REGULAR PAPER

Enigmatic floral structures in *Alternanthera*, *Iresine*, and *Tidestromia* (Gomphrenoideae, Amaranthaceae). A developmental homology assessment

Alexander Vrijdaghs[1,2,*], Hilda Flores-Olvera[3] & Erik Smets[1,4]

[1]Ecology, evolution and biodiversity conservation KU Leuven, Kasteelpark Arenberg 31, P.O. Box 2435, B-3001 Heverlee (Leuven), Belgium
[2]Botanic Garden Meise, Nieuwelaan 38, 1860 Meise, Belgium
[3]Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Apdo. Postal 70-367, México D.F. CP 04510, México
[4]Naturalis Biodiversity Center, P.O.Box 9517, 2300 RA Leiden, The Netherlands
*Author for correspondence: alexander.vrijdaghs@bio.kuleuven.be

Background and aims – Amaranthaceae comprise the former Chenopodiaceae, *Beta*, and core-Amaranthaceae. Particularly in the Gomphrenoid clade, most species have flowers with an 'androecial tube'. On its rim and alternating with the stamens, so-called (pseudo-)staminodia occur. However, neither their origin nor function have been clarified. We aimed to determine the nature of both the 'androecial tube' and pseudostaminodia in species of three genera: *Iresine*, *Alternanthera* and *Tidestromia*. In addition, we also aimed to document the development of the gynoecium.

Methods – Scanning electron microscopic (SEM) and light microscopic (LM) observations of the floral development.

Key results – Five individual sepal primordia appear in quincuncial order, followed by the appearance of five individual stamen primordia opposite them. At semi-maturity, the stamens are raised by the formation of a tube below them, with at its inside surface a glandular zone. Alternating with the stamens, appendages develop from individual primordia on the rim of the androecial tube. In bisexual/female flowers, from a primary gynoecium primordium, secondarily, an annular gynoecium wall primordium is formed, surrounding a central zone from which a single ovule develops. At maturity, the gynoecium stands on a gynophore. In *Alternanthera microcephala*, the gynophore may become large, sometimes developing appendages.

Conclusions – Our hypothesis that the androecial tube develops from an underlying annular intercalary meristem is supported by the absence of an annular androecium primordium and the late development of the androecial tube. The 'pseudostaminodes' are appendages of the androecial tube. Their often conspicuous appearance and the glandular inside surface of the androecial tube suggest an animal(insect)-based pollination syndrome. We consider the androecial tube, the staminal ring in flowers of *Beta vulgaris* and the 'fused filaments' in some Chenopodioideae as morphological homologues. We suggest that the development of the gynoecium wall might be decoupled from the development of the ovule.

Key words – Androecial tube, appendages, floral development, gynophore, insect-based pollination syndrome, morphological homology, floral nectaries, (pseudo-)staminodia, scanning electron microscopy.

INTRODUCTION

In the APG classification, the former families Amaranthaceae (Moquin-Tandon 1849, Bentham & Hooker 1880, Schinz 1893) and Chenopodiaceae (Bentham & Hooker 1880) were merged into a single family Amaranthaceae (APG 2009, Stevens 2001 onwards). Within this family, core-Amaranthaceae or Amaranthaceae *sensu stricto* correspond to a subclade (Kadereit et al. 2003, Müller & Borsch 2005), consisting of of herbs, shrubs or trees with centers of diversity in the neotropics, tropical and southern Africa, and Australia (see among others Heywood et al. 2007).

Amaranthaceae *sensu stricto* are subdivided into an Amaranthoideae clade characterised by tetrasporangiate anthers

and a Gomphrenoideae clade characterised by disporangiate anthers (Schinz 1893, Endress & Stumpf 1990), opening with a single longitudinal slit. Sánchez-del Pino et al. (2009) confirmed the monophyly of the Gomphrenoideae. However, (sub-)tribes that were earlier delimited using inflorescence and floral (androecial) characters showed to be poly- or paraphyletic. According to the same authors, Gomphrenoideae consists of a basal, so-called iresinoid clade (*Irenella*, *Iresine*, *Woehleria*), sister to a clade with a alternantheroid branch (*Alternanthera*, *Pedersenia* and *Tidestromia*) and a core-gomphrenoid branch (fig. 1). *Irenella* and *Woehleria* are monotypic endemics of respectively Ecuador and Cuba. In contrast, *Iresine* (fig. 2A–D, H) is one of the most species-rich genera in Amaranthaceae with c. 45 species mainly distributed in the Americas, with its center of diversity and endemism in Mexico (Zumaya et al. 2013), although some species are distributed in tropical Africa. Core-Gomphrenoids occur in America, the Caribbean, Africa and Australia. *Alternanthera* (fig. 2I) consists of about 100 species mainly in the New World. It is characterised by an invariable floral structure, which contrasts with the a large variation in vegetative characters. *Tidestromia* (fig. 2E–G, J) comprises eight species in Mexico and the south-western USA (Sánchez-del Pino & Motley 2010).

The inflorescences of Amaranthaceae *sensu stricto* are terminal or axillary, spiciform or capitate, sometimes compound racemes or panicles (see for example Eliasson 1988, Townsend 1993 and Acosta et al. 2009). Eliasson (1988) mentioned clusters of flowers that he called 'cymules' consisting of a terminal flower and lateral flowers in its bracteoles. The terminal flower (and its cluster) is subtended by a bract.

Flowers are usually inconspicuous and have three to five perianth parts with opposite stamens in the same number. Payer (1857) called the in quincuncial order appearing perianth parts 'sepals', insisting on the absence of a corolla. Eliasson (1988), Judd et al. (1999), and Borsch (2007) called the perianth parts in Amaranthaceae 'tepals'. Flores-Olvera et al. (2008, 2011) used the term 'perianth part'. However, following Payer (1857) and several more recent authors of the German school such as Rohweder (1967, 1970), Rohweder & Huber (1974) and Urmi-König (1981), who considered the perianth in Caryophyllales to have lost the corolla, we will further call the perianth parts 'sepals'.

The stamens are "free or often united at the base in a tube from which petaloid appendices arise between the stamens in some genera" (Heywood et al. 2007: 29). The superior ovary is two or three-carpellate and unilocular with in most species a single centrally placed ovule. For this and other reasons,
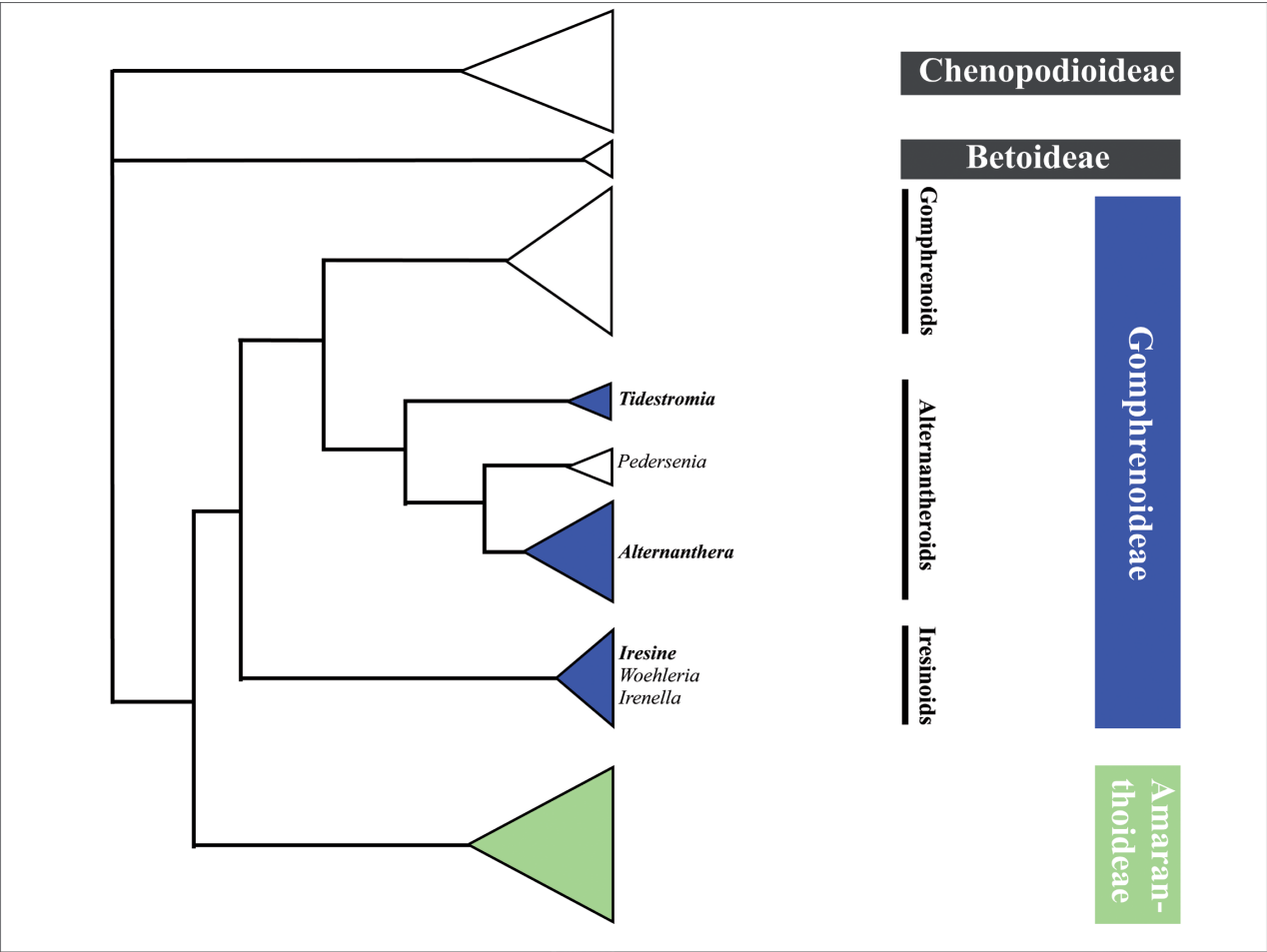

