# A developmental model for the corolla in Rubiaceae.
# Cryptic character states in corollas of the Spermacoceae alliance

**Alexander Vrijdaghs[1,2*], Petra De Block[2], Brecht Verstraete[1,2],
Inge Groeninckx[1], Erik Smets [1,3,4] & Steven Dessein[2]**

[1]Plant Conservation and Population Biology, Institute of Botany & Microbiology KU Leuven, Kasteelpark Arenberg 31 P.O.Box 2435, BE-3001 Leuven, Belgium
[2]Botanic Garden Meise, Nieuwelaan 38, BE-1860 Meise, Belgium
[3]Naturalis Biodiversity Center, P.O.Box 9517, NL-2300 RA Leiden, The Netherlands
[4]Hortus Botanicus, Leiden University, P.O.Box 9500, NL-2300 RA Leiden, The Netherlands
*Author for correspondence: alexander.vrijdaghs@bio.kuleuven.be

**Background and aims** – In Rubiaceae, most flowers have a tubular corolla. Earlier studies showed that a tubular corolla may develop from an annular primordium or result from postgenital fusion of petals. Often stamen-corolla tubes are formed, concurring with epipetaly. Moreover, flowers with a fenestrated tubular corolla occur. The scattered distribution of species with corolla splits in Rubiaceae suggests a multiple origin. We intended to answer the following questions: (1) should the tubular corollas studied be assigned to a single or to several character states?; and (2) are the corolla splits studied homologous? We investigated the corolla development in five species of the Spermacoceae alliance with corolla splits in comparison with a *Spermacoce* species without.

**Methods** – Floral developmental studies using scanning electron microscopy (SEM) and light microscopy (LM).

**Results** – In *Spermacoce*, the tubular corolla with epipetalous stamens originates from an annular primordium. In *Sacosperma* and *Pentas*, the tubular corolla develops similarly with the stamens fixed basally. In *Paederia* and *Pentodon*, individual petal primordia originate on a ring wall. Next, stamen primordia appear adaxially on the ring wall. Then, the bases of the petals fuse partially, resulting in fenestrated tubular corollas. In *Sacosperma* and *Pentas*, corolla splits are actively formed in an initially closed tubular corolla.

**Conclusions** – The tubular corollas of the species studied in *Spermacoce, Sacosperma* and *Pentas* are early sympetalous. Those in *Paederia* and *Pentodon* are late sympetalous. The tubular corolla results from a combination of three developmental processes: the formation of (1) a stamen-corolla tube, (2) a corolla tube *sensu stricto*, and (3) postgenital fusion of petals. The final form of the tubular corolla and the stamen insertion depend on the relative activity of these processes. Consequently, similar looking tubular corollas may constitute different character states. The corolla splits studied constitute two different cryptic character states in respectively *Sacosperma*/*Pentas* and *Paederia/Pentodon*.

**Key words** – Annular intercalary meristem, (cryptic) character states, corolla splits, fenestrated corolla, stamen-corolla tube, corolla tube *sensu stricto*, floral development, postgenital fusion, Rubiaceae, SEM.

## INTRODUCTION

Flowers in most Rubiaceae have an actinomorphic tubular corolla with four or five corolla lobes, also called 'petals' (e.g. Vaes et al. 2006). Previous developmental studies suggest that tubular corollas, though morphologically homologous and similar in appearance, may result from different developmental processes (e.g. Payer 1857, Erbar & Leins 1996b, Ronse Decraene & Smets 2000). Moreover, in several more or less distantly related species, the flowers show longitudinal splits in the corolla tube. The development of the tubular corollas and the morphological homology of corolla splits are central in this study.

### Rubioideae and the Spermacoceae alliance

Within Rubiaceae, Bremer & Eriksson (2009) recognised three subfamilies, namely Cinchonoideae, Ixoroideae and

Rubioideae, whereas Robbrecht & Manen (2006) only recognised two, Cinchonoideae and Rubioideae, with Ixoroideae assigned to the rank of 'supertribe' within the subfamily Cinchonoideae.

Rubioideae are characterised by the presence of raphides (calcium oxalate crystals) and a valvate corolla. The c. 21 tribes of the Rubioideae fall into a basal grade and two major groups: the Psychotrieae alliance (*sensu* Razafimandimbison et al. 2008, Rydin et al. 2009a = supertribe Psychotriidinae *sensu* Robbrecht & Manen 2006; see also fig. 1) and the Spermacoceae alliance (*sensu* Rydin et al. 2009b = supertribe Rubiidinae *sensu* Robbrecht & Manen 2006; see also fig. 1). The Psychotrieae alliance is predominantly woody, with fleshy fruits and a single ovule per locule. The Spermacoceae alliance is herbaceous or woody, with fleshy or dry fruits and many to one ovules per locule (Robbrecht & Manen 2006).

Our study group consists of species of *Paederia*, *Pentas*, *Pentodon*, *Sacosperma*, and *Spermacoce* (table 1), all classified in the Spermacoceae alliance, subfamily Rubioideae, which is similar in both abovecited classifications (fig. 1). We also compare our current observations with those of *Canephora madagascariensis* (De Block & Vrijdaghs 2013), which belongs to the tribe Octotropideae, subfamily Ixoroideae *sensu* Bremer & Eriksson (2009) or Cinchonoideae-Ixoridinae *sensu* Robbrecht & Manen (2006) (fig. 1).

Flowers in Rubioideae typically have a (usually inconspicuous) calyx tube with well-developed calyx lobes (sepals), a conspicuous actinomorphic tubular corolla with corolla lobes, epipetalous stamens with dorsifixed, introrse anthers and an inferior ovary. Heterostyly is frequently present (Robbrecht 1988).

## Tubular corollas

Most flowers in Rubiaceae have a 'tubular sympetalous corolla'. Such a corolla tube is considered to be the result of congenital fusion of petal and stamen primordia (e.g. in *Spermacoce*, Vaes et al. 2006; see also in *Canephora*, De Block & Vrijdaghs 2013: figure 10), or of postgenital fusion of individual petal primordia, as in the rubioid genera *Asperula*, *Galium* and *Rubia* (Payer 1857). According to Payer (1857), in these three genera, stamen primordia originate after initiation of the petal primordia. The stamen primordia are free and alternate with the petal primordia, later to become connate at their bases. More recent research showed that in case of epipetalous stamens, the tubular corolla consists of a stamen-corolla tube below the insertion of the filaments upon the corolla, and a corolla tube *sensu stricto* above the insertion of the filaments up to the bases of the corolla lobes (Erbar 1991, Erbar & Leins 1996a, 1996b, Ronse Decraene & Smets 2000, De Block & Vrijdaghs 2013). Based on developmental studies, Erbar & Leins (1996a, 1996b) defined 'early sympetaly' and 'late sympetaly' as distinct developmental character states for tubular corollas. Ronse Decraene & Smets (2000), based on a developmental study in *Galopina* (Rubioideae), suggested that an early sympetalous ring develops into a stamen-corolla tube. Moreover, in their opinion, early sympetaly concurs with a concave floral apex in contrast to late sympetaly, which concurs with a convex floral apex.

When using the term '**corolla tube *sensu stricto***', we mean the part of the tubular corolla situated above the insertion points of the (epipetalous) stamens. The corolla tube *sensu stricto* does not include postgenitally fused bases of the corolla lobes. The term '**stamen-corolla tube**' refers to the part of a tubular corolla below the insertion points of the epipetalous stamens.

## Floral cups and gynoecial hypanthium

In most Rubiaceae, the gynoecium is inferior (e.g. Robbrecht 1988, Groeninckx et al. 2007). Robbrecht (1988: 74) stated that the "rubiaceous hypanthium consists of only gynoecial and axial tissue". De Block & Vrijdaghs (2013), in a developmental study in *Canephora madagascariensis*, combined the views of Ronse Decraene & Smets (2000) with the floral cup theory of Leins (2000) and Leins & Erbar (2010: 102). De Block & Vrijdaghs (2013) argued in favour of a hypanthium originating from an annular intercalary meristem *sensu* Leins & Erbar (2010: 102: figure 99/6 or 7), explaining why at early developmental stages the floral apex becomes concave and why the ovary is inferior. It was concluded that the hypanthium also comprises the meristems from which later on the calyx, corolla and androecium develop. In none of the previously mentioned studies, basal corolla splits were considered.

## Corolla splits

Though not commonly present in Rubiaceae, tubular corollas with splits or fenestrated tubular corollas were reported in species of several more or less distantly related genera in both subfamilies (*sensu* Robbrecht & Manen 2006) of Rubiaceae. In the genera *Heinsia*, *Mussaenda*, *Pseudomussaenda* (tribe

**Table 1 – Species studied and voucher data.**

| Species | Collectors | N° | Locations/Herbarium |
|---|---|---|---|
| *Paederia farinosa* (Baker) Puff | *De Block et al.* | 911 | Madagascar /BR |
| *Paederia thouarsiana* Baill. | *De Block et al.* | 663; 2453 | Madagascar /BR |
| *Pentas lanceolata* (Forssk.) Deflers | *De Block* | 1550 | BR cultivated/BR |
| *Pentodon pentandrus* Vatke | *Groeninckx et al.* | 58 | Madagascar /BR |
| *Sacosperma paniculatum* (Benth.) G.Taylor | *Dessein et al.* | 1687 | Gabon /BR |
| *Spermacoce occultiseta* Harwood | *Harwood* | 1123 | Australia /BR |

Mussaendeae, subfamily Cinchonoideae-Ixoridinae *sensu* Robbrecht & Manen 2006 or subfamily Ixoroideae *sensu* Bremer & Eriksson 2009; see also Alejandro et al. 2005 and fig. 1), corolla splits, when present, occur far above the base of the tubular corolla. In the subfamily Rubioideae, species with flowers with basal corolla splits are reported in **Pentas lanceolata** of tribe Knoxieae (e.g. see Fukuoka 1979: figure 12F; for recent taxonomic changes of the genus, see Kårehed & Bremer 2007) and in **Paederia** of tribe Paederieae (Puff & Igersheim 1991). We observed fenestrated corollas in both species of **Pentodon**. In **Sacosperma paniculatum** of tribe Spermacoceae *sensu lato*, corolla splits were reported by Excell (1944: 219). Corolla splits were also reported in species of *Coelospermum, Damnacanthus, Gynochthodes, Mitchella* and *Morinda* of tribe Morindeae (Robbrecht 1988, Robbrecht et al. 1991, Johansson 1988 & 1994, see also Razafimandimbison et al. 2008 & 2009 for the most recent changes of tribal delimitation and fig. 1) and in *Pagameopsis* (*incertae sedis*; Piesschaert et al. 2001). The species with corolla splits studied in this paper belong to the genera in bold. The

presence of corolla splits in species of different clades raises questions about their origin – are they morphologically homologous? – and, consequently, about the formation of the tubular corolla itself.

## Aims

By means of a floral developmental study, we aim to unravel and compare the developmental complexity of the tubular corollas in five species of four genera with fenestrated corollas within the subfamily Rubioideae, compared with one rubioid species without corolla splits. Our research questions are: (1) should the tubular corollas in the species studied be assigned to a single or to several character states? and (2) are the corolla splits in the four genera homologous?

