolla-calyx tube instead of individual primordia. The pairwise sequence of appearance of the perianth lobes may be a consequence of spatial constraints.

The adaxial part of the central floral cup in flowers of *Dialypetalanthus* consists of an annular androecial meristem or primary androecium primordium, from which multiple individual stamen primordia originate in a centripetal succession, eventually resulting in two ‘whorls’ of in total more or less 10 stamens. Hence, polyandry and no epipetaly sensu stricto, though the position of the stamens on a stamen-corolla-calyx tube can be considered as another character state of epipetaly (see also Table 3).

The inferior gynoecium in flowers of *Dialypetalanthus* develops like in all other Rubiaceae studied, but no gynoecial nectary surrounding the base of the single style is developed.

The inflorescence in *Dialypetalanthus* is characterised by a pairwise pattern of appearance of all lateral parts, including these of the perianth, whereby each pair of leaf-like structures is positioned at 90° with respect to the preceding (or following) one (decussate arrangement).

Mussaendopsis beccariana has a corolla consisting of five free petals and an androecium consisting of five free stamens. Hence, no polyandry occurs, neither does epipetaly. In contrast, the floral ontogeny in *P. exsertus* concurs with that in all other flowers studied in Rubiaceae.

We are now aware of floral ontogenetically well-documented cases of a switch in the development of floral whorls from a limited number of individual primordia to a primary annular meristem/primordium from which many individual parts originate secondarily.

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