Figure 1 – Simplified cladogram of Amaranthaceae *sensu stricto* based on Sanchez-del Pino et al. (2009). In dark blue are highlighted the genera studied.
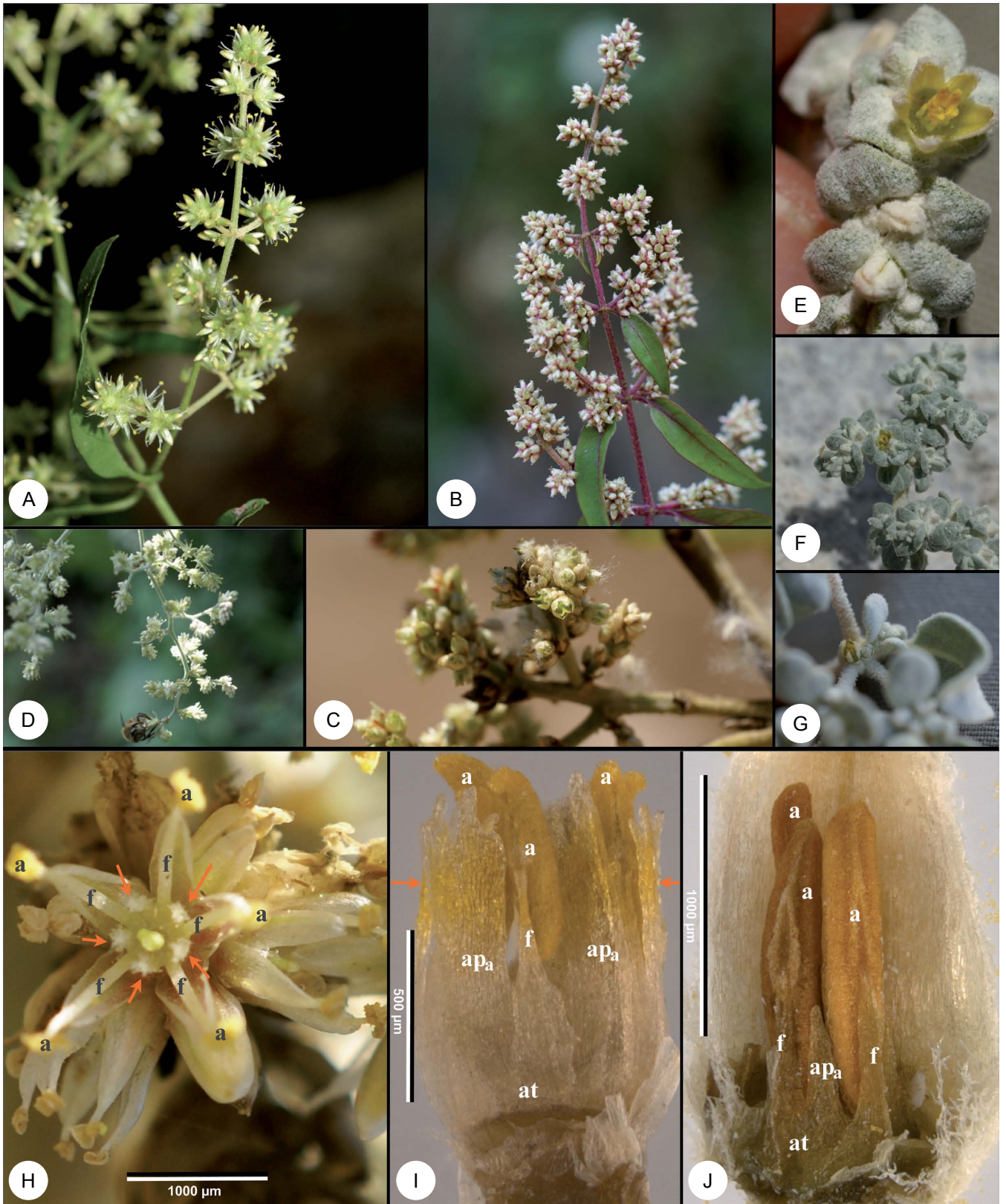
Figure 2 – Photographs of species studied. A, male inflorescence of *Iresine hebanthoides*; B–C, female inflorescence of *I. hebanthoides* and detail; D, inflorescence of *I. latifolia*; E–F, flower and inflorescence of *Tidestromia rhizomatosa*; G, inflorescence of *T. sufruticosa*; H, male flower of *I. hebanthoides* with conspicuous androecial tube appendages (arrowed); I, flower of *Alternanthera microcephala* with conspicuous androecial tube appendages. The filaments and appendages show an accumulation of bright yellow colour (arrowed); J, flower of *T. rhizomatosa*, with unconspicuous appendages of the androecial tube.

Eliasson (1988) considered Amaranthaceae to be a typical core-centrospermous family, Centrospermae are now called Caryophyllales (APG 2009, Stevens 2001 onwards).

Floral developmental studies in Amaranthaceae – Few such studies have been carried out. Payer (1857) investigated flowers in three genera; *Alternanthera*, *Amaranthus*, and *Celosia*. In *Alternanthera tenella*, he described the inflorescence as a spike with bisexual flowers in the axil of a bract. Each flower has two bracteoles. Opposite the sepals, five free stamens originate simultaneously, which "immediately become connate" (Payer 1857: 320), forming a cup around the gynoecium. In Gomphrenoideae, the anthers are disporangiate. The androecial cup grows and forms five bulges alternating with the filaments. These bulges develop in to serrated ribbon-like structures. About the origin of the gynoecium, Payer (1857) wrote that initially a single carpel primordium appears opposite sepal one. The first carpel primordium becomes quite large, then fusing with the two other carpels (opposite sepals two and three), it forms a bag-like structure, which Payer called "sac ovarien [ovarian bag]" (Payer 1857: 319–320). On page 320, Payer (1857) described the further development of the gynoecium as follows: "the developing bag-like structure subsequently forms a single style with on top a stigma. Meanwhile, the distal part of the receptacle, which is more and more surrounded by the gynoecial bag-like structure, becomes enveloped by two layers, transforming itself into a campylotropous ovule on a long funiculus".

Enigmatic structures in the flowers of Gomphrenoideae – The androecium morphology in 'iresinoid' flowers is variable; for example flowers in *Irenella* have one (rarely two) stamens and a low 'androecial tube' without appendages (Eliasson 1987). In contrast, flowers in *Whoeleria* show appendages of the androecial tube, which were described as staminodia positioned at the places of hypothetical stamens (Eliasson 1988). In *Iresine* the usually (functionally) unisexual flowers occur in dioecious plants. According to Eliasson (1987, 1988), functionally male flowers usually have five stamens with slender filaments widening towards the bases, which are 'connately fused' forming a shallow cup. Alternating with the filaments, five lobes are present on the rim of the cup. Centrally in this 'staminal cup', a rudimentary, pillar-shaped gynoecium occurs. Functionally female flowers have five rudimentary stamens positioned on a similar 'staminal cup', also alternating with five lobes. Centrally in this cup, a fertile gynoecium is present with a superior ovary and short style with two stigma branches. The five lobes of the 'staminal cup' are usually described as 'pseudostaminodia'. Eliasson (1988) proposed to use the term 'interstaminal appendages' instead of 'pseudostaminodia' for these structures, in order to distinguish them from 'filament appendages', which are lateral appendages of filaments. However, Eliasson (1971, 1987, 1988) and other authors such as Clemants (2003) and Borsch (2007) also frequently used the term 'pseudostaminodia'. Interstaminal appendages can vary from ligulate and laciniate (*Alternanthera*) to triangular (*Tidestromia*) or they can appear as wide lobes on a rudimentary staminal cup (some *Iresine* species). According to Eliasson (1988), in the genus *Guilleminea*, the perianth forms a tube at the base, which is adnate to the staminal tube. However, in most genera, there are five free hypogynous sepals (oc-

casionally four, three or even a single perianth part occurs). Eliasson (1988) considered the staminal cup to be a result of a fusion process between the filaments. Eliasson (1988) suggested that the appendages in *Gomphrena* and several other core-gomphrenoid genera, including *Alternanthera*, are homologous, in despite of the fact that he interpreted the appendages in *Gomphrena* in a different way ("apical parts of deeply notched filaments with the anthers attached in the notch"; Eliasson 1988: 243).

Aims of this study

The main objective of this study is to address the following questions: (1) Does the floral developmental pattern of the species studied concur with the pattern in *Beta vulgaris* (Flores-Olvera et al. 2008) and in the core-Chenopodioideae (Flores-Olvera et al. 2011)? (2) What is the nature of the 'androecial tube' in *Iresine* and in *Alternanthera* and *Tidestromia*? (3) What is the nature of the so-called pseudostaminodes in the species studied? (4) Does the developmental pattern of the gynoecium concur with the one in *Beta vulgaris* and in the core-Chenopodioideae? The present study is part of a larger project envisaging among others the investigation of similar floral structures within and beyond Amaranthaceae.

MATERIAL AND METHODS

Partial inflorescences of species of *Alternanthera*, *Tidestromia*, and *Iresine* were collected in the field (table 1) and immediately fixed in 70% ethanol. The inflorescences were dissected in 70% ethanol under a Wild M3 stereo microscope (Leica Microsystems AG, Wetzlar, Germany) equipped with a cold-light source (Schott KL1500; Schott-Fostec LLC, Auburn, New York, USA).

Scanning electron microscopy

The material was prepared for critical point drying by dehydration in alcohol series. It was washed twice with 70% ethanol, each time for five minutes. Subsequently, the material was placed in a mixture (1:1) of 70% ethanol and DMM (dimethoxymethane) for five minutes. Eventually, the material was transferred to pure DMM for 20 minutes. Critical point drying was done using liquid CO_2 with a CPD 030 critical point dryer (BAL-TEC AG, Balzers, Liechtenstein). The dried samples were mounted on aluminium stubs using Leit-C and coated with gold with a SPI-Module™ Sputter Coater (SPI Supplies, West-Chester, Pennsylvania, USA). Scanning electron microscope (SEM) images were obtained with a JEOL JSM-6360 (JEOL Ltd., Tokyo) at the section of Ecology, Evolution and Biodiversity Conservation (KU Leuven).