The species studied (table 1) all belong to the Spermacoceae alliance (fig. 1) and were selected based on availability and phylogenetic position. Our observations are also compared with those from a previous study in *Canephora* (De
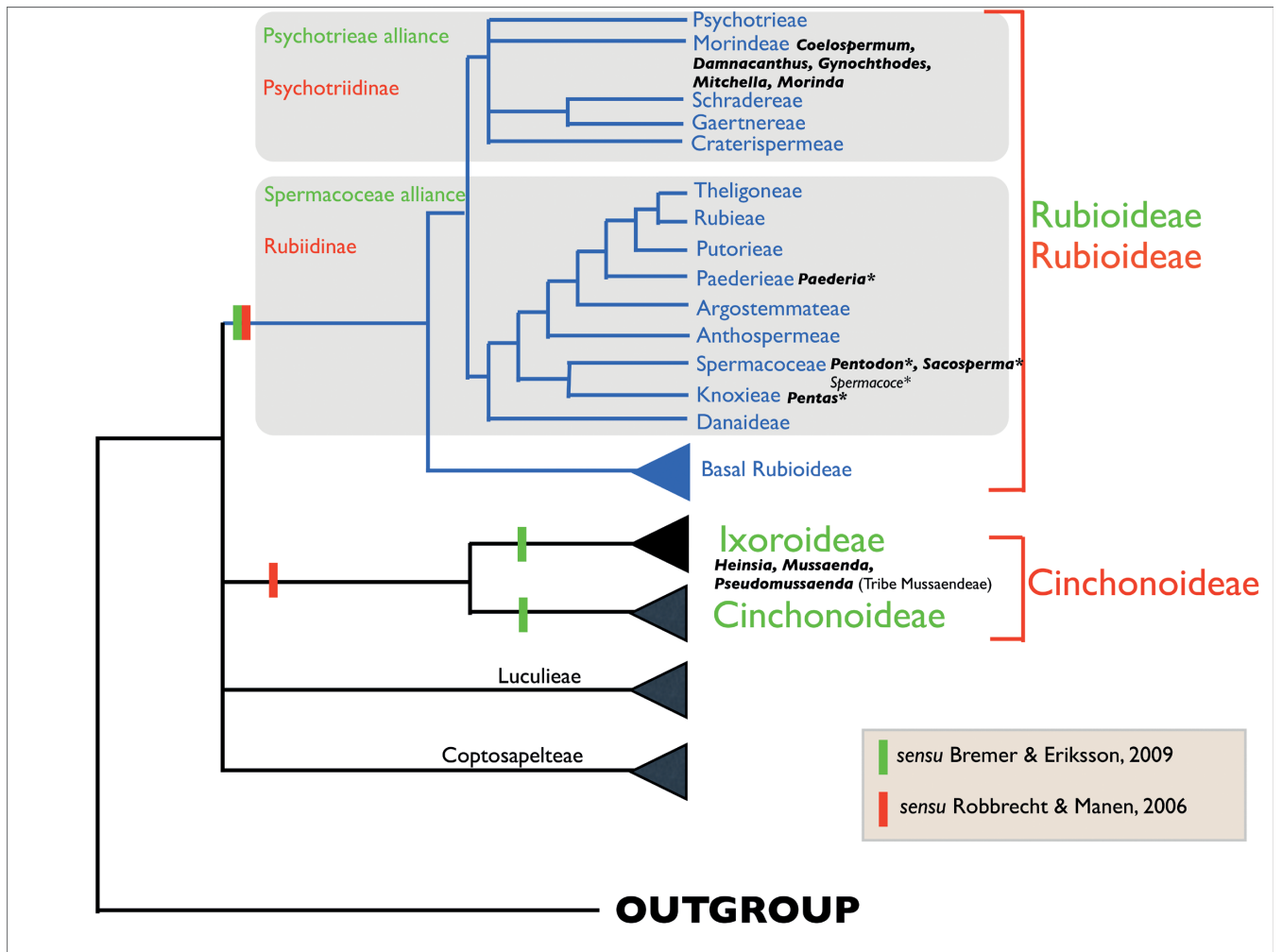


**Figure 1** – Simplified cladogram of Rubiaceae after both Bremer & Eriksson (2009) and Robbrecht & Manen (2006). Bremer & Eriksson recognised three subfamilies, Rubioideae, Ixoroideae and Cinchonoideae, whereas Robbrecht & Manen recognised only two subfamilies, Rubioideae and Cinchonoideae, the latter comprising Cinchonoideae and Ixoroideae *sensu* Bremer & Eriksson. In bold, genera with flowers with corolla splits. Genera indicated with an asterisk (*) are comprised in this study.

Block & Vrijdaghs 2013), tribe Octotropidae, subfamily Cinchonoideae-Ixoridinae *sensu* Robbrecht & Manen (2006).

## MATERIAL AND METHODS

### Plant material

Flowers of the species studied were collected in the field or cultivated in the greenhouses of the Botanic Garden Meise (table 1) and subsequently fixed in 70% ethanol. The material was dissected in 70% ethanol under a Wild M3 (Leica Microsystems AG, Wetzlar, Germany) stereo microscope equipped with a cold-light source (Schott KL1500, Schott-Fostec LLC, Auburn, NY, USA).

### Scanning electron microscopy (SEM)

After preparation, the material was washed twice with 70% ethanol for 5 min. Subsequently, it was placed in a mixture (1/1) of 70% ethanol and DMM (dimethoxymethane) for 5 min. Next, the material was transferred to pure DMM for 20 min. Then, the material was $CO_2$ critical point dried using a CPD 030 critical point dryer (BAL-TEC AG, Balzers, Liechtenstein). With the aid of Leit-C, the dehydrated samples were mounted on aluminium stubs and subsequently coated with gold with a SPI-Module™ Sputter Coater (SPI Supplies, West-Chester, PA, USA). Images were obtained on a Jeol JSM-6360 (Jeol, Tokyo) at the laboratory of Plant Conservation and Population Biology (KU Leuven).

### Light microscopy (LM)

Previously prepared material was dehydrated through a graded ethanol series and subsequently embedded in KULZER's Technovit 7100 (based on HEMA, hydroxyethyl-methacrylate). With a rotation microtome Leica RM2135 with disposable blades (Leica DB80L), 7 μm thick sections were cut. These were stained with 0.05% toluidine blue in aq. dest. LM images were obtained using an Olympus BX51 microscope equipped with a Color View Soft Imaging System camera.

## RESULTS

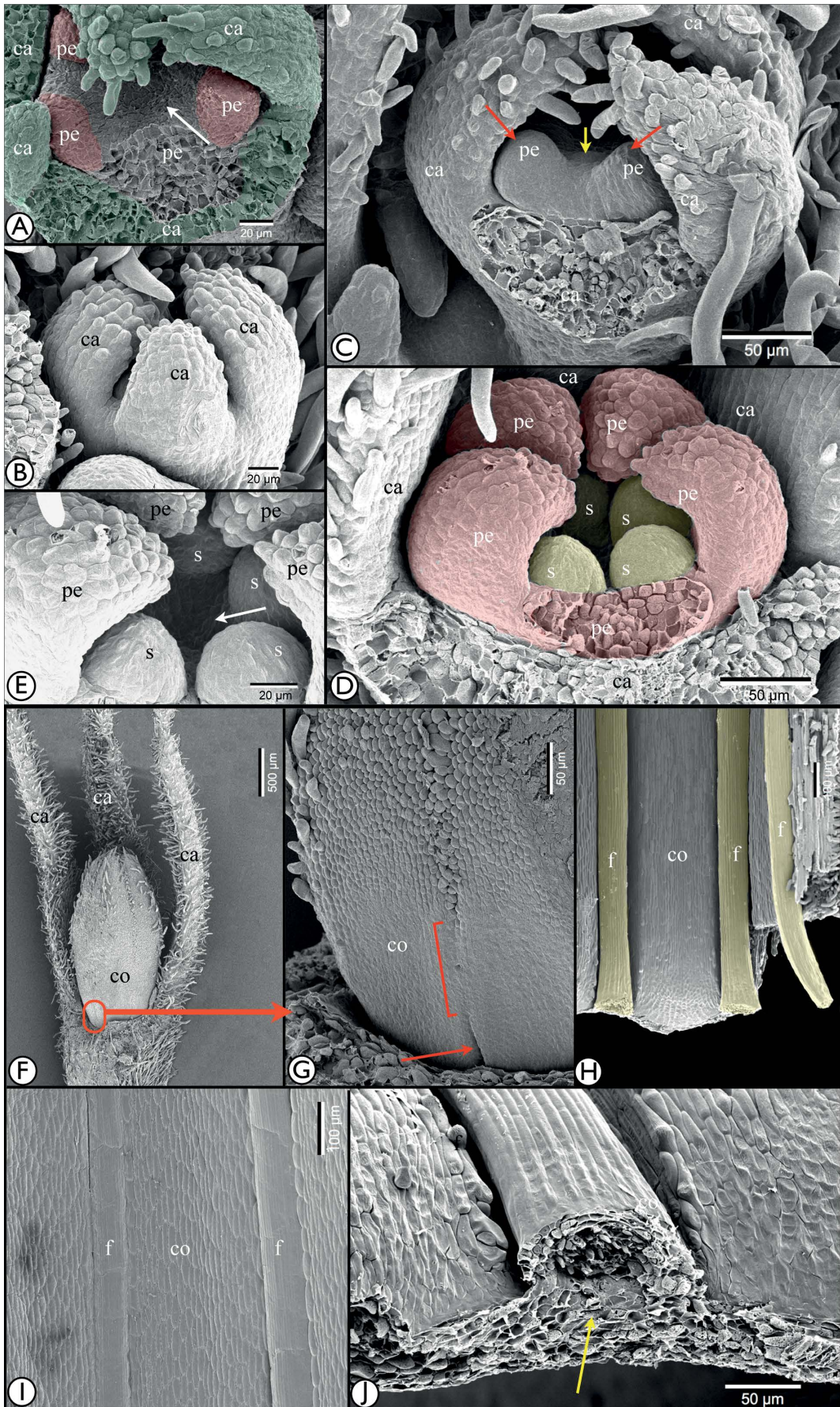Our conclusions are mainly based on SEM and LM observations, of which a selection is presented (figs 2–9). Below follows a detailed description of our observations. We preferred to describe the floral development in a logical order (first developmental stages, tubular corolla and corolla splits, androecium, gynoecium; the figures of respectively *Paederia/Pentodon* and *Pentas/Sacosperma* are grouped together because of similar developmental patterns) instead of strictly following the sequence of the figures. For *Paederia* and *Pentodon*, we use the term 'petal' instead of 'corolla lobe', which is justified in the Discussion.

### Floral development in *Paederia* (figs 2, 3 & 9C, G)

**Early floral development** – The SEM observations of *Paederia farinosa* and *P. thouarsiana* are presented complementarily. An undifferentiated flower primordium first develops a calyx with five, more or less equally developing calyx lobes (fig. 2A & B). The calyx lobes entirely cover the inner part of the flower (fig. 2B). Next, alternating with the calyx lobes, individual petal primordia appear on the annular collar of a now concave floral apex (fig. 2A). Subsequently, five individual stamen primordia originate (fig. 2C), alternating with the corolla lobes. The petals and stamens develop simultaneously around the center of the flower (fig. 2D), where two carpelary zones become visible (fig. 2E). Meanwhile, the calyx lobes become narrow and hairy, highly protruding above the corolla (fig. 2F).

**Tubular corolla and corolla splits** – Fusion between the individual petals takes place, resulting in a tubular corolla (fig. 2G). At the base of this tube, incomplete fusion between the corolla lobes results in small splits (fig. 2G). At early developmental stages, in the basal, adaxial part of the corolla, from the midvein of each (fused) petal towards the edges, long hairs originate (fig. 3B & G). More or less simultaneously, around the corolla splits, papillate cells appear on the abaxial (fig. 3A) and adaxial side of the splits (figs 3C, D, G & 9G). Subsequently, the tubular corolla elongates and the adaxial basal hairy zone is raised to a higher position (fig. 3B, E, G & H).