Light microscopy

The material was dehydrated using a graded ethanol series. Next, it was embedded in KULZER's Technovit 7100 (based on HEMA, hydroxyethyl-methacrylate). Seven μm sections were made with a rotation microtome Leica RM2135 equipped with disposable blades (Leica DB80L). The sections were stained with a 0.1% solution of toluidine blue in

Table 1 – Species studied and voucher data.
* DFCU, Distrito Federal Ciudad Universitaria

Species	Collected by	Location	Date	Voucher
Alternanthera microcephala (Moq.) Schinz	T Borsch H Flores-Olvera H Ochoterena A Torres	Mexico, Veracruz	9 Mar 2012	*Borsch et al.* 5351 (B, MEXU)
Iresine hebanthoides Suess.	T Borsch H Flores-Olvera H Ochoterena A Torres	Mexico, Querétaro	12 Mar 2012	*Borsch et al.* 5415 (B, MEXU)
Iresine herbstii Hook.	H Flores-Olvera	Mexico, DFCU*	22 Nov 2011	*Flores et al.* 1637 (MEXU)
Iresine latifolia (M. Martens & Galeotti) Benth. & Hook.	H Flores-Olvera	Mexico, DFCU*	22 Nov 2011	*Flores et al.* 1638 (MEXU)
Iresine orientalis G.L.Nesom	T Borsch H Flores-Olvera H Ochoterena A Torres	Mexico,Querétaro	11 Mar 2012	*Borsch et al.* 5410 (B, MEXU)
Tidestromia rhizomatosa I.M. Johnst.	H Flores-Olvera M Moore H Ochoterena N Douglas	Mexico,Coahuila	15 Aug 2011	*Flores et al. 1629* (TEX, MEXU)

aq. dest. Images were made using an Olympus BX51 microscope equipped with a Color View Soft Imaging System camera at the National Botanical Garden of Belgium. Permanent slides were deposited at UNAM and KU Leuven.

RESULTS

Floral development in *Iresine*

We examined inflorescences of *I. hebantoides*, *I. herbstii*, *I. latifolia* and *I. orientalis*. The developmental sequence from primordium to mature functionally male and functionally female flower of *I. latifolia* is presented. As for *I. herbstii*, only the development of functionally male flowers is shown. Some developmental stages of the functionally female and hermaphroditic flowers of *I. hebantoides* and *I. orientalis*, respectively, are also presented.

In *I. latifolia*, flowers are organised in cymosely branched clusters, occurring on axes which are densely covered by trichomes (fig. 3A). Each flower is subtended by a bract and has a short pedicel with two opposite bracteoles (figs 3A–B & 8A–D). At the earliest developmental stages, large amounts of trichomes appear on the main axis, pedicel and bracteoles (fig. 3A). The floral primordium is dome shaped. First, the posterior (abaxial) sepal primordium appears (fig. 3C), followed by two anterior (adaxial) ones (fig. 3D), and a fourth and fifth in quincuncial order (fig. 3E–F). At this stage, opposite each sepal, a stamen primordium appears (figs 3E–F & 8A–B). Simultaneously, the dome-like floral apex flattens, forming a primary gynoecium primordium (figs 3E & 8B–C). Next, a secondary annular gynoecium wall primordium is formed. Surrounded by the developing gynoecium wall, a single ovule is subsequently formed on a central placenta (figs 3F–G & 8D). Later on, the developing gynoecium wall, at this stage forming an immature ovary, envelops the ovule (fig. 3H & 8E), subsequently developing a style

and two stigmas. Until the closure of the ovary, the stamens develop free from each other (fig. 3H) and there is no androecial tube or staminal ring (figs 3F & 8D). With the ovary closing, beneath the stamens an annular tube is developed from the receptacle (figs 3K & 8E), the stamens remaining free from each other (fig. 3H). Until this stage, the flowers seem bisexual. However, during further development, the flowers become functionally unisexual by the development of the gynoecium, with the stamens remaining vestigial (fig. 3I), or by the development of the stamens with the gynoecium remaining underdeveloped (fig. 3M & O). A fertile gynoecium consists of a superior, unilocular ovary, a style and two stigma lobes covered with papillate cells (fig. 3I). The ovule is campylotropous (figs 3J & 8E). The tube below the stamens is formed in both functionally male and female flowers. Simultaneously with the closure of the ovary and the appearance of a floral cup beneath the stamens, the stamens differentiate into filament and anther (fig. 3K). The inside surface of the cup below the stamens (further called androecial tube) surrounds the gynoecium, obtaining numerous large stomata (fig. 3L) and becoming glandular. In *I. latifolia* as well as in the other species of *Iresine* studied, on the rim of the androecial tube, alternating with the stamens, irregularly shaped appendages appear (figs 3K, L & N, 4C–E & 5A–C & F). Meanwhile, in functionally male flowers, the stamens grow to different heights. Each stamen consists of a more or less long filament and a dorsifixed, introrse and disporangiate anther which opens with a single longitudinal slit (fig. 3O). The underdeveloped gynoecium consists of a sterile ovary with a blunt style on which the stigma branches do not develop (fig. 3O).

In *I. herbstii*, the inflorescence consists of an open main axis with many lateral flowers, the older proximal, the new ones originating below the apical part of the axis, each subtended by a bract (fig. 4A). Conspicuous trichomes are pre-
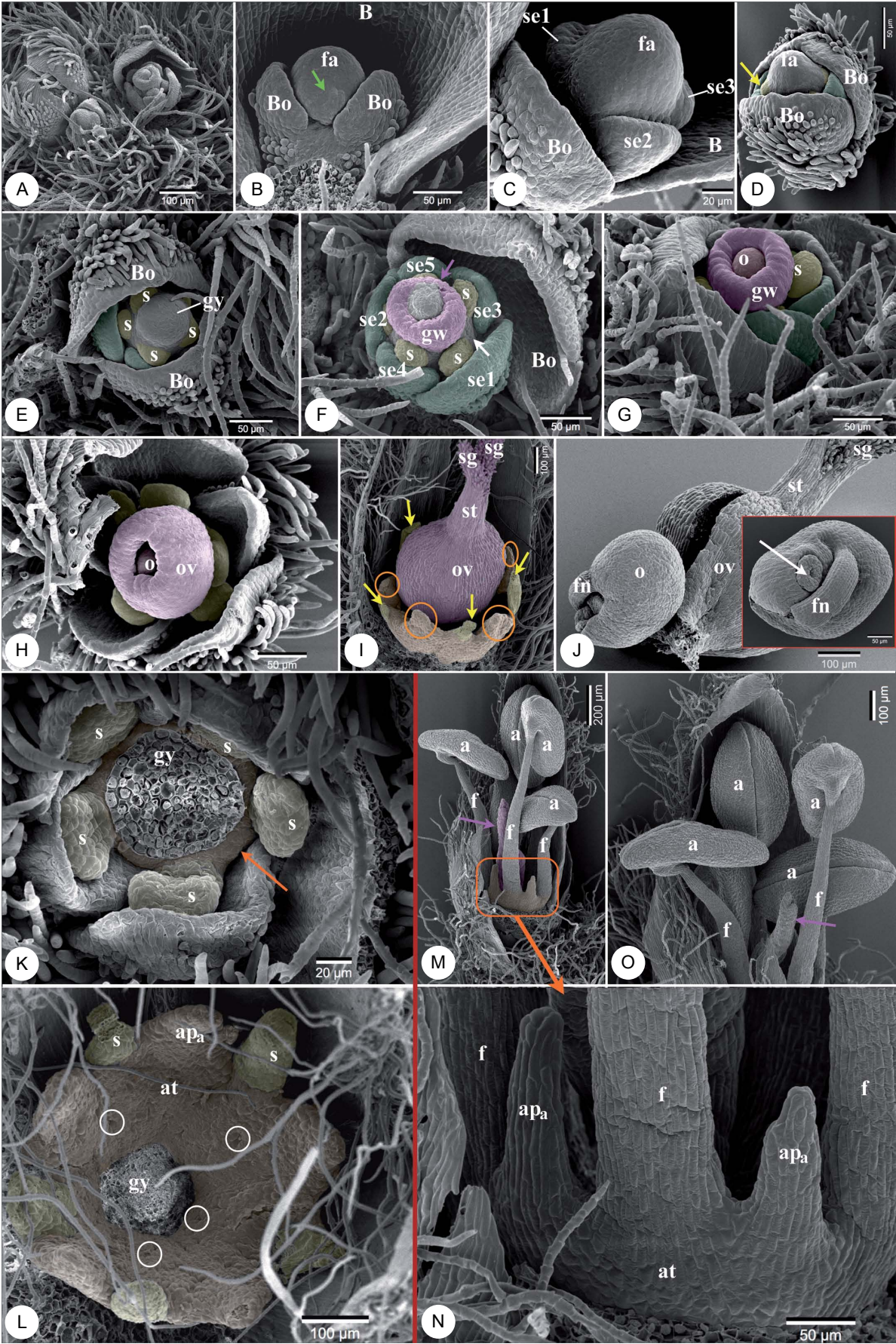
◄ **Figure 3** – SEM images of the floral development in *Iresine latifolia*. A, flower cluster on an axis densely covered with trichomes, with flowers at different developmental stages; B, adaxial view of a flower primordium protected by two bracteoles. A first sepal primordium is appearing (arrowed); C, developing flower with three sepal primordia; D, successive stage, with stamen primordia appearing (arrowed); E, developing flower with five stamen primordia opposite the sepals, and floral apex differentiating into gynoecium primordium; F, successive stage with annular gynoecium wall primordium surrounding an undifferentiated central part. At one side (arrowed), the ring is less separated from the central part; G, successive stage with immature, open ovary consisting of a bag-like gynoecium wall surrounding a developing single, central ovule; H, successive stage with the ovary closing; I, semi-mature functionally female flower with underdeveloped stamens (arrowed) upon an androecial tube with appendages (encircled). The gynoecium consists of an ovary and a single style with two papillose stigma lobes; J, opened ovary with campylotropous ovule. In frame: view of the ovule from the other side; K, top view of a developing flower with removed gynoecium. The androecial tube (arrowed) is appearing below the stamens; L, same view as in 'K' of flower at semi-mature stage. The androecial tube appendages alternate with the stamens. The inside part of the cup has many large stomata (some of them encircled); M, semi-mature functionally male flower with stamens of different heights. Androecial tube appendages are encircled. The gynoecium is underdeveloped (arrowed). The dorsifixed anthers are disporangiate with a longitudinal slit; N, detail of the androecial tube and its appendages; O, detail of anthers around an underdeveloped style/stigmas (arrowed).