**Androecium** – At early developmental stage, the stamens are positioned upon the hypanthium (fig. 2D). At later stages, it becomes clear that the filaments remain connected with the tubular corolla when the latter is removed (figs 2H, J & 3C, F–H). With the tubular corolla increasing in height, the filaments lengthen accordingly (fig. 3F–H). At later developmental stages, more distally, the filaments lay in a groove
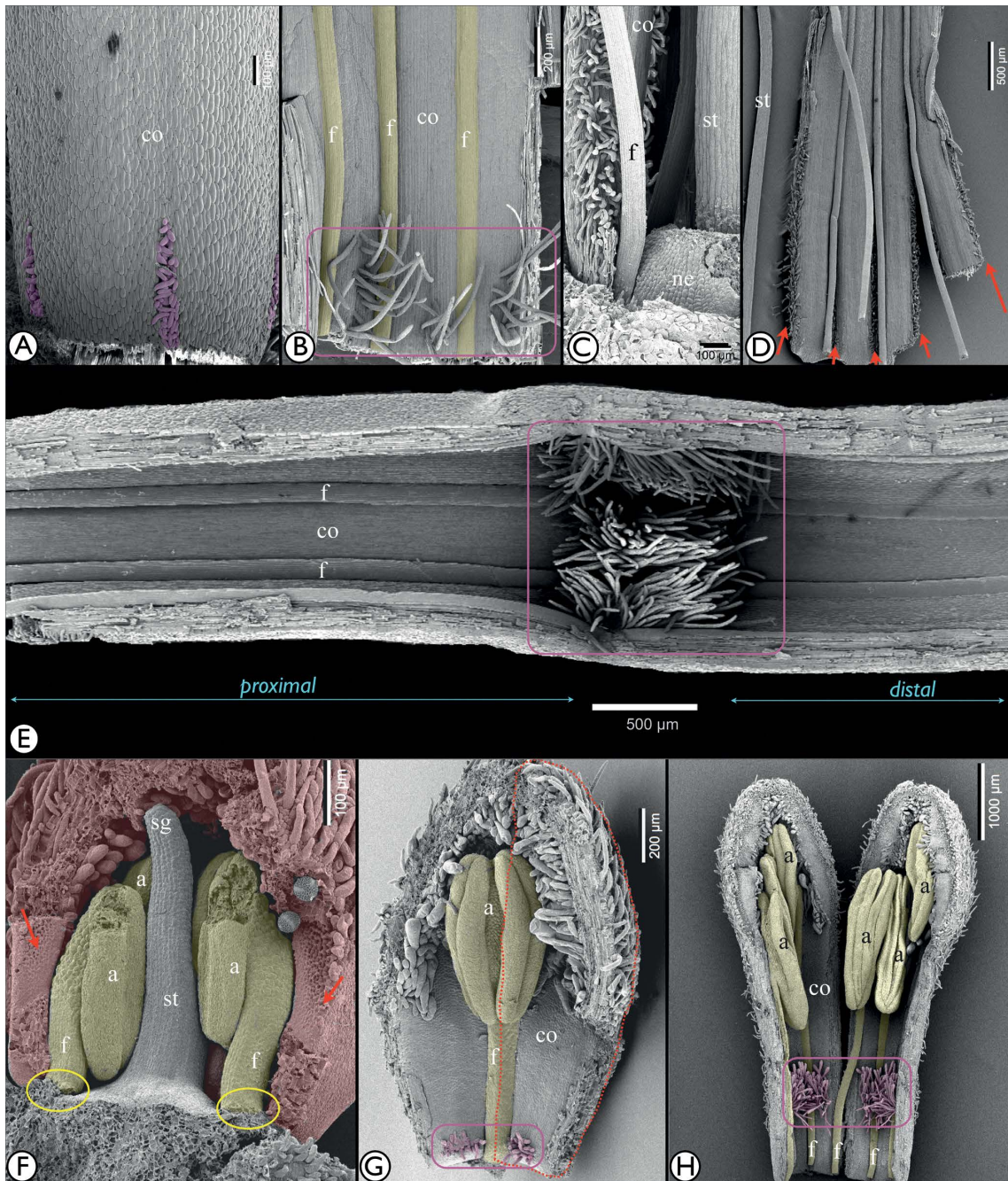
► **Figure 2** – SEM micrographs of the early floral and corolla development in *Paederia thouarsiana* (A) and *P. farinosa* (B–J): A, flower at early developmental stage with five developing calyx lobes (green) and alternating with them, five petal primordia (red); B, the calyx lobes develop, enveloping the inner part of the flower; C, lateral view of a successive stage with the calyx lobes enveloping the inner part of the flower. One calyx lobe is removed and an originating stamen primordium (yellow arrow) is visible through the opening in between two petals (red arrows); D, lateral view of equally developing corolla lobes (red). Alternating with them, five individual stamen primordia (yellow) adaxially appear, of which four are visible; E, detail of D, showing the depression in the centre of a concave floral apex (arrowed); F, lateral view of a developing flower, with two sepals removed. Fusion of the petals results in a tubular corolla (co) with small splits at the base of each seam between two petals, and distally free parts of the petals = corolla lobes. At this stage, the pubescent calyx consists of five slender calyx lobes upon a rudimentary calyx tube; G, detail of a corolla split (arrowed) and of the fusion (bracket) between two petals; H, view of the basal adaxial surface of the corolla at a later developmental stage, with free filaments, each covering a corolla split and the seam between two petals; I, view of a more distal part of the adaxial surface of the corolla, where each filament is sunk in a groove between two petals; J, view from below the insertion point (arrowed) of a filament. The bases of filament and corolla adhere to each other (arrowed).
Abbreviations: ca, calyx (lobe); co, tubular corolla; f, filament; pe, petal (primordium); s, stamen (primordium). Colour code: green, calyx; red, corolla; yellow, androecium.

**Figure 3** – SEM micrographs of the corolla development in *Paederia farinosa* (A–E) and in *P. thouarsiana* (F–H): A, lateral view of the abaxial basal part of a corolla of a developing flower. Short, papillate cells (purple) are formed along the rims of the splits. The outer surface of the corolla is smooth and seamless; B, idem as in A, seen from the adaxial side. Basally, at the midvein of each corolla lobe, long hairs originate (encircled). The filaments are loosely embedded in the seams between two petals; C, successive developmental stage. View of the free basal part of a filament and a hairy rim of a corolla split. Part of the nectary and style is visible; D, basal adaxial surface of a nearly mature tubular corolla, with five splits (arrowed) and four out of five filaments visible; E, large part of the adaxial surface of a nearly mature corolla. The hairs originally growing on the midvein of the corolla lobes (see B) now form a whorl of hairs in the middle of the tube (encircled). Above this whorl of hairs, in the distal part of the tubular corolla, the filaments are more or less fused with the seams between the corolla lobes. Proximally, below the hair whorl, the filaments are free; F, lateral view of the superior part of a developing flower, showing corolla, stamens, style and one of the two stigmas. Insertion of the filaments (encircled) is in the hypanthium, the filaments are free from the corolla. At this stage, the corolla lobes are not completely fused (arrowed); G, developing stamen (yellow) with introrse anther. At the base of the corolla lobes, hairs (purple, arrowed) originate. Encircled with a red dotted line is one single corolla lobe; H, view of the adaxial surface of a young corolla with stamens. The filaments differ in height and are loosely embedded in the seam between two corolla lobes. The hairs of G (purple, encircled) are now raised to the middle of the tubular part of the corolla.

Abbreviations: a, anther; co, corolla; f, filament; ne, nectary; sg, stigma; st, style. Colour code: red, corolla; yelllow, androecium.
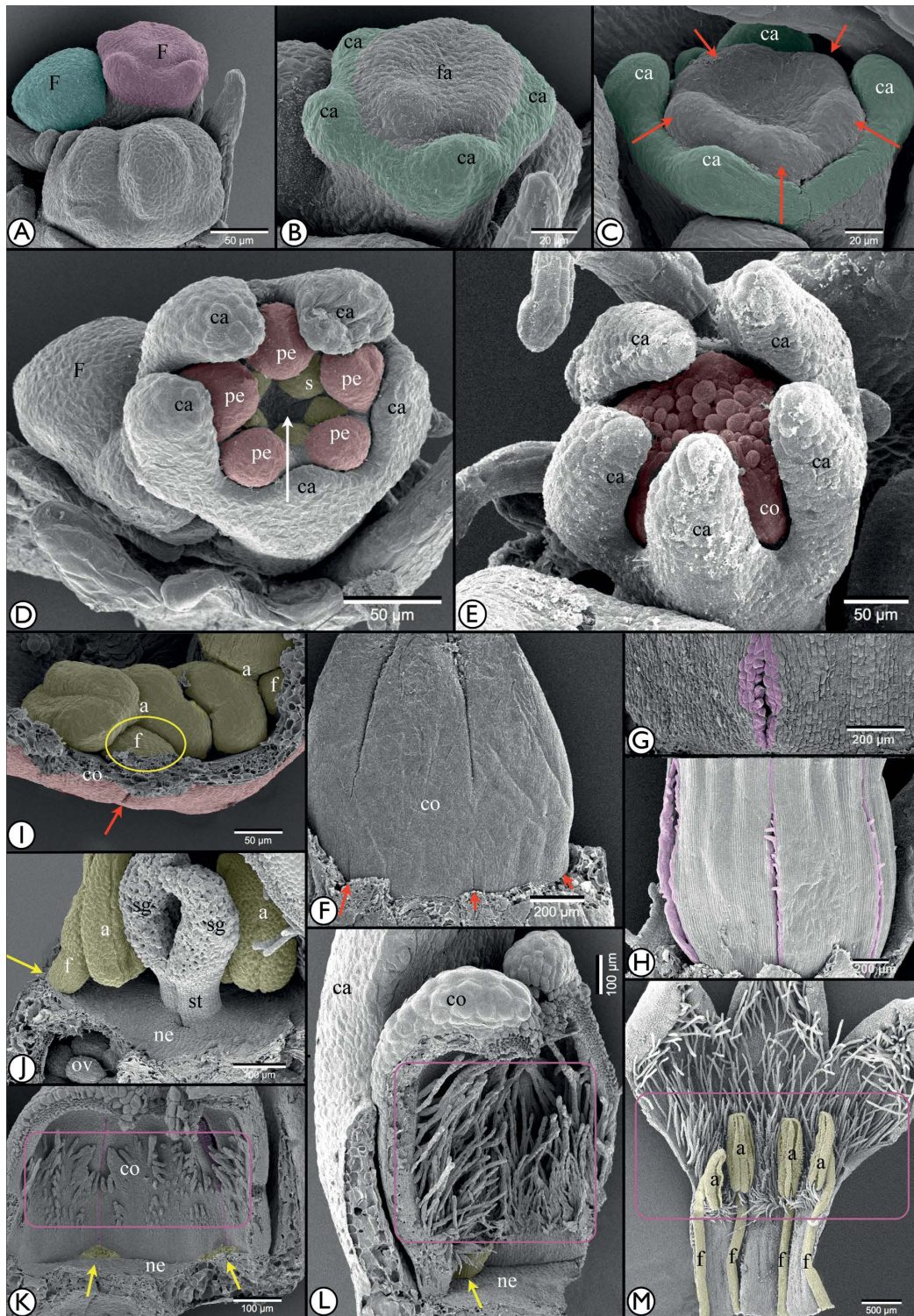
**Figure 4** – SEM micrographs of the early floral and corolla development in *Pentodon pentandrus*: A, cluster of flowers at successive early developmental stages; B, detail of the terminal flower of A; C, next developmental stage; D, five petals alternate with the calyx lobes; E, developing flower with the petals enveloping the inner part of the flower; F, adaxial view of a developing corolla with partially fused corolla lobes; G–H; lateral, adaxial view of successive stages of the development of corolla and corolla splits; I, view from below of a developing stamen; J, view of a developing pistil and stamens surrounding it; K, adaxial view of a part of a corolla with stamens removed; L–M, the tubular corolla elongates from the base.

Abbreviations: a, anther; f, filament; F, flower primordium; fa, floral apex; ca, calyx (lobe); co, corolla (lobe); ne, nectary; pe, petal; sg, stigma; st, style. Colour code: green, calyx; red, corolla; yellow, androecium.

See appendix at the end of this paper for a more detailed caption of this figure.

with some postgenital coherence *sensu* Endress (2006), but everywhere remaining distinguishable from the corolla tube (figs 2H, I, 3B, D, E & 9C). Anthers are tetrasporangiate, dorsifixed and introrse (fig. 3F–H). In semi-mature flowers of *P. thouarsiana*, the filaments are unequal in length (fig. 3H).

### Floral development in *Pentodon pentandrus* (figs 4 & 9D, H)

**Early floral development** – Initially, the floral apex is disciform (fig. 4A). Next, it becomes concave and five unequal calyx lobe primordia appear (fig. 4A & B). More or less simultaneously, on the collar of the concave floral apex, five individual petal primordia arise, alternating with the developing calyx lobes (fig. 4B & C). Subsequently, five individual stamen primordia surrounding the central depression originate, alternating with the petals (fig. 4D). Meanwhile, the calyx lobes develop more or less at the same rate (fig. 4D & E). The petals develop equally and envelop the inner part of the flower (fig. 4E).

**Tubular corolla and corolla splits** – Next, the petals fuse partially, forming a tubular corolla bearing five free corolla lobes (fig. 4F). At the base of each seam between two petals, corolla splits are visible (fig. 4F & G). The entire corolla, including the splits, starts elongating (fig. 4K–M). The cells bordering the corolla splits become papillate (fig. 4G & H). From the adaxial side, initially, the basal corolla splits are not visible (fig. 4K), but the seams between the corolla lobes are. On the inner surface of the fused corolla lobes, hairs are formed in the zone in between the insertion points of the filaments and the corolla lobes (fig. 4K). Later on, the tubular corolla elongates, gradually raising corolla lobes and hairy zone (fig. 4K–M), so that at anthesis, the hairy zone is at the same height as the anthers (fig. 4M).

**Androecium** – The filaments are inserted opposite the corolla splits (fig. 4I), seemingly in the hypanthium though having the same origin as the tubular corolla (fig. 4I–L). Above their insertion point, the filaments are free from the corolla (figs. 4K & 9H). The anthers are tetrasporangiate, dorsifixed and introrse (fig. 4I, J & M). In the centre of the flower, surrounded by the anthers, a short, initially bilobed style and two long stigma branches develop (fig. 4J). The filaments, which, above the splits are loosely fused to the corolla tube (figs 4M & 9D), elongate accordingly with the tubular corolla (fig. 4M). At anthesis, the released pollen grains stick in the hairs surrounding the proximal parts of the anthers.

### Floral development in *Sacosperma paniculatum* (figs 5, 6A–I & 9A, E) and in *Pentas lanceolata* (figs 6J–L, 7A–E & 9B, F)

In the two species, the general floral development is similar. The images presented for *P. lanceolata* focus only on the development of the corolla splits.

**Early floral development** – At the rim of an initially disciform flower primordium, five (rarely four) individual sepal primordia originate (fig. 5A & B). These initially develop at different rates (fig. 5C & D). Next, alternating with the sepals, on the collar of the now concave floral apex, five (rarely four) individual corolla lobe primordia appear (fig. 5C). The

corolla lobes develop equally, covering the inner parts of the flower (fig. 5D & E). At the abaxial side of the corolla, immediately below the corolla lobes, a basal tube of less than ten cell layers high becomes visible (figs 5E, F & 7D). From inside the flower, this basal corolla tube is also visible (fig. 6B).

**Tubular corolla and corolla splits** – The basal corolla tube, together with the partially fused corolla lobes on its top, forms a compound tubular corolla. The seams between the lobes remain visible from the dorsal side (figs 6D & 7D). Above the insertion zone of the filaments in the basal corolla tube, a small opening appears (figs 6A, B, J–L & 7B, C), which initially was not present (fig. 7A; not shown in *Sacosperma*). Later on, each opening develops into a corolla split (figs 6F, G & 9E, F). In *P. lanceolata*, this opening can sometimes also be seen from the abaxial side (fig. 7D). Around (along the corolla splits) and to a lesser degree upon the base of each filament, papillate cells originate (fig. 9E & F). Early in the development of the corolla, on the inner surface of the corolla lobes, hairs originate in three longitudinal rows per corolla lobe, one along the midrib and two left and right of the midrib (figs 6D, E & 7B). These hairs gradually extend, eventually occupying the whole surface of the fused part of the corolla lobes. Next, the zone in between the base of the tubular corolla and the hairy part of the fused corolla lobes elongates, raising the hairy zone to a higher level in the tubular corolla (fig. 6E–G). At (semi-)maturity, these hairs form a dense whorl at the base of the corolla lobes, just below the anthers (fig. 6H & I).

**Androecium** – At the earliest stages immediately after the appearance of the corolla lobe primordia, no stamen primordia are visible (fig. 5C). Later on, epipetalous stamens are present though inserted in the basal part of the tubular corolla (figs 6B–D & 7A–C, E). The anthers are tetrasporangiate, dorsifixed and introrse (fig. 5G). With the longitudinal growth of the tubular corolla, the filaments elongate accordingly and adhere to the corolla, laying in grooves with a certain degree of postgenital coherence (figs 6E–G & 9A, B). In the course of the further development, the bases of the filaments opposite the splits and their distal parts remain free (figs 6C, H, I, 7A & 9E, F). The bases of the anthers are surrounded by the hairy zone, now at the top of the tubular corolla (fig. 6I).