Abbreviations: a, anther; at, androecial tube; ap$_a$, androecial tube appendage; B, bract; Bo, bracteole; f, filament; fa, floral apex; fn, funiculus; gy, gynoecium primordium; gw, gynoecium wall (primordium); o, ovule; ov, ovary; s, stamen primordium; se, sepal; sg, stigma; st, style. Colour code: green, perianth; yellow, androecium; orange, androecial tube; purple, gynoecium.

sent, though large parts of the surfaces of the axis and bracts are glabrous. A young, developing flower seems to be hermaphrodite, but a closer look reveals that the developing gynoecium only consists of a stylar structure. The stamens originate individually (fig. 4B), but are soon raised by the development of an androecial tube. On the rim of the androecial tube, alternating with the filaments, bulges originate from which appendages of the androecial tube develop (fig. 4C–D). The inner part of the rather small cup formed by the androecial tube and its appendages becomes covered with papillate cells. Around the base of the stylar structure, a depression is formed (fig. 4E & G). At maturity, the dorsifixed, introrse, and disporangiate anthers open with a longitudinal slit (fig. 4G), showing a tapetum covered with orbicules and small (diameter approximately 10 μm) pantoporate pollen grains (fig. 4F). No ovary is formed and the stylar structure gets its final club-like shape with a slightly papillose distal part (fig. 4G). In (semi-)mature female flowers of *I. hebanthoides*, only remnants of the stamens can be seen on a massive androecial tube with large, irregularly shaped appendages (fig. 5A–C). The gynoecium consists of an ovary and a short style with two long stigma branches of which the inner surface is covered with papillate cells (fig. 5A & D). The cup-shaped androecial tube is glandular at its inside surface, showing large stomata (fig. 5C). Hermaphroditic flowers of *I. orientalis* show a similar cup with appendages like those in *I. hebantoides* (figs 5E & F, 8G & M). The inside surface of this androecial tube is also glandular, with large stomata (fig. 5G). Mature anthers contain pantoporate pollen of the same size as in *I. hebantoides* (fig. 5H).

Floral development in *Alternanthera* and *Tidestromia*

In *Alternanthera microcephala*, the inflorescence consists of an open main axis. In the axil of each new bract, a flower originates (fig. 6A). First, two opposite bracteoles are formed (fig. 6B). Then, an adaxially positioned (posterior) first sepal primordium becomes visible (fig. 6B). In a young developing flower, five free stamens appear, each opposite a sepal (fig. 6C). At this stage, the floral apex is still undifferentiated (fig. 6C). Next, a gynoecium is formed in a similar way as in *Iresine* (fig. 6C–D). Meanwhile, the stamens enlarge and the sepals develop unequally (fig. 6D–E). Subsequently, the

stamens differentiate into an anther with filament. Already at this stage, the development of one or more of the stamens may deviate, resulting in the formation of staminodium-like structures (figs 6F & 7C). Simultaneously, the gynoecium wall develops from the base, forming a bag-like structure enveloping the central ovule (figs 6E–F & H & 7A). Concurrently with the previous stages, bulges appear upon the flower receptacle, positioned in between the filaments, from which interstaminal structures develop (fig. 6G–H). The stamens and interstaminal structures are raised by the formation of a tube below them (further called androecial tube; fig. 6H–K). Simultaneously, the interstaminal structures develop into lobed leaf-like appendages of the androecial tube. At this stage, the gynoecium consists of an ovary, style and an annular stigmatic zone with papillate cells (figs 6H–I & 7B–D). The stigma protrudes slightly above the stamens. On the inside surface of the androecial tube, an annular nectary appears, surrounding the base of the gynoecium (figs 6L & 7F).

In *Tidestromia rhizomatosa*, the developmental pattern concurs with that in *A. microcephala* (fig. 6M–Q). Like in *A. microcephala* (fig. 6D), the formation of the annular gynoecium wall is asymmetrical (fig. 6N). At a later stage, two stigma lobes are formed on the top of the bag-like gynoecium wall, one of the stigmas lobes is somewhat larger than the other (fig. 6O). At (semi-)maturity, the appendages of the androecial tube are triangular (fig. 6P). The gynoecium now consists of a swollen ovary, a short style and two papillose stigma lobes (fig. 6Q).

In *A. microcephala*, at semi-maturity, a gynophore is present which, depending on the flower examined, may be more or less developed (fig. 7D). Within the ovary, a large, campylotropous and bitegmic ovule is formed (fig. 7E). In some flowers, the gynophore grows out into a stalk with more than half of the height of the actual gynoecium (fig. 7G). Rarely, appendages resembling the androecial tube appendages are present upon the gynophore, protruding in between the ovary and androecial tube (fig. 7G–H). In modified stamens, the anthers become flat structures with few, conspicuous trichomes on inner and outer surfaces (fig. 7I–J).

Additional observation

In all flowers with mature anthers, the tapetum was rippled, covered by a layer of orbicules (figs 4F & 5H).
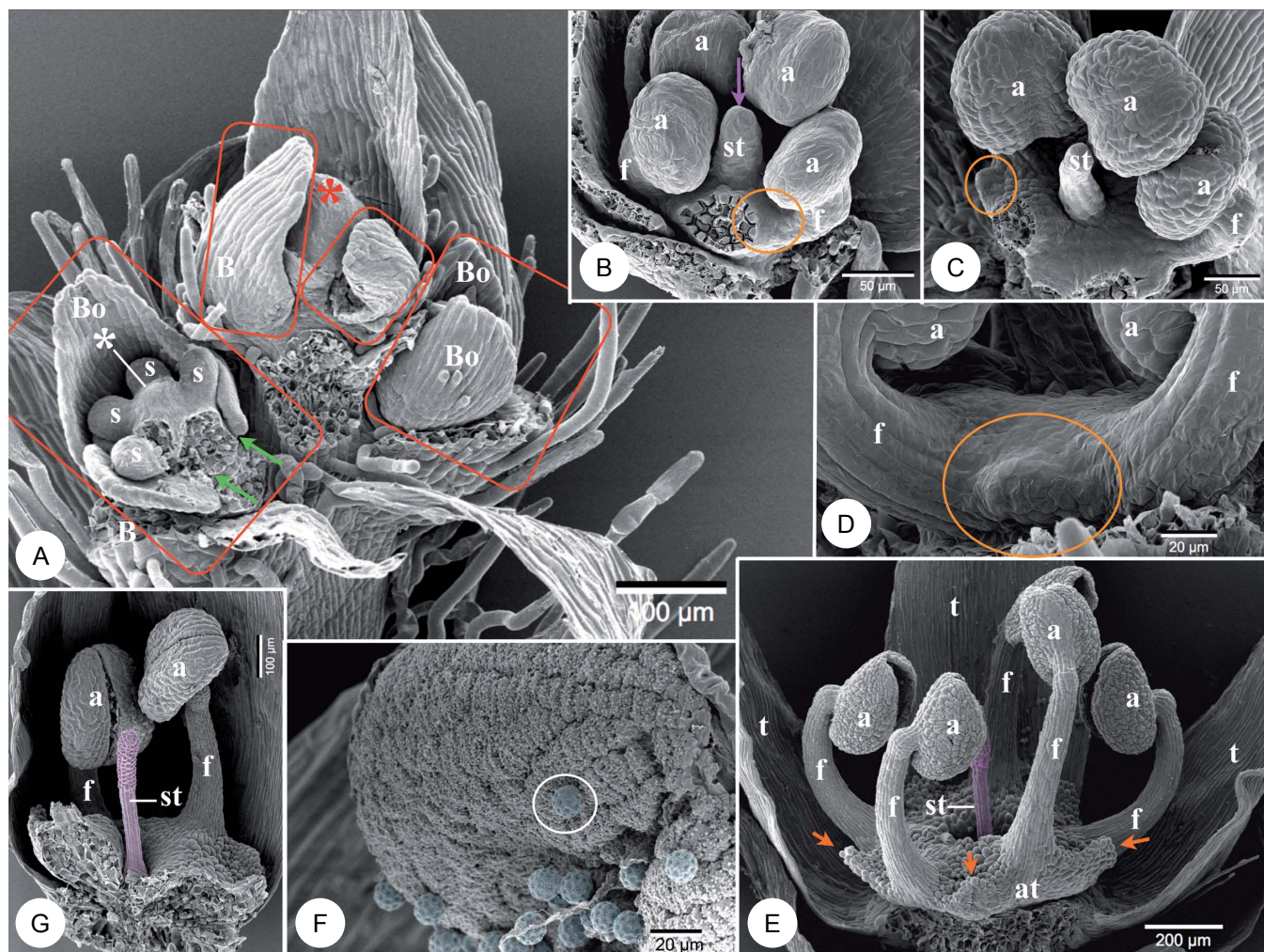
▲ **Figure 4** – SEM images of the development in functionally male flowers of *Iresine herbstii*. A, distal part of indeterminate inflorescence axis with below the apex flowers (encircled) at different developmental stages, each flower subtended by a bract, and standing on a pedicel with two opposite bracteoles. The flower at the left hand side shows four stamen primordia (one removed) and a conical floral apex. The sepals are free at the base (arrows); B, lateral view of a developing flower with the androecial tube originating (one stamen removed). At this stage, no appendages are visible (encircled) in between the stamens. From the floral apex, a massive stylar structure originates (arrowed); C–D, successive stage. Primordia of androecial tube appendages (encircled) appear alternating with the filaments; E, the androecial tube and its appendages (arrowed) become papillose. Around the stylar structure a depression is formed; F, opened anthers with the tapetum, covered with orbicules. Pollen grains (one encircled) have a diameter of approximately 10 μm and are of the Iresine-type *sensu* Borsch (1998); G, the stylar structure is club-shaped with the distal part slightly papillose.
Abbreviations: a, anther; at, androecial tube; B, bract; Bo, bracteole; f, filament; s, stamen primordium; se, sepal; st, style; *, floral apex. Colour code: purple, stylar structure. Blue, pollen.

► **Figure 5** – SEM images of floral developmental stages in female flowers of *Iresine hebanthoides* (A–D) and bisexual flowers in *I. orientalis* (E–H). A, nearly mature functionally female flower. The ovary is surrounded by a massive androecial tube with large appendages, the stamens are underdeveloped. The style is terminated by two filiform stigma lobes with the inner sides papillose; B, detail of the androecial tube and appendages from 'A'; C, view of the androecial tube glandular cup with large stomata (encircled). The gynoecium is removed; D, detail of the stigma lobes; E, developing flower with dorsifixed, introrse anthers with a single longitudinal slit and disporangiate. On top of the gynoecium, an annular, slightly bilobed stigmatic zone is developing; F, lateral view of a mature flower. The gynoecium is standing on a gynophore (arrowed) and has a short style with an annular, slightly bilobed and papillose stigma; G, detail of the glandular inner part of the androecial tube (gynoecium removed), with large stomata (encircled); H, detail of a tapetum with orbicules and pollen grains (10 mm diameter) of the Iresine-type *sensu* Borsch (1998).
Abbreviations: a, anther; at, androecial tube; ap$_a$, androecial tube appendage; f, filament; gy, gynoecium; ne, nectary; ov, ovary; s, stamen/ staminodium; se, sepal; sg, stigma; st, style. Colour code; blue, pollen; orange, androecial tube; purple, gynoecium; yellow, (underdeveloped or modified) stamens.
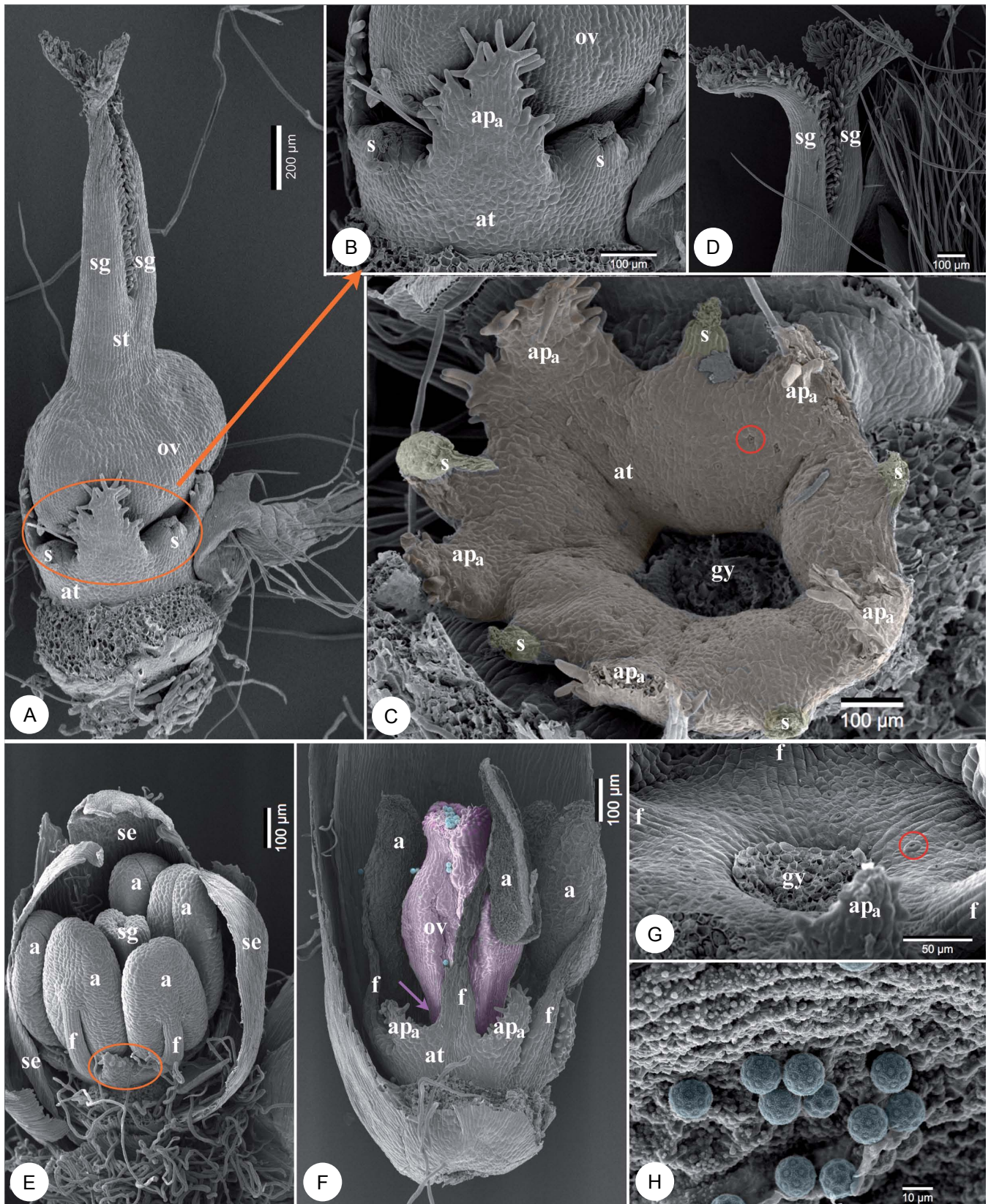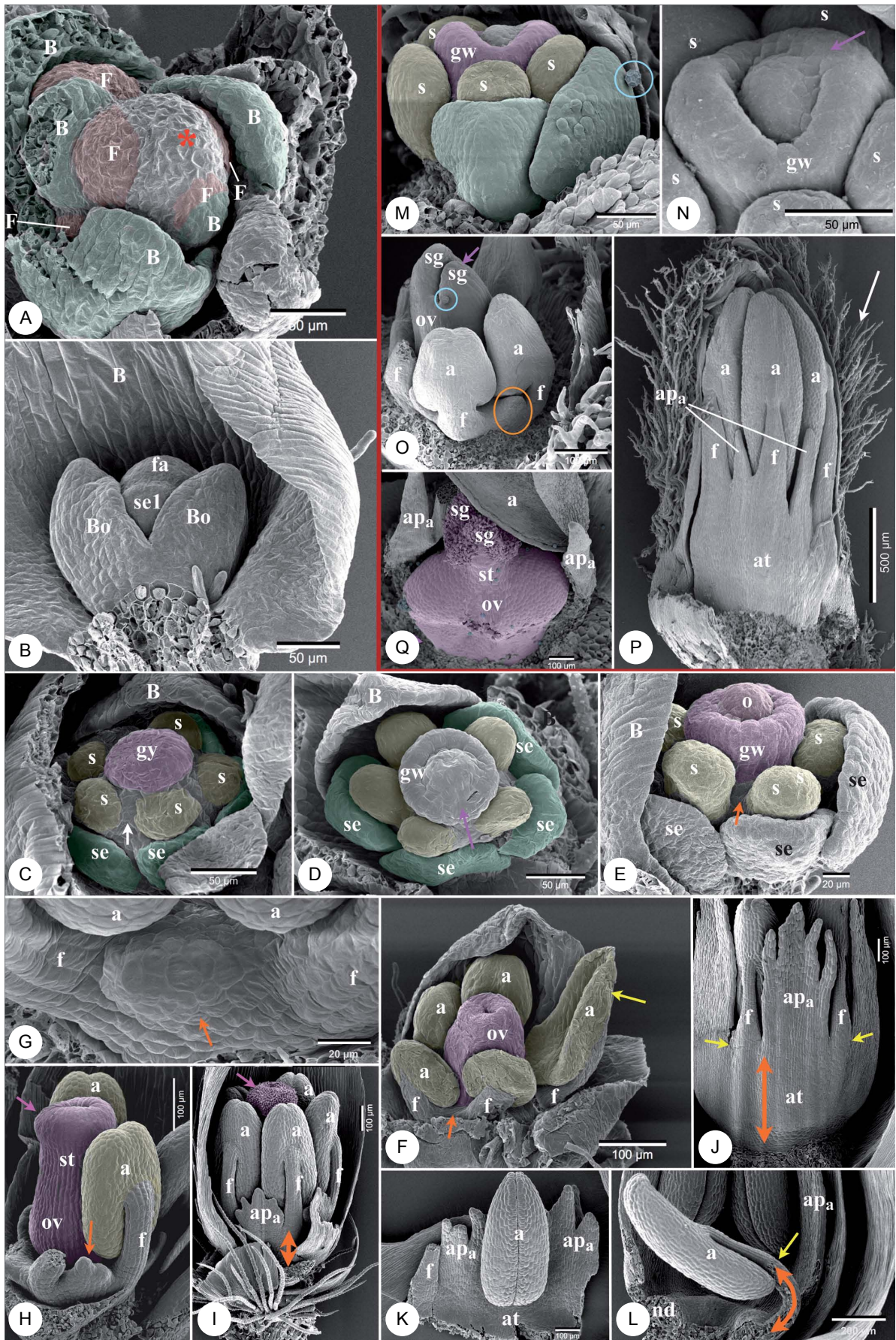
◀ **Figure 6** – SEM images of floral development in flowers of *Alternanthera microcephala* (A–L) and of *Tidestromia rhizomatosa* (M–Q). A, distal part of an indeterminate inflorescence axis with bracts below the apex, each subtending a flower primordium; B, abaxial view of a pedicel with two basally fused opposite bracteoles and a conical floral apex with a first sepal primordium; C, lateral view of a developing flower with five free sepals, opposite them five stamen primordia and centrally a primary gynoecium primordium; D, successive stage with an annular gynoecium wall primordium. At one side, the ring is less conspicuously separated from the floral apex (arrow); E–H, successive stages. The gynoecium wall develops into a bag-like structure surrounding a central zone which is transformed into ovule. Initially, no appendages are visible in between the stamens (orange arrow in 'E'). The stamens differentiate into filament and (modified, yellow arrow in 'F') anther; G–H, successive stages; below the stamens, an androecial tube develops with meristematics bulges (arrowed) alternating with the stamens. These develop into androecial tube appendages. Anthers are dorsifixed, introrse, and disporangiate. The gynoecium now consists of an ovary, short style, and annular stigmatic zone (purple arrow); I–L, the androecial tube and its appendages increase in height. The ligulate-laciniate appendages become as tall as the stamens; L, the basal inside part of the androecial tube forms a nectary. A section of the androecial tube is visible (double orange arrow) stamen; M–N, a gynoecium wall is formed, initially with one side less conspicuously separated from the floral apex (arrowed); O, early androecial tube with appendage primordia (encircled). Two unequal stigma lobes are formed (arrow); Q, mature gynoecium, consisting of a swollen ovary upon a gynophore (arrowed), a short style, and two short papillose stigma lobes; P, lateral view of a semi-mature flower with triangular androecial tube appendages. The outside of the sepals is densely covered with long, sometimes branched trichomes.

Abbreviations: a, anther; at, androecial tube; ap_a, androecial tube appendage; B, bract; Bo, bracteole; F, flower primordium; f, filament; fa, floral apex; gy, gynoecium primordium; gw, gynoecium wall primordium; ne, nectary; o, ovule; ov, ovary; s, stamen primordium; se, sepal; sg, stigma; st, style; * (asterisk red), apex of inflorescence axis. Colour code: green, perianth; yellow, androecium; purple, gynoecium; blue, pollen.