### Floral development in *Spermacoce occultiseta* (figs 7F–H & 8)

Initially, the floral apex is disciform with an irregular rim where calyx lobe primordia originate (fig. 7F). Next, the floral apex becomes concave. Its collar consists of an annular corolla primordium (fig. 7F & G). Calyx lobe primordia become distinct (fig. 7G & H). Simultaneously, the annular corolla primordium forms four bulges (fig. 7H) alternating with the sepal primordia, which develop into corolla lobes (fig. 8A). The annular corolla primordium grows up from the base, forming a tube without splits (fig. 8A–E), raising the corolla lobes. Meanwhile, conspicuous trichomes appear on the top of the corolla lobes (fig. 8A). Initially, no stamen primordia can be observed (fig. 7H), but at a somewhat later stage, stamens are visible (fig. 8B). The elongating
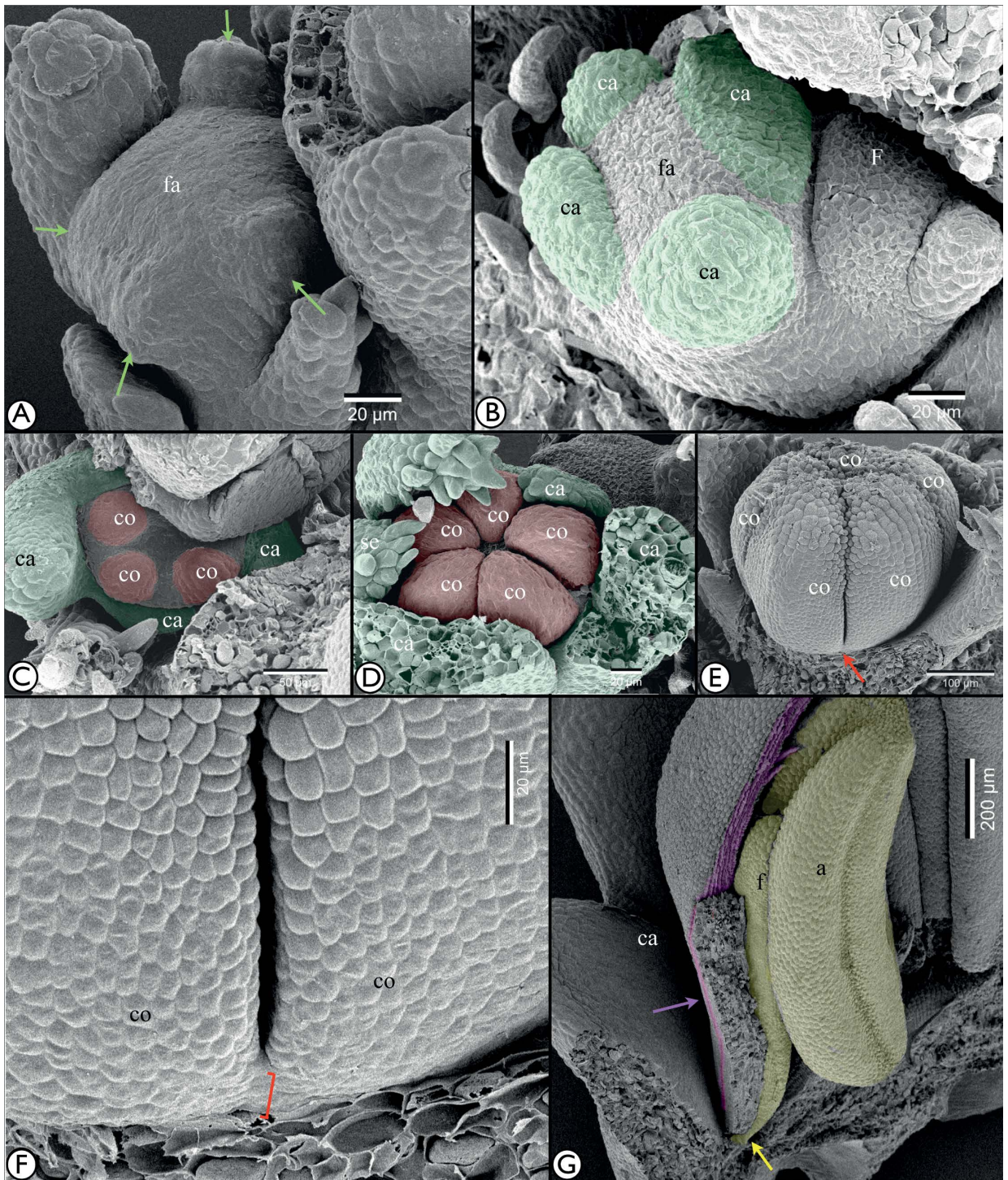
**Figure 5** – SEM micrographs of the early floral development in *Sacosperma paniculatum*: A, disciform flower primordium with the first calyx lobe primordia (arrowed) appearing; B, detail of part of a flower cluster with a flower primordium ('F') and a flower at early developmental stage (left) with four calyx lobe primordia (usually five) on the rim of the disciform floral apex; C, detail of developing flower with unequally developing calyx lobes and three out of five visible individual corolla lobe primordia on the rim of the now concave floral apex; D, apical view of a developing flower with at this stage five unequal calyx lobes and five equally developing corolla lobes covering the inner part of the flower; E, next developmental stage, view of the abaxial surface of the corolla. The free corolla lobes are raised on a rudimentary corolla tube (arrowed); F, detail of the basal corolla tube, 4–5 cell layers thick (hooked); G, lateral view of a part of the corolla with a stamen. The stamen is fixed at the base, in the tubular corolla (yellow arrow). The proximal part of the corolla lobes fuse, though at the abaxial surface of the corolla, the seam between two corolla lobes remains visible (purple, arrowed). The anther is dorsifixed and introrse. Abbreviations: a, anther; ca, calyx (lobe); co, corolla lobe; F, flower primordium; f, filament; fa, floral apex. Colour code: green, calyx; red, corolla; yellow, androecium.
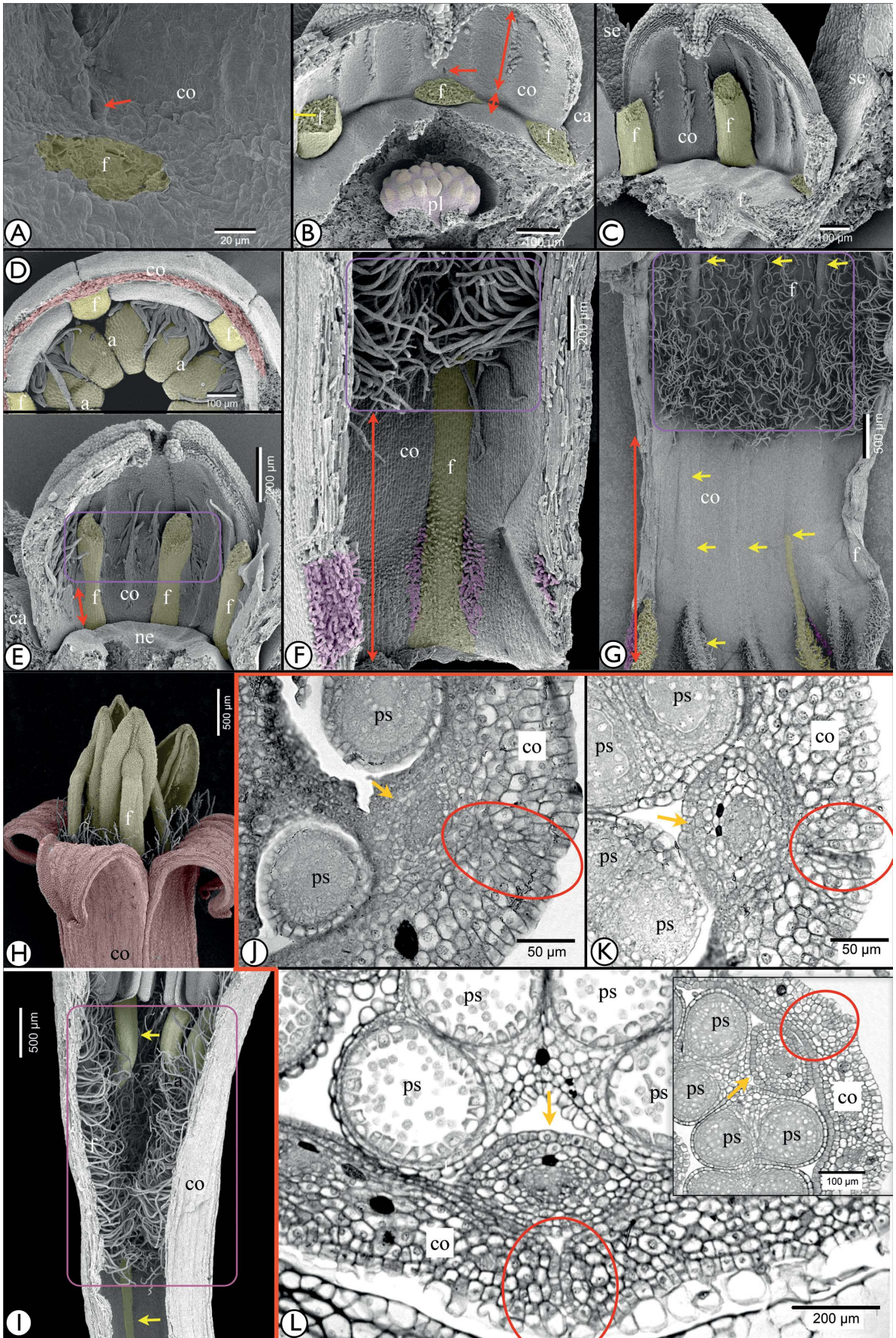
◄ **Figure 6** – SEM micrographs of the corolla development in *Sacosperma paniculatum* (A–I) and LM images of the initial split formation in *Pentas lanceolata* (J–L): A–C, lateral view of the adaxial side of the corolla at successive stages in a developing flower. The corolla is tubular and above the insertion of the filaments, a small opening appears (arrowed in A and B). The stamens are inserted in the basal part of the tubular corolla (small double arrow in B), situated below the fused corolla lobes (large double arrow in B); B–C, along the midrib of each corolla lobe and left and right of it, three rows of hairs appear; D, successive stage. View from below the tubular corolla. The filaments are fixed in the basal part of the tubular corolla (red). Abaxially, grooves corresponding with seams between two corolla lobes remain visible; E & G, successive stages, the initially three rows of hairs extend, forming a massive hairy zone (encircled), which is raised by the elongation of the tubular corolla below it (red double arrows); F & G, at the bases of the filaments, and around the corolla splits, papillate cells (respectively yellow and purple) are formed; G, at semi-maturity, the filaments (yellow arrows indicate one filament at different heights in the corolla) are embedded in the corolla, except their basal and distal parts. The basal part of each filament is opposite a corolla split; H & I, at maturity, the anthers protrude above the corolla, with the free distal parts of the filaments and the lower part of the anthers hidden in a dense whorl of hairs; J, transverse section through tubular corolla above the insertion of the filament, in a young flower bud; K, idem, somewhat higher than in J through the initiation of a split; L, idem, somewhat higher than in K. The image in the inlay is a section above the initiation of the same split. Yellow arrows indicate a filament opposite a zone where split formation is initiated.

Abbreviations: a, anther; ca, calyx; co, corolla (lobe); f, (scar of a) filament; ne, nectary; pl, placenta; ps, pollen sac; se, sepal. Colour code: red, corolla; yellow, androecium; purple, papillate cells in corolla.

tubular corolla lifts up the insertion points of the filaments (fig. 8B–E), which until maturity maintain their relative position in between and just below the bases of two adjacent corolla lobes (fig. 8G). During the development of the flower, corolla appendages appear (fig. 8C & E). Meanwhile, dorsifixed, introrse anthers on short filaments have been formed (fig. 8D–F) and in the basal part of the corolla lobes and the zone immediately below the insertion points of the filaments, there is a massive growth of moniliform hairs (fig. 8D–G). In the basal part of the corolla tube, no hairs are present. The filaments are inserted in between and just below the bases of two adjacent corolla lobes (fig. 8E) and form a continuous tissue with the tubular corolla. Below their insertion points, a single vascular bundle supplies each filament (figs 7I & 8H).

DISCUSSION

In this section, in order to keep the text readable, we refer to 'Canephora', 'Paederia', 'Pentas', 'Pentodon', 'Sacosperma' and 'Spermacoce' instead of the species studied belonging to these genera [respectively *Canephora madagascariensis* (De Block & Vrijdaghs 2013) and table 1]. We do not intend to generalise our findings.

**The structural origin of the corolla and androecium**

**Corolla** – Corolla and androecium originate from a similar annular intercalary meristem. De Block & Vrijdaghs (2013) demonstrated that in *Canephora* the calyx lobes, a well-developed stamen-corolla tube, the androecium, a short corolla tube *sensu stricto*, and eventually the calyx tube originate successively from annular meristematic tissue from which initially the hypanthium itself was formed. A similar floral developmental pattern is found in *Spermacoce* (figs. 7F–H & 8). As a consequence, we infer that in *Canephora* and *Spermacoce*, the tubular corolla results from two developmental processes: the formation of a stamen-corolla tube and the development of a corolla tube *sensu stricto*. In *Spermacoce*, the corolla tube *sensu stricto* remains rudimentary.

From our observations in *Sacosperma* and *Pentas* (figs 5–7), we deduce that a similar situation occurs as in *Spermacoce* and *Canephora*, albeit in other proportions: in flowers of these species, the stamen-corolla tube is only a few cell layers high (figs 5F, 6B & 7D). The stamens de-

velop from the rudimentary stamen-corolla tube and remain attached to it (figs 6B–D & 7A–C, E). The major part of the tubular corolla is formed by a corolla tube *sensu stricto* developing from an annular intercalary meristem in between the insertion of the stamens and the corolla lobes; this explains why the hairy zone at the base of the corolla lobes in *Sacosperma* is raised (figs 6E–G, I & 7B). During the floral development, filaments and corolla tube *sensu stricto* grow at an equal rate, the filaments adhered (see below) to the proximal part of the tubular corolla and remaining discernable even in mature flowers.