DISCUSSION

Floral development in *Iresine*, *Alternanthera* and *Tidestromia*; appendages of androecial tube and gynophore

Early developmental stages – Based on our observations, we argue that the following developmental processes result in the particular floral structure of all flowers studied: (1) the floral apex becomes conspicuously dome-shaped (figs 3C–F & 4A–B for *Iresine*; fig. 6C–E for *Alternanthera*), with the stamen primordia laterally positioned upon the dome. (2) an annular meristem below or connecting the primordia of the stamens (congenital fusion) forms an androecial tube/annular nectary (figs 3L & 4E & 5C for *Iresine*; figs 6L & 7F for *Alternanthera*, *Tidestromia* not shown). Simultaneously, a gynoecium with gynophore is formed (fig. 5A–B for *Iresine*; fig. 7B & D for *Alternanthera*). As in many Caryophyllales, the nectary seems to be associated with the androecial floral parts (see for example Ronse Decraene & Smets 1991, Vanvinckenroye & Smets 1996), although an axial origin of the androecial tube and nectary cannot be ruled out (cf. Smets 1986, Smets & Cresens 1988); see also below. The origin and quincuncial aestivation of the perianth and the origin of the stamens opposite the sepals in flowers of all species studied concur with Payer's (1857: 320; plate 74) description of the early development in flowers of *A. tenella* (see also figs 3A–C & 8A–B).

The androecial tube – Androecial tubes occur in many Caryophyllales, often also connected with the perianth (Rohweder 1967, 1970, Rohweder & Huber 1974). Urmi-König (1981) described in *Obione sibirica* (Atripliceae) a similar androecial tube as in the species we studied, though without appendages. Flores-Olvera et al. (2008, 2011) observed respectively the appearance of a 'staminal ring' in *Beta vulgaris* and fusion of the basal parts of the filaments in *Chenopodium album* and *Atriplex hortensis*. Androecial tubes with a nectary occur also in not related taxa such as Oxalidales and the Rhizosporaceae alliance (Matthews & Endress 2002, 2011).

In the three Amaranthaceae species studied by Payer (1857), in *B. vulgaris* (Flores-Olvera et al. 2008), in *C. album* and *A. hortensis* (Flores-Olvera et al. 2011) and in the species studied here (e.g. figs 3F, 6C & 7A), the stamens develop from individual primordia. Based on our observations in Amaranthaceae *sensu lato*, 1. no fusion processes occur between the stamens, 2. development of an androecial tube from the base, lifting up the stamens (figs 3I, K, L & N, 4B–E & 8D–E for *Iresine*, fig. 6F–K for *Alternanthera*, *Tidestromia* not shown), we hypothesise that the androecial tube develops from an intercalary ring meristem below the stamens, similar to the underlying intercalary ring meristems from which other floral cups or tubes originate, as explained by Leins & Erbar (2010). Consequently, we do not follow Payer (1857: 320), who stated that the free stamen primordia "soon become connate".

The ring meristem from which the androecial tube develops may be the result of congenital fusion of the stamen primordia. Or alternatively, the androecial tube may result from the development of a receptacular (axial) tube from an underlying intercalary meristem, which, when developing, raises the stamens. Our results do not allow us to interpret the nature of the meristematic ring from which the androecial tube originates. To do so, a thorough developmental-histological study is needed. In the following paragraph, we explain why we nevertheless favour the hypothesis of a receptacular origin of the androecial tube.

In his study of Caryophyllaceae, Rohweder (1967) described filaments of, among others, *Silene dioica*, each with a diameter increasing downwards until they unite in a 'Staminaltubus' (staminal tube), which is basally united with the petals. Rohweder (1967) distinguished between 'Kelchstaubblätter' and 'Kronstaubblätter', referring to respectively an outer whorl of five stamens opposite the sepals and an inner whorl of five stamens opposite the petals. In an obdiplostemonous condition, according to Rohweder (e.g. 1967) common in Caryophyllaceae, the inner whorl is positioned more outwards resulting in a seemingly single whorl of ten stamens. Moreover, the stamens opposite the petals are fused
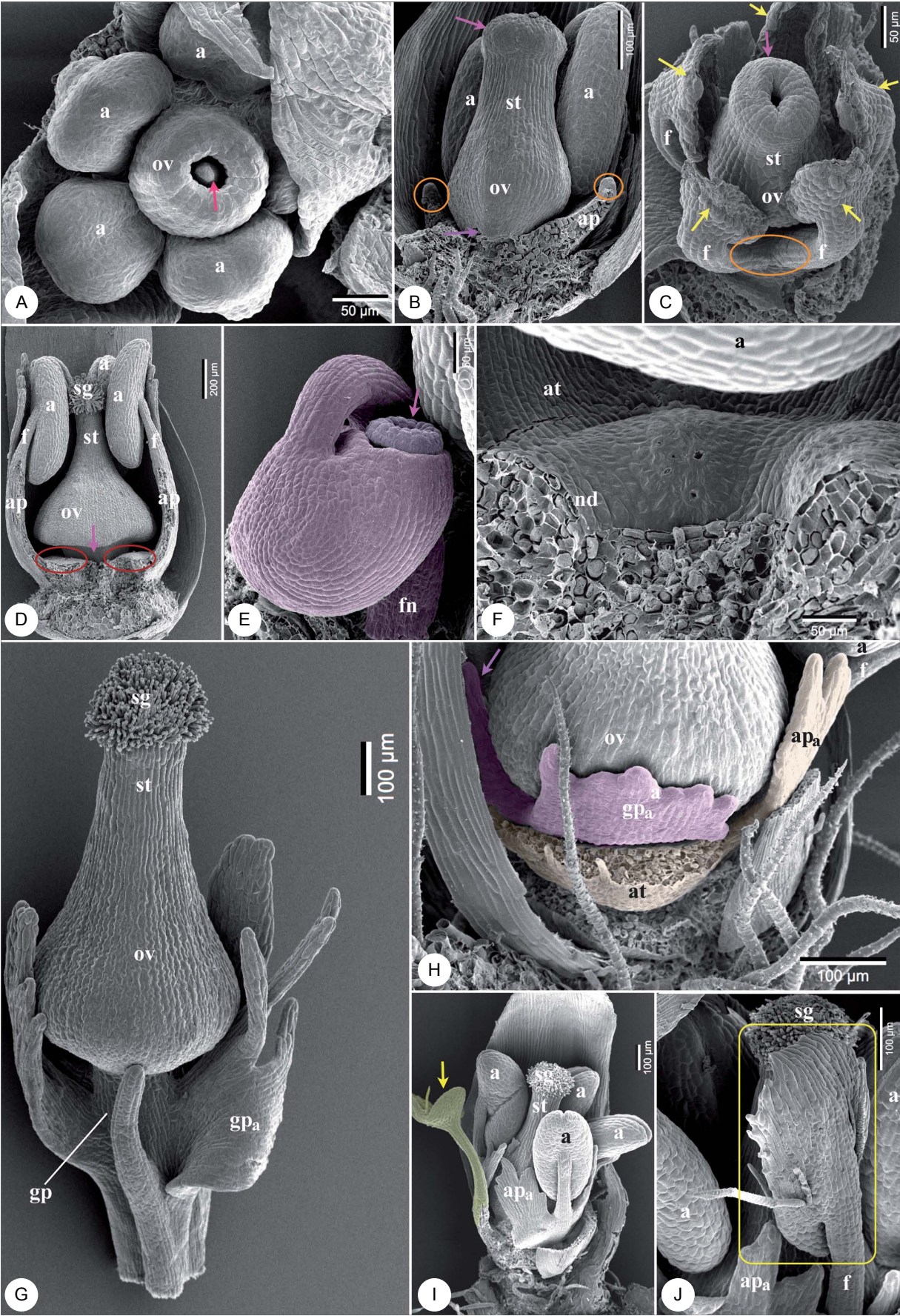
◀ **Figure 7** – SEM images of details of the development in *Alternanthera microcephala*, including modifications of androecium and gynoecium. A, apical view of a nearly closed ovary with still visible developing ovule (arrow). Anthers and filaments are being formed; B, the androecial tube and a gynophore (arrow) simultaneously develop; C, *de facto* functionally female flower because of modified, sterile anthers (arrowed in yellow). An annular stigma around a central opening is formed. In this flower, there is no androecial tube neither androecial tube appendages; D, the gynoecium is raised upon a gynophore (arrowed), surrounded by a nectary formed by the inside of the androecial tube (encircled); E, campylotropous bitegmic ovule with long, curved funiculus, micropyle indicated by an arrow; F, detail of a nectary; G, developing pistil standing on a high gynophore with appendages similar to those of the androecial tube; H, detail of the base of a gynoecium, of which the gynophore is hidden by the androecial tube. In between androecial tube and ovary, appendages of the gynophore protrude; I, semi-mature flower with modified stamen (in yellow); J, detail of modified anther (encircled).

Abbreviations: a, anther; at, androecial tube; ap_a, androecial tube appendage; f, filament; fa, floral apex; fn, funiculus; gp, gynophore; gp_a, gynophore appendage; ne, nectary; o, ovule; ov, ovary; sg, stigma; st, style. Colour code: yellow, androecium; orange, androecial tube; purple, gynoecium.

with the corolla. In Caryophyllales, including Amaranthaceae *sensu lato*, it is assumed that the corolla is lost (e.g. Payer 1857, Rohweder & Huber 1974, Urmi-König 1981). If with the corolla also the five 'Kronstaubblätter' disappeared, the appendages can be considered to be novel structures. The appendages would then result from a reorientation of the intercalary meristem from which in a putative ancestor a corolla-androecial tube originated (like in Caryophyllaceae; e.g. Rohweder 1967) towards the formation of an androecial tube/appendages with new functions, respectively nectary (figs 6L, 7F, 8F & G) and pollination (fig. 2 as illustration for putative pollinator attraction). Alternatively, the 'Kronstaubblätter' were not lost along with the corolla but evolved into appendages. If so, the appendages are modified 'Kronstaubblätter', hence staminodia. Their position, alternating with the stamens in a single whorl, can be understood from the putative ancestral obdiplostemonous condition. Both cases would correspond to a receptacular origin of the annular meristem from which the androecial tube develops.

Flores-Olvera et al. (2011) stated that in *Chenopodium album* and *Atriplex hortensis* (Chenopodioideae), the bases of the stamens fuse postgenitally. This was based on the observation that initially free stamens become fused later on. However, postgenital fusion implies epidermal fusion ("interdigitation of epidermal cells"; Endress 2006: 29), in contrast to congenital fusion, which implies meristem fusion (Hagemann 1973, Endress 2006). This was not investigated by the authors. But like in *Beta vulgaris* (Flores-Olvera et al. 2008) and in the species studied here, it seems probable that the 'fusion of the filaments' is due to the formation of a small androecial tube from a basal ring meristem. In the same publication, a similar misinterpretation was made in the perianth of *Chenopodium bonus-henricus*, where individual primordia develop into sepals which appear fused at the base towards maturity of the flower. Though not investigated by us, in view of the common tendency in Chenopodioideae to have a congenitally fused perianth, it seems more probable that also in *C. bonus-henricus* the fused part of the perianth developed from a ring meristem resulting from congenital fusion of the sepal primordia. Flores-Olvera et al. (2011) described an annular perianth primordium in *Dysphania ambrosioides*, which actually provides direct evidence for congenital fusion of the sepal primordia.

The appendages of the androecial tube – Are these staminodes? Our LM data do not provide evidence that the androecial tube appendages have a staminal origin; fig. 8F shows that only five vascular bundles are present in the androecial tube, each opposite a sepal and hence vascularising a stamen. About the formation of the androecial tube appendages, our results concur with those of Payer; like in *A. tenella*, the 'pseudostaminodia' in the species studied originate relatively late in the course of the floral development (all floral organs are present) from a primordial bulge upon the rim of the early androecial tube (figs 3K & 4D for *Iresine*; fig. 6G & O for *Alternanthera/Tidestromia*). According to Endress (2008), reduced organs can appear late in the development and be increased in number. This implies that the appendages may reflect the putatively lost second whorl of stamens. But even if this were the case, the result (for the Gomphrenoideae species studied) indicates that the potential of the meristem present in the floral apex was reoriented towards the development of structures with new functions and morphologies (the androecial nectariferous tube and the showy appendages). Therefore, we assume that the appendages of the androecial tube develop from remaining meristematic zones forming bulges on the rim of the androecial tube. These bulges appear where there is free space, hence alternating with the stamens.

Regarding the function of the androecial tube appendages, it is probable that they perform one or more of the typical staminode functions described by Ronse Decraene & Smets (2001). At least in flowers of *I. hebanthoides* (fig. 2H), their bright white and conspicuous shiny appearance contrasting with the yellow sepals with reddish base suggests attraction of pollinators. This is also the case in *A. microcephala*, where the appendages and filaments are bright yellow (fig. 2I) contrasting with red perianth sepals. The pollen grains we observed (figs 4F, 5F & H, 6M & O) have a diameter of around 10 µm as described by Borsch (1998; see also Eliasson 1988). They seem to stick easily to surfaces (e.g. figs 4F, 5F & H, 6M & O). In *I. hebanthoides* and *A. microcephala*, a possible function of the appendages may be to collect pollen and to brush it onto insects that look for nectar in the cup formed by the androecial tube. In contrast, since the appendages in *T. rhizomatosa* are inconspicuous (figs 2J & 6P), our 'pollen brush' hypothesis cannot be generalised. Anyhow, based on our observations of the androecial tube and its appendages, we hypothesise that the origin and morphology of the androecial tube appendages are additional functional adaptations to animal (probably insect) pollination (fig. 2) just like the nectariferous structures at the inner basal part of the androecial tube. Ecological studies to test our insect pollination syndrome hypothesis are needed.
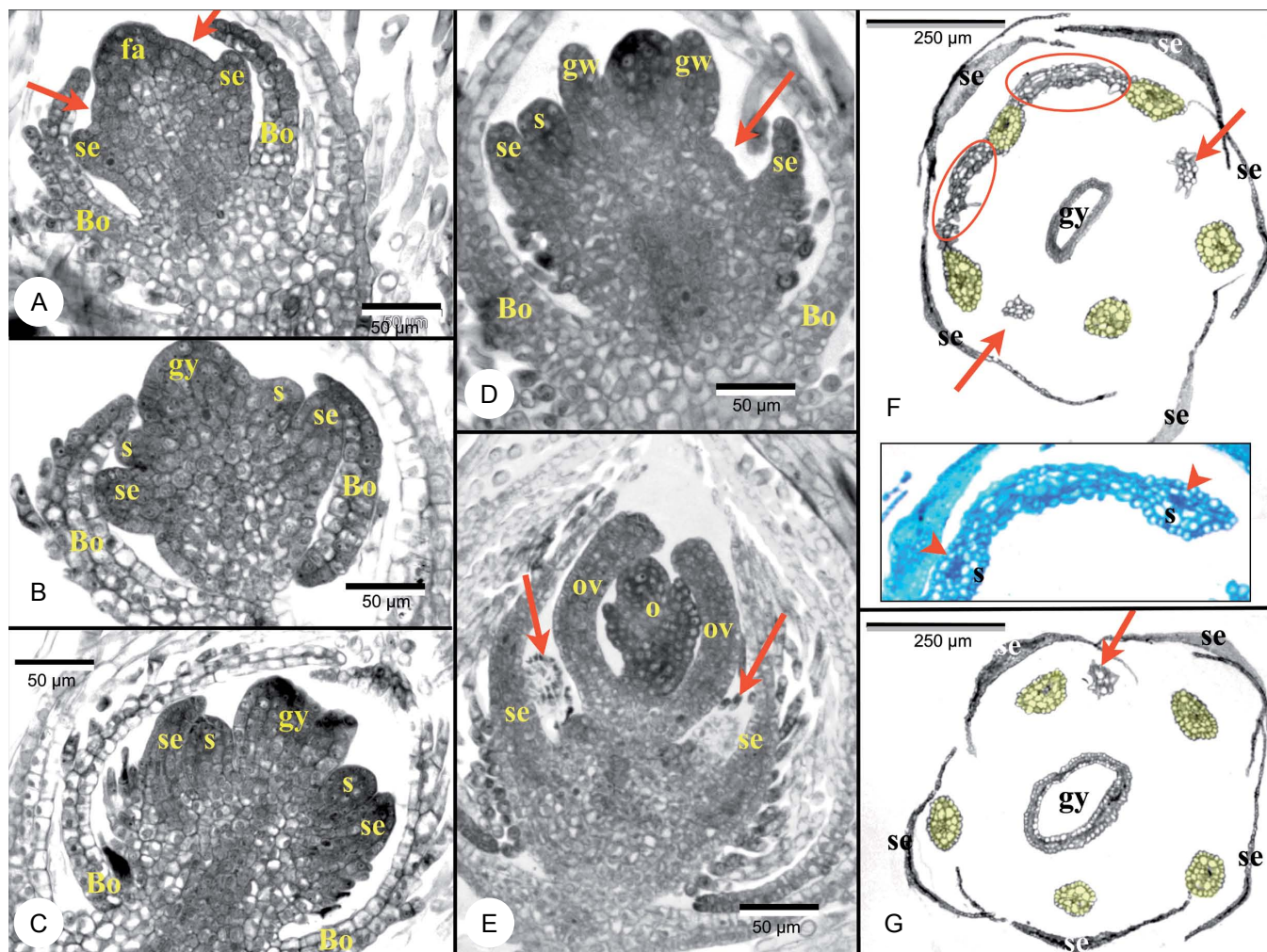
Figure 8 – LM images of longitudinal sections through developing flowers of *Iresine latifolia* (A–E), and a transversal section through the upper part of a mature androecial tube of *I. orientalis* (F, G). A, very young floral bud with the sepals originating; B–C, successive stages with the stamens present, and a primary gynoecium primordium being formed; D, successive stage. The primary gynoecium primordium is differentiated into a gynoecium wall primordium surrounding a central zone. The stamens are directly inserted on the dome-shaped receptacle, there is no staminal ring (arrow); E, successive stage. The androecial tube is appearing, and the the developing gynoecium wall is enclosing the immature, unilocular ovule; F, quincuncial aestivation of the sepals, with stamens opposite to them (yellow), each with one vessel (arrowed in the window). In the androecial tube, between the stamens, no vessels are present (encircled and detail in window); G, transverse section above the androecial tube. Arrowheads in F & G show appendages of the androecial tube. In F, the zone of the androecial tube in between two stamens is encircled. The inserted box shows a detail.

Abbreviations: at, androecial tube; Bo, bracteole; fa, floral apex; gy, gynoecium; o, ovule; ov, ovary (gynoecium) wall; s, stamen; se, sepal.

In *Alternanthera microcephala*, regularly one, several or all five stamens in a flower develop a flattened structure on top of the filaments instead of proper anthers. These flattened structures have one or few very large, conspicuous trichomes (fig. 7C & J). We do not know the function of the modified anthers. The samples used for this study were collected in a disturbed area, and perhaps the modified anthers result from an abnormal floral development, something which is sometimes seen in plants growing in a disturbed area or in gardens.

Gynoecium – Our observations of the origin of the gynoecium partially concur with those of Payer (1857); in his plate 74/7, Payer (1857) drew a similar initiation pattern of the gynoecium in *A. tenella* as we observed in all species stu-

died (see also figs 3F for *I. latifolia*; 6D for *A. microcephala*; 6M–N for *T. rhizomatosa*). In contrast to Payer (1857: 321), however, in all species studied we observed that the primary gynoecium primordium secondarily develops an annular gynoecium wall primordium, surrounding a central part from which the ovule is later formed (figs 3E–H & 6C–F; see also fig. 8B–D). Indeed, initially the ring is more developed on one side (figs 3F & 6D), but in our opinion, carpel primordia are never formed, neither at the stages just before the ring appears (figs 3E & 6C), nor afterwards. Perhaps its initial irregular appearance reflects the three-carpellary evolutionary origin of the ring structure. Note that we did not observe individual carpel primordia in *Beta vulgaris* (Flores-Olvera et al. 2008), neither in several species of Chenopodiaceae (Flores-Olvera et al. 2011). Endress (2006: 108) mentioned

that in "a few more advanced groups [of angiosperms], carpel closure is delayed, as compared to the ovules, and the young ovule(s) is (are) visible before closure", naming caryophyllids and Chenopodiaceae among others. However, in the species presented here, as well as in earlierly studied species of the former Chenopodiaceae (Hakki 1971, Flores et al. 2008, 2011), the annular gynoecium wall primordium develops into an open bag-like structure surrounding the ovule. Like Payer (1857), we consider this open bag-like structure to be an immature ovary, since it has all the properties of a unilocular ovary. Closure of the ovary occurs when the growing gynoecium wall forms a style.

Endress (2006: 39) considered ring meristems as an innovation of flexibility or an escape of constraints in the particular case of the androecium of polystemonous flowers: first an annular primary androecium primordium is formed, followed by the appearance of carpel primordia. Only subsequently, individual stamen primordia secondarily appear. Vrijdaghs et al. (2009) and Reynders et al. (2012) suggested a similar decoupling of the ovule development and the development of the gynoecium wall (ovary wall, style and stigma branches) in Cyperoideae (Cyperaceae), because of the common presence of an annular gynoecium wall primordium. We suggest that also in the species we studied in Amaranthaceae *sensu lato*, the development of the gynoecium wall may be decoupled from the development of the single ovule. In Amaranthaceae, like in Cyperaceae, such a decoupling of the development of gynoecium wall and ovule may explain the fast development of the single ovule, while the immature ovary is not yet closed. However, at this stage of knowledge of the development of the gynoecium in Amaranthaceae, our hypothesis lacks support from developmental and anatomical data and is only based on the similarity of the development of the gynoecium with that in Cyperaceae. We envisage a thorough study of the development of the gynoecium in Amaranthaceae *sensu lato*.