In *Paederia* and *Pentodon*, some observations (figs 2J & 4I) suggest that also in these species, corolla and androecium have a common origin in an annular intercalary meristem just below the base of the tubular corolla but in contrast to *Sacosperma* and *Pentas*, no stamen-corolla tube is developed. The tubular corolla is formed by two processes: first fusion of the basal parts of the corolla lobes and next formation of a corolla tube *sensu stricto*, which develops from a meristem in between the insertion of the stamens and the fused corolla lobes (see fig. 10); again, like in *Sacosperma* and *Pentas*, this explains why the hairy zone at the base of the corolla lobes is raised (figs 3B, E, G, H & 4K–M). Filaments and corolla tube *sensu stricto* grow at an equal rate. The filaments remain discernable throughout the development. Anatomical observations of Puff & Igersheim (1991: 68–69, figure 10G, H) show "postgenital fusion" of filaments and corolla tube in *Paederia pospischilii* K.Schum. "for more than three quarters of their length [not at the level of the corolla splits], in the region between two adjacent, fused segments but strands of filament-like tissue remain clearly discernable". Puff & Igersheim considered the situation in *Paederia* to be "intermediate between that of flowers with basally attached long-filamented stamens and flowers with truly (sub)sessile stamens [...]. In the latter [case], common in numerous Rubiaceae, no strands of filament-like tissue are discernible on the interior of the corolla tube below the point of separation of the "true" filaments" – this situation is found in *S. occultiseta* (see figs 7I & 8H). Above the insertion points of the filaments, Puff & Igersheim's (1991) observations concur with ours in *Paederia/Pentodon* and *Sacosperma/Pentas* with clearly discernable filaments (fig. 9A–D), providing supplementary evidence that this part of the tubular corolla is a
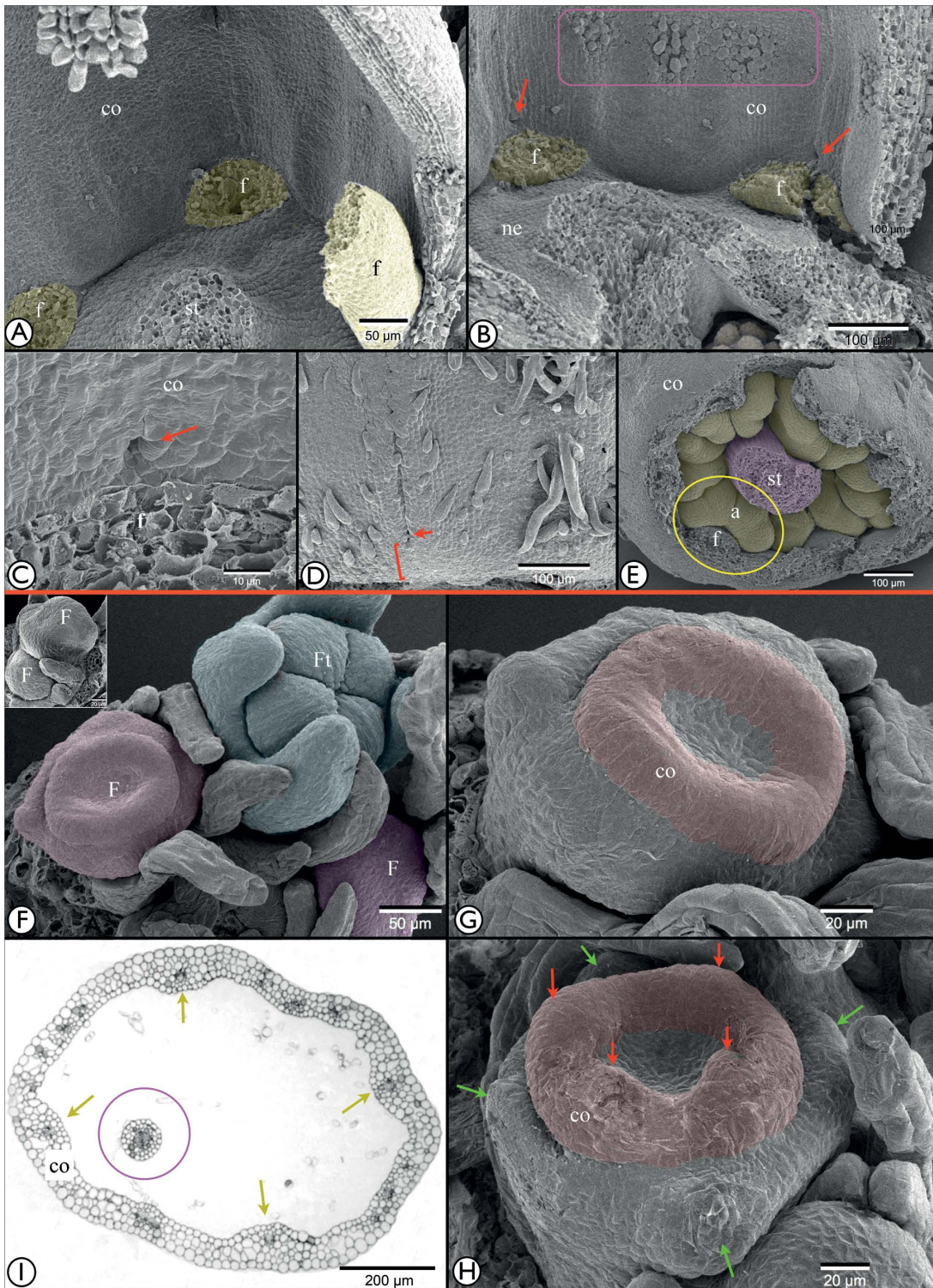
**Figure 7** – Corolla development in *Pentas lanceolata* (A–E) and early developmental stages in *Spermacoce occultiseta* (F–I): A, adaxial view of a corolla at early developmental stage; B, idem as in A, somewhat later in the development; C, detail of the opening (arrowed) above the insertion point of the filaments (scar); D, abaxial view of the developing corolla; E, view from below the tubular corolla; F, view of a cymosely branched cluster of flowers at different, early developmental stages; G, flower at very early developmental stage; H, successive stage; I, transverse section below the insertion points of the filaments through a tubular corolla of a developing flower.

Abbreviations: a, anther; co, corolla (lobe); f, filament; F, flower (primordium); Ft, terminal flower; ne, nectary; st, style. Colour code: green, calyx; red, corolla; yellow, androecium.

See appendix at the end of this paper for a more detailed caption of this figure.
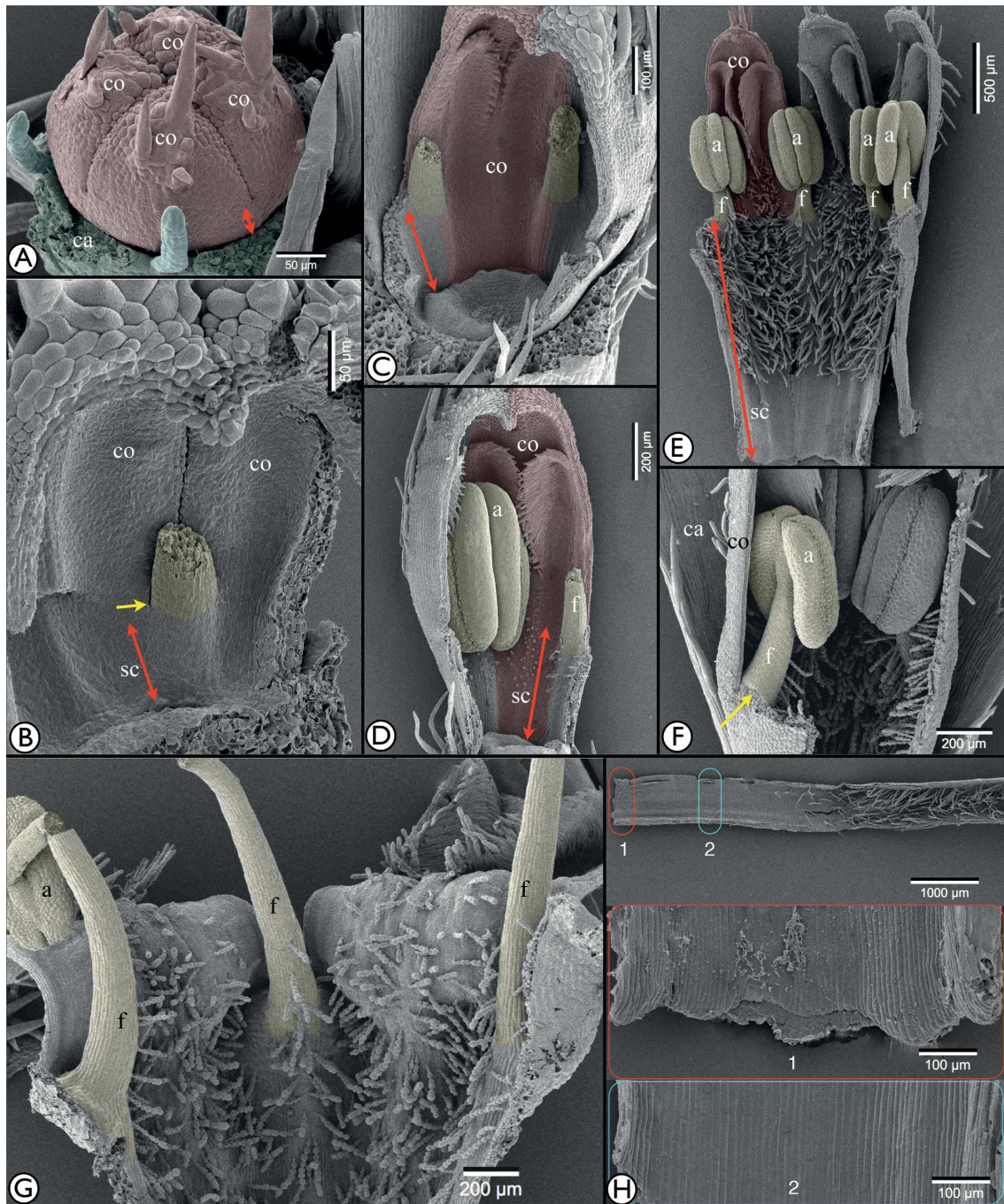
**Figure 8** – SEM micrographs of the development of corolla and androecium in *Spermacoce occultiseta*: A, apical view of a developing flower. On the corolla lobes (red), large trichomes appear. Between the scars of the removed calyx lobes, colleters are visible (blue); B, successive developmental stage. Adaxial view of a part of the corolla with a filament (yellow, arrowed, anther removed) inserted at the base of two corolla lobes. The zone below the insertion of the filaments is a stamen-corolla tube (indicated by a red double arrow); C–E, successive stages, with the stamen-corolla tube developing from its base, raising the stamens and corolla lobes to a higher position (indicated by a red double arrow). In red, the part of the corolla corresponding to a single corolla lobe. Hairs originate in the zone around the insertion points of the filaments and are raised together with the stamens (compare D and E). At the adaxial side of the corolla lobes, appendices appear; E, semi-mature corolla with a large zone of moniliform hairs immediately below the corolla lobes (of which one is coloured in red). Each corolla lobe has two appendages, each covering one theca of an anther of two neighbouring stamens; F, detail of the attachment (arrowed) of a stamen at the base of two adjacent corolla lobes (one removed, only the scar is visible); G, adaxial view of the throath of a mature corolla with elongated filaments inserted at the base of two adjacent corolla lobes; H, adaxial view of a mature tubular corolla with details of the basal zone (1, encircled in red) and of a zone higher up (2, encircled in blue). The LM-image in figure 7 is a transverse section in this zone. Abbreviations: a, anther; ca, calyx; co, corolla (lobe); f, filament; sc, stamen-corolla tube. Colour code for A–H: green, calyx; red, corolla; yellow, androecium.
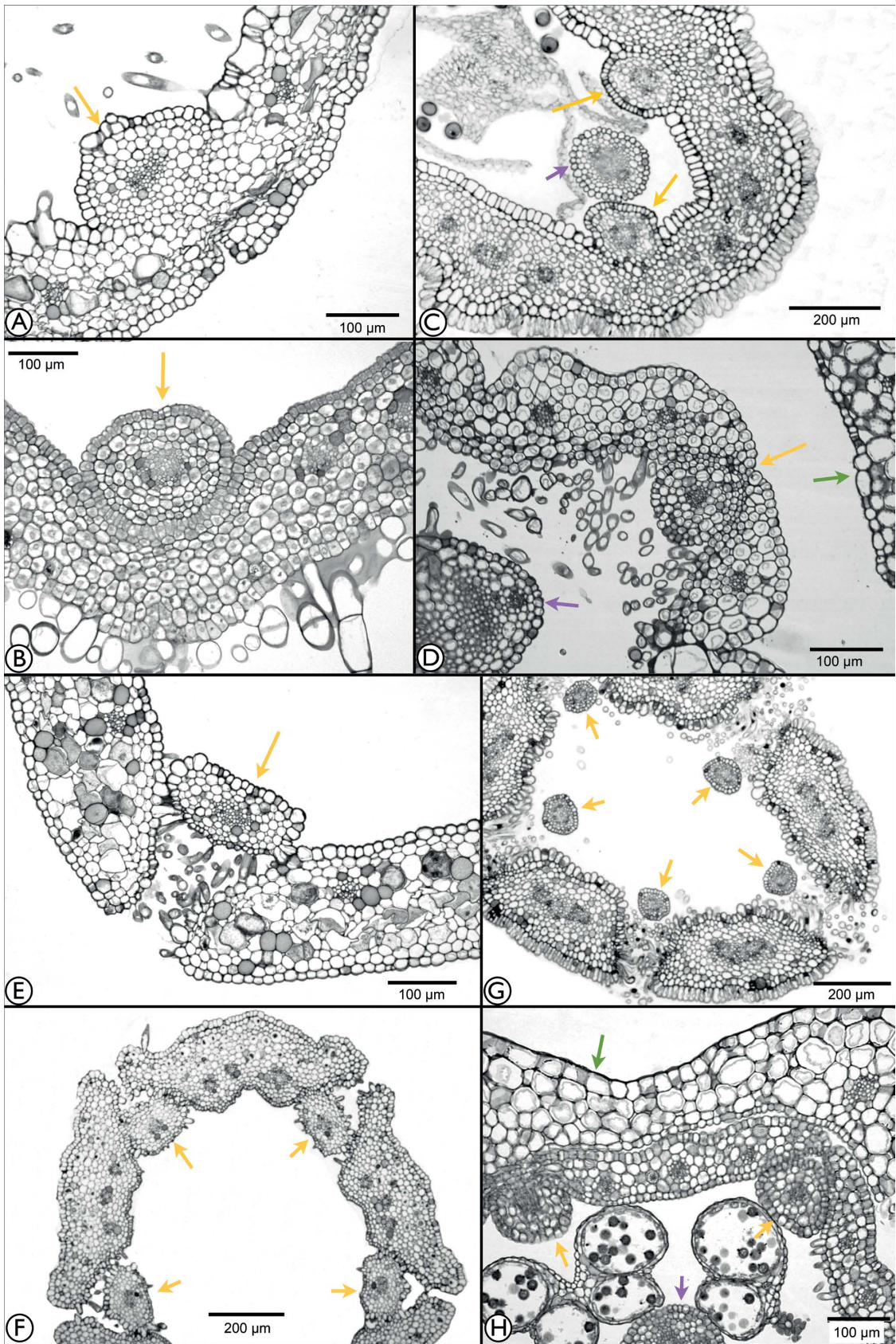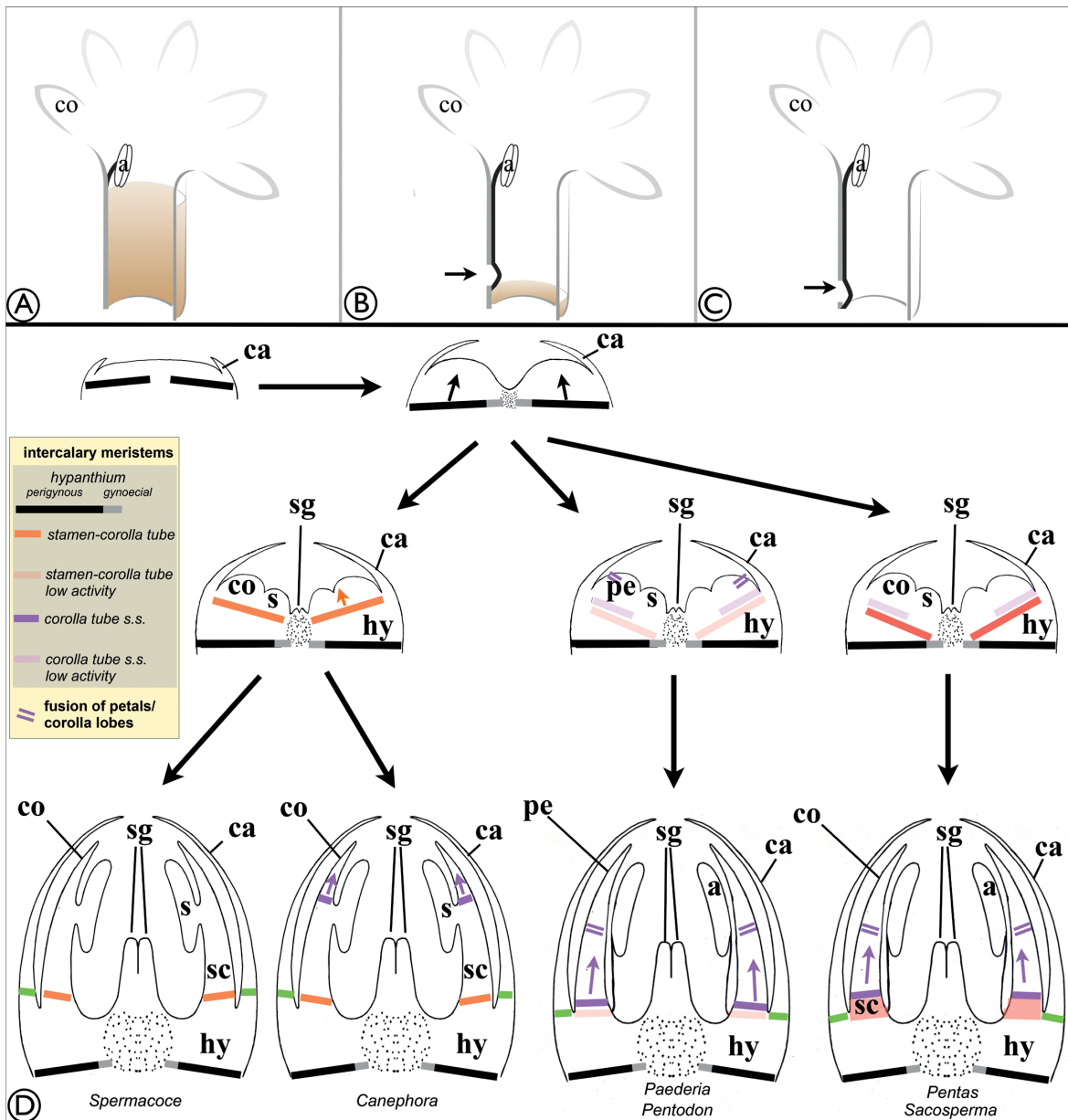
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**Figure 9** – LM images of transverse sections half way the height of the tubular corolla (A–D) and in the zone of the corolla splits (E–H) in developing flowers: A & E, *Sacosperma paniculatum*; B & F, *Pentas lanceolata*; C & G, *Paederia farinosa*; D & H, *Pentodon pentandrus*. H is a section through a young flower bud. Green arrow; calyx; yellow arrow, filament; purple arrow, style.

corolla tube *sensu stricto* combined with postgenitally fused bases of the petals respectively corolla lobes.

**Congenital fusion, postgenital coherence and underlying annular intercalary meristems** – In our opinion, tubular structures originating from an annular meristem are not 'fused' in a literal sense. Therefore, we do not advocate the use of the expression 'congenitally fused petals'. However, from an evolutionary point of view, a floral tube usually is a result of synorganisaton of floral organs of the same kind, of

the same whorl. "Primordia of organs are confluent so that the organs have a shared base from the beginning. Longitudinal growth is by intercalary elongation (zonal growth) of the shared basal part" (Endress 2006: 29; about meristematic fusion, see also Hagemann 1973). The development of a stamen-corolla tube and a corolla tube *sensu stricto* concur with Endress' (2006) definition of 'congenital coherence'. According to Endress (2001), sympetaly is a key-innovation in flowers of the asterids, resulting from a combination of meristem fusion of the petal primordia and elongation from



**Figure 10** – Schematic presentation of corolla and androecium in the genera studied + *Canephora* (De Block & Vrijdaghs 2013; A–C) and schematic outline after Leins & Erbar (2010: 102) of the development of tubular corollas from underlying intercalary meristems in the species studied (D): A, *Canephora/Spermacoce*; B, *Pentas/Sacosperma*; C, *Paederia/Pentodon*; D, developmental models for *Canephora/Spermacoce*, *Paederia/Pentodon*, and *Pentas/Sacosperma*. Colour code for A–C: black, filament; brown, stamen-corolla tube. Black arrow indicates a corolla split.
Abbreviations: a, anther; hy, hypanthium; ca, calyx lobe; co, corolla (lobe); pe, petal; s, stamen; sc, stamen-corolla tube; sg, stigma lobe. The dotted zones indicate the (developing) inferior ovary.

an intercalary meristem. In addition, postgenital fusion of the corolla lobes, which each develop from an individual 'primordium', is possible.

As for Gentianales, already in 1983, based on an anatomical study, Nishino concluded that: "The lower portion of the corolla tube is formed by the elongation of the common base of petal and stamen primordia, resulting in the formation of the epipetalous condition of stamens..." (Nishino 1983: 223). Nishino actually described what we now call a stamen-corolla tube. The stamen-corolla tube (in Rubiaceae) is an example of synorganisation between two adjacent whorls. Consequently, according to our observations in all species studied including *Canephora* (De Block & Vrijdaghs 2013), we interpret the tubular corolla as a compound organ consisting of two or three main parts, each with its own origin: a stamen-corolla tube developing from an annular primordium (which may be considered to be the result of a congenital fusion between all petal primordia and all stamen primordia) concurring with epipetaly, a corolla tube *sensu stricto* originating from an annular intercalary meristem or from an annular primordium (which can only be considered to be the result of a congenital fusion of petals when the stamens are not epipetalous) and possibly a distal part resulting from a fusion of initially free corolla lobes or petals, hence "postgenitally coherent" (Endress 2006: 29). Therefore, we think that in this context, using the term 'congenital fusion' is confusing and does not contribute to a better understanding of the development of the tubular corolla.

**Corolla lobes or petals?** − In the context of the above explanation, we can approve the term 'petals' for corolla lobes, as it was used by Vaes et al. (2006) in their study of the corolla appendages in *Spermacoce*, since one can consider their position as raised by the formation of a stamen-corolla tube below them. However, in this paper, we used the more neutral term 'corolla lobes': in *Canephora*, *Pentas, Sacosperma*, and *Spermacoce*, the term 'corolla lobes' refers to petals raised by the development of a stamen-corolla tube and/or a corolla tube *sensu stricto* below them. In addition, in *Pentas* and *Sacosperma*, the bases of the corolla lobes may be postgenitally fused (the seam between them is visible at earlier developmental stages, see e.g. fig. 7D). In *Paederia* and *Pentodon*, the term 'corolla lobes' refers to the distal free parts of postgenitally fused petals. Consequently, in these genera, we used the term 'petals' for the corolla parts at developmental stages before postgenital fusion.

**Epipetaly** − The length of the stamen-corolla tube determines the insertion point of the stamens. If no stamen-corolla tube is formed, the stamens are inserted in the hypanthium. If an intercalary stamen-corolla tube meristem is present but not growing out (as is the case in the *Paederia* and *Pentodon* species studied here), the stamens are inserted basally, sharing a same hypanthial structural origin with the corolla with as a possible symptom that the filaments and the base of the corolla adhere to each other in the hypanthium (e.g. fig. 2J). If a stamen-corolla tube is formed, the stamens are epipetalous. The height of the stamen-corolla tube in combination with the extent to which a corolla tube *sensu stricto* is formed and/or the bases of the corolla lobes fuse postgenitally determines where the filaments separate from the tube (like in *Canephora*/*Spermacoce*, fig. 8; or like in *Pentas*/*Sa-*

*cosperma*, figs 5 & 6). This is an example of Troll's (1948, 1959) principle of variable proportions. Figure 9A-D shows different degrees of postgenital coherence between the filaments and the tubular corolla in between the corolla splits and the corolla lobes in respectively *Sacosperma*, *Pentas*, *Paederia* and *Pentodon*.

**Early and late sympetaly** − Early sympetaly was first defined by Erbar (1991) and later on again by Erbar & Leins (1996a, 1996b) and Leins & Erbar (2010: 96) as follows: "Petals arise on a ring primordium and are connected already at initiation; stamen primordia originate in front of the interprimordial connections**.**" In contrast, late sympetaly is the result of postgenital fusion of the petals. Ronse Decraene & Smets (2000) nuanced the definitions of Erbar (& Leins) stating that early sympetaly concurs with the formation of a concave floral apex at early developmental stage, while late sympetaly concurs with a convex floral apex. Moreover, Ronse Decraene & Smets (2000: 166) discussed the possibility of "a stamen-petal tube [stamen-corolla tube] in the sense of a basal meristematic zone", which according to them was left out in the discussion of Erbar & Leins. In all species studied here, a concave floral apex was formed and evidence is present for a basal meristematic zone contributing to the initiation of both corolla and androecium. In *Spermacoce* (fig. 7F−H) and *Canephora* (De Block & Vrijdaghs 2013), the stamen-corolla tube originates from a ring primordium, thus constituting a straightforward example of early sympetaly. In *Pentas* and *Sacosperma*, the situation is similar to the one in *Spermacoce* and *Canephora*, albeit with a very short stamen-corolla tube. However, in *Paederia* and *Pentodon*, the situation is less clear: no stamen-corolla tube is formed but the filaments and the base of the tubular corolla tend to adhere to each other (figs 2J & 4M). Moreover, five petals originate from individual primordia situated on the annular rim of an, at that stage, concave floral apex (figs 2A & 4C) and subsequently the stamen primordia appear at the adaxial side of the annular rim (cfr. Ronse Decraene & Smets 2000) and "in front of the interprimordial (= interpetalous) connections" (Erbar & Leins 2011: 96; see also Erbar & Leins 1996a, 1996b, and figs 2C & 4D). These observations suggest early sympetaly. However, the tubular corolla results from a combination of postgenital fusion of the basal parts of the petals and elongation. The elongation cannot be due to an underlying intercalary meristem in the initial ring wall, because in such case, the corolla splits present in both species (see below) should be raised to a higher than basal position. Therefore, we assume that a corolla tube *sensu stricto* is formed from a meristem situated above the splits. The annular rim of the concave floral apex consists of a common meristem for corolla and androecium, which does not develop into a stamen-corolla tube. Consequently, in *Paederia* and *Pentodon*, we have late sympetaly, in spite of the initially concave floral apex.

**Conclusions for the corolla** − All tubular corollas studied are homologous due to a same structural origin and relative position in the flower, in spite of the fact that they reach maturity through combinations of three major developmental processes which, depending on the species studied, occur at variable proportions. It actually is the difference in developmental processes that makes them distinct character states of

the character 'tubular corolla'. The distinct character states are cryptic, because at first sight, the mature tubular corollas seem to be similar. Only after thorough (ontogenetic) investigation, one can distinguish them as distinct character states. A wide spectrum study in the entire family of the development of the tubular corolla is ongoing, in order to determine (1) whether the here observed developmental patterns are consistently present and (2) whether the proportions in which they are active during the development of the tubular corolla are clade specific.

### Corolla splits

In the species studied, corolla splits develop in two different ways. In *Sacosperma* and *Pentas*, corolla splits appear actively in the basal part of an initially closed tubular corolla (figs 6A, B, J−L & 7A−D). Later, conspicuous papillate cells develop around the splits and at the bases of the filaments (fig. 6F & G). This phenomenon of papillate cells around the splits and on the filaments at the level of the splits, is also observed in *Paederia* (fig. 3A) and *Pentodon* (fig. 4G & H) where in contrast to *Sacosperma* and *Pentas*, the corolla splits result from an incomplete fusion of the corolla lobes (figs 2G & 4F). In all species studied, the corolla splits occur at the same relative position in the flower/tubular corolla. As a consequence, we consider them to be morphological homologues. However, since splits can be formed by two different developmental patterns, we distinguish two character states of the character 'corolla splits': the *Paederia/Pentodon* type splits and the *Pentas/Sacosperma* type splits. The two character states are called cryptic, since the two different developmental processes result in seemingly similar corolla splits.

### Additional observations

**Calyx** − In the flowers of the species studied, like in most other Rubiaceae, the gynoecium is inferior due to the formation of a hypanthium. The calyx, corolla and androecium originate from the hypanthium and therefore their ontogenies are closely related. This particularly complicates the determination of the origin of the calyx, which could be an explanation for Payer's (1857) observation that the calyx in *Asperula*, *Galium* and *Rubia* appears after the formation of corolla and androecium, which on first sight seems in contrast to our observations in *Paederia*, *Pentodon*, *Sacosperma* and *Spermacoce*, as well as in *Canephora* (De Block & Vrijdaghs 2013). However, in our opinion, the meristem from which the hypanthium is formed also comprises the meristem from which the calyx originates (De Block & Vrijdaghs 2013). This may explain why initially calyx lobes appear in an irregular succession on the rim of the, at that stage, rather disciform floral apex (figs 4A, B, 5A−C & 7F−H). Later on, a calyx tube *may* grow out from the underlying annular intercalary meristem (below the calyx lobes). As for *Asperula*, *Galium* and *Rubia*, Payer (1857) described the calyx as a circular rim without, at any developmental stage, calyx lobes. We, like Payer, also observed that if a calyx tube is developed (it usually remains very short; e.g. fig. 2F), this happens late in the floral development after the appearance of corolla and androecium. Therefore, our observations of the development of the calyx and those of Payer (1857) concur,

corroborating our view that the calyx originates from an annular intercalary meristem in the hypanthium.

**Corolla appendices** − In *S. occultiseta*, corolla appendices appear in the course of the development of the corolla lobes (fig. 8C−E). A thorough study of this particular phenomenon in *Spermacoce* was done by Vaes et al. (2006).

**Unequal filament development** − In *Paederia thouarsiana*, the filaments develop unequally (fig. 3H), resulting in different filament lengths. According to Puff & Igersheim (1991), this phenomenon is rare in Rubiaceae. However, it is common in *Paederia*, with as exceptions *P. bojeriana* (A.Rich. ex DC.) Drake and according to our observations also *P. farinosa*. According to Robbrecht (1988: 86), 'unequal filaments' can be found in five other rubiaceous genera.

### CONCLUSIONS

In all the species studied, including *Canephora madagascariensis*, the tubular corolla is a **compound structure** resulting of a **combination of three major developmental processes**: (1) the development of a stamen-corolla tube from an underlying annular intercalary meristem; (2) the development of a corolla tube *sensu stricto* from an annular intercalary meristem; and (3) postgenital fusion of the basal part of the corolla lobes. Consequently, similar looking tubular corollas may constitute different, cryptic character states.

The position of the stamens depends on proportion of the contribution of each of the developmental processes:
- in *Spermacoce occultiseta* and *Canephora madagascariensis*, process 1 is very active in combination with little activity of process 2 and probably no activity of process 3. Consequently, the stamens are fixed just below the bases of the corolla lobes.
- in *Sacosperma paniculatum* and *Pentas lanceolata*, process 1 is little active whereas process 2 is very active. Consequently, the stamens are fixed in the basal part of the tubular corolla.
- in the two *Paederia* species studied and in *Pentodon pentandrus*, process 1 is inactive, process 3 is active possibly in combination with process 2. Consequently, the fixation points of the filaments are in the hypanthium, though there is adherence to the fixation area of the corolla.

*Canephora madagascariensis*, *Pentas lanceolata*, *Sacosperma paniculatum* and *Spermacoce occultiseta* are early sympetalous. In the two species of *Paederia* studied and in *Pentodon pentandrus*, no stamen-corolla tube is formed. The tubular corolla results from partial postgenital fusion of the petals in combination with elongation. Therefore, we consider the tubular corolla in *Paederia* and *Pentodon* to be late sympetalous, at least in the species studied here.

The corolla splits described here are morphological homologues, but constitute two different, cryptic character states: splits resulting from incomplete fusion of the petals (*Paederia* and *Pentodon*) and splits resulting from an active split forming process (*Pentas* and *Sacosperma*) in an initially closed tubular corolla wall. We call the splits in respectively *Paederia/Pentodon* and *Pentas/Sacosperma* cryptic character states, since they may easily be mistaken for one single character state.

In following studies, we intend to extend our investigation of the corolla to the entire family, with special interest for the other genera where corolla splits occur.

## ACKNOWLEDGEMENTS

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**Appendix** – Detailed captions of figs 4 & 7 appearing in this paper.


**Figure 4** – SEM micrographs of the early floral and corolla development in *Pentodon pentandrus*: A, cluster of flowers at successive early developmental stages; in purple, the terminal flower (most developed) and in blue a yet undifferentiated lateral flower primordium; B, detail of the terminal flower of A, five calyx lobes (green) are already visible. The floral apex becomes concave with petal primordia appearing; C, next developmental stage, with five individual petal primordia (arrowed) on the rim of the concave floral apex; D, five petals alternate with the calyx lobes. Alternating with the petals, at the adaxial side of the collar of the concave floral apex, individual stamen primordia originate, surrounding a hollow centre (arrowed); E, developing flower with the petals enveloping the inner part of the flower; F, adaxial view of a developing corolla with partially fused corolla lobes. At the base of the tubular corolla, the fusion between the corolla lobes is not complete (arrowed), resulting in splits; G–H; lateral, adaxial view of successive stages of the development of corolla and corolla splits. Along the rims of the splits, extending towards the fusion seam between two corolla lobes, papillate cells originate (purple lines); I, view from below of a developing stamen (yellow) at early developmental stage with the base of the filament (encircled) adhering to the base of the corolla. Petals are fused forming a corolla tube (red). A basal corolla split (arrowed) is visible abaxially. The filament is inserted opposite and below the basal split; J, view of a developing pistil and stamens surrounding it. The insertion point of the stamens is on the hypanthium (arrowed for the left stamen); K, adaxial view of a part of a corolla with stamens removed. The scars of the insertion points of the filaments are arrowed. The fusion joint is visible (vertical purple lines). Sticky hairs are formed in a zone (encircled) above the basal part of the tubular corolla; L–M, the tubular corolla elongates from the base, simultaneously with the filaments (arrowed). The sticky hairs develop into numerous moniliform hairs, and the hairy zone as a whole (encircled) is raised by the elongation of the tubular corolla.
Abbreviations: a, anther; f, filament; F, flower primordium; fa, floral apex; ca, calyx (lobe); co, corolla (lobe); ne, nectary; pe, petal; sg, stigma; st, style. Colour code: green, calyx; red, corolla; yellow, androecium.


**Figure 7** – SEM micrographs of the corolla development in *Pentas lanceolata* (A–E) and of the early developmental stages in *Spermacoce occultiseta* (F–I), including one LM image (I): A, adaxial view of a corolla at early developmental stage, stamens and style removed. The lower part of the corolla is tubular, the seam between two corolla lobes hardly visible; B, idem as in A, somewhat later in the development. Above the insertion point of the filaments, a small opening is formed (arrowed). Along the midrib of the corolla lobes, and left and right of it, hairs originate (encircled). These develop into a hairy zone, which is raised with the elongation of the tubular corolla (not shown); C, detail of the opening (arrowed) above the insertion point of the filaments (scar); D, abaxial view of the developing corolla. Fusion seams are visible between the corolla lobes. A tubular corolla (hooked) appears below the fused corolla lobes. At the lowest point of the fusion seam, the above-mentioned opening is sometimes also visible from the abaxial side (arrowed); E, view from below the tubular corolla. One stamen is encircled, with the filament fixed in the basal tubular corolla; F, view of a cymosely branched cluster of flowers at different, early developmental stages from undifferentiated flower primordium (dark purple) over early flower with first floral organ primordia appearing (light purple) to developing terminal flower (blue). In the inlay, a flower primordium (below) and a developing flower with convex floral apex and appearing calyx lobe primordia are visible; G, flower at very early developmental stage, with developing calyx lobes and an annular corolla primordium surrounding a now concave floral apex; H, successive stage. Four corolla lobe primordia (red arrows), alternating with the calyx lobes (green arrows), become visible; I, transverse section below the insertion points of the filaments through a tubular corolla of a developing flower. Where filaments are supposed to be present, the tubular corolla is somewhat thicker because of the presence of a staminal vessel (arrowed). Encircled in purple is the single style. See also figure 8H.
Abbreviations: a, anther; co, corolla (lobe); f, filament; F, flower (primordium); Ft, terminal flower; ne, nectary; st, style. Colour code: green, calyx; red, corolla; yellow, androecium.