In *I. herbstii*, flowers are functionally unisexual. At first sight, the male flower seems bisexual. However, at the position of the gynoecium, an immediately closed relatively long style-like structure is developed (fig. 4A–C, E & G). Where two stigma lobes are expected, this structure has a mace-shaped, slightly papillose top (fig. 4G). Around its base, the inner basal part of the androecial tube is glandular-like in the other species studied. We do not know whether this structure is functional or not, and if functional, what the function might be.

In the (semi-)mature flowers of all species studied, though less conspicuous in *Iresine*, a gynophore is present surrounded by the annular nectariferous zone at the base of the androecial tube (figs 3L, 7B, D & F). In *A. microcephala,* the height of the gynophore varies from nearly inconspicuous to more than half the length of the entire pistil (fig. 7D & G).

More enigmatic appendages – In *Alternanthera*, we found a few exceptional flowers of wild plants collected in a disturbed environment with appendages on the gynophore. The morphology of the gynophore appendages is similar to the appendages of the androecial tube (fig. 7G–H). This suggests that both structures may share a common underlying genetic programme for their particular morphology. In this context,

we refer to Viaene et al. (2009) who studied the influence of B class gene duplications (*AP3* and *PI*) on the morphology of petals and stamens in basal asterids. Based on studies of *Petunia* (Solanaceae), Vandenbussche et al. (2004) suggested that in asterids, retained *PI* duplications result in diversification of the B-function, which they hypothesised to be correlated with diversification of corolla and androecium through fusion events.

Developmental patterns in Amaranthaceae sensu stricto, Betoideae, and Chenopodieae

Integrating the results of previous investigations of *Beta vulgaris* (Flores-Olvera et al. 2008) and in the former Chenopodiaceae (Flores-Olvera et al. 2011) with our current observations in the context of the current molecular phylogenetic hypotheses, we provide a preliminary hypothesis of evolutionary trends in floral structure and development in Amaranthaceae *sensu lato* (table 2). In general, the family has (1) an ontogenetic sequence from perianth to gynoecium; (2) a perianth consisting of five or two sepals, quincuncially aestivated, originating from free sepal primordia. In the species studied in Chenopodieae and in *Beta vulgaris*, the sepals tend to be fused congenitally; (3) an androecium usually consisting of five stamens (sometimes less) originating from free stamen primordia, with an androecial tube in *B. vulgaris* and Gomphrenoideae and some species in Chenopodioideae (in literature called 'staminal ring', 'androecial tube' and 'fused filaments' respectively), which develops after the appearance of the stamen primordia. Moreover, Gomphrenoideae have appendages on the androecial tube and disporangiate anthers; (4) a gynoecium wall developing from an annular primordium surrounding a single, campylotropous ovule. Only later in the development (simultaneously with the differentiation of the stamen primordia into filament and anther; e.g. fig. 7A), the initially open ovary is closed. A style is present in all species, except *C. bonus-henricus*. Two to four stigma lobes or branches are formed and in *Alternanthera*, the stigma is annular.

In all species studied, the gynoecium is superior and in the Gomphrenoideae, a gynophore is present. In some flowers of *Alternanthera,* the gynophore can become very large and have appendages similar to the appendages of the androecial tube. In *B. vulgaris*, the gynoecium becomes secondarily semi-inferior due to the formation of a perigynous hypanthium late in the floral development. In functionally male flowers of *I. herbstii*, the gynoecium consists of a sterile stylar structure, of which the function remains enigmatic. In Chenopodioideae, sex determination varies from extremely flexible (*Atriplex*) to stricty unisexual (*Spinacia*). In Amaranthaceae *sensu stricto*, some flexibility in sex determination occurs in *Iresine*, while in the other species studied and in *B. vulgaris* bisexuality prevails. In Chenopodioideae, in particular in female flowers, there is a tendency to develop two modified sepals while the three other sepals remain underdeveloped. In *B. vulgaris*, the two bracteoles of a terminal flower each subtend a lateral flower, forming cymosely branched units. In the Amaranthaceae *sensu stricto* studied, all flowers are subtended by a bract and have very short pedicels with two opposite bracteoles.

Table 2 – Comparison of floral and floral developmental characters in Amaranthaceae *sensu lato*.
Based on Flores-Olvera et al. (2008, 2011) and new findings presented here.
Abbreviations: ap, appendage; bas. fused, basally fused; free, freely originating; late fus., fusion at later developmental stage; Q, quincuncial; var, variable in size; 1L and 2L, di- and tetrasporangiate (uni- and bilocular); +, present; -, absent. * ring primordium is less conspicuous at one side, as also reported by Payer (1857).

	Perianth			Androecium				Gynoecium				Floral sex
	parts	primordium	aestivation	stamens	primordium	anther	androecial tube	gynoecium wall primordium	style	stigmas	gynophore	
Chenopodioideae (former Chenopodiaceae without *Beta*)												
Dysphania	5	annular	Q	1-5	free	2L	-	annular	+	2	-	bisexual functionally male
Chenopodium album	5	free, +late fus.	Q	5	free +late fus.	2L	-	annular	+	2	-	bisexual functionally female
Atriplex	2/5	free, bas. fused	Q	5	free	2L	-	annular	+	2	-	bisexual functionally unisexual strictly female
Spinacia	5	free, bas. fused	Q	2-5	free	2L	-	annular	+	2	-	strictly unisexual
Chenopodium bonus-henricus	5	free, +late fus.	Q	5	free	2L	-	annular	-	2	-	bisexual
Betoideae (formerly within Chenopodiaceae)												
Beta vulgaris	5	free	Q	5	free	2L	+ ap-	annular	-	3	-	bisexual
Gomphrenoideae (main subclade in former Amaranthaceae = Amaranthaceae *sensu stricto*)												
Iresine	5	free	Q	5	free	1L	+ ap+	annular*	+	2	+	bisexual functionally unisexual
Alternanthera/	5	free	Q	5	free	1L	+var ap+	annular*	+	annular	+	bisexual
Tidestromia	5	free	Q	5	free	1L	+ ap+	annular*	+	2	+	bisexual

CONCLUSIONS

Based on species of Amaranthaceae *sensu lato* studied by us, and on data from literature, we conclude that: (1) the general floral developmental pattern of Gomphrenoideae is similar to that earlier found by us (Flores-Olvera et al. 2008, 2011) in Betoideae and Chenopodioideae. With the exception of Chenopodioideae, flowers in Amaranthaceae *sensu lato* stand on a short pedicel with two opposite bracteoles; (2) the androecial tube in gomphrenoid flowers is homologous with the 'staminal ring' in *B. vulgaris* and with the basally fused zone of the filaments in some species in Atripliceae, developing from an underlying annular meristem, which we consider to be axial; (3) the highly variable appendages of the androecial tube develop each from a primordium on the rim of the early androecial tube, alternating with the filaments.

In our opinion, gomphrenoid androecial tubes and their appendages result from a reorientation of parts of the ancestral meristematic potential in the floral apex into androecial tubes, coupled with new functions such as attraction of pollinators and/or contributing to the pollination process. Support for our hypothesis is provided by the following observations: the inner base of the cup formed by the androecial tube is glandular, and at least in *Iresine hebanthoides* and *Alternanthera microcephala*, the androecial tube appendages are conspicuous and brightly coloured. Regarding the nature of the appendages, our anatomical data do not support that appendages are staminodes.

And (4) in all species studied in Amaranthaceae *sensu lato*, the gynoecium wall develops from a ring primordium, surrounding a central zone in which a single campylotropous ovule develops. We hypothesise that the evolutionary realisation of an annular gynoecium wall primordium liberated

the gynoecium development of the constraints of a carpelary organisation. As a consequence, the gynoecium wall and the ovule develop independently. In the gomphrenoid species studied, the unequally formed annular gynoecium wall primordium may still reflect the carpellary origin of the ring primordium. In order to determine whether the development of the gynoecium wall is decoupled from the development of the ovule(s), an anatomical/developmental study of gynoecia in Amaranthaceae *sensu lato* is envisaged.

Finally, for the first time, the presence of gynophores in Amaranthaceae *sensu lato* is observed. The exceptional presence of appendages of the gynophore in *Alternanthera* invites for further investigation of this phenomenon.

ACKNOWLEDGMENTS

LITERATURE CITED

Acosta J.M., Perreta M., Amsler A., Vegetti A.C. (2009) The flowering unit in the synflorescences of Amaranthaceae. The Botanical Review 75: 365–376. http://dx.doi.org/10.1007/s12229-009-9037-9

APG (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161: 105–121. http://dx.doi.org/10.1111/j.1095-8339.2009.00996.x

Bentham G., Hooker J.D. (1880) Genera Plantarum Vol. 3: 20–43. London, Lovell Reeve.

Borsch T. (1998) Pollen types in the Amaranthaceae. Morphology and evolutionary significance. Grana 37: 129–142.

Borsch T. (2007) Amaranthaceae. In: Stevens W.D., Ulloa C., Pool A., Montiel O.M. (eds) Flora de Nicaragua: 56–83. St. Louis MO, Missouri Botanical Garden.

Clemants S.E. (2003) Iresine. In: Flora of North America Editorial Committee (ed.) Flora of North America Vol. 4: 454–456. New York, Oxford University Press.

Eliasson U.H. (1971) Amaranthaceae. In: Wiggins I.L., Porter D.M. (eds) Flora of the Galapagos Islands 18. Stanford, Stanford University Press.

Eliasson U.H. (1987) Amaranthaceae. In: Harling G., Anderson L. (eds) Flora of Ecuador no. 28. Gothenburg: Department of Systematic Botany, University of Gothenburg; and Stockholm: the Section for Botany, Museum of Natural History.

Eliasson U.H. (1988) Floral morphology and taxonomic relations among the genera of Amaranthaceae in the New World and the Hawaiian Islands. Botanical Journal of the Linnean Society (London) 96: 235–283. http://dx.doi.org/10.1111/j.1095-8339.1988.tb00683.x

Endress P.K. (2006) Angiosperm floral evolution: morphological developmental framework. Advances in Botanical Research 44: 1–61. http://dx.doi.org/10.1016/S0065-2296(06)44001-5

Endress P.K. (2008) The whole and the parts: relationships between floral architecture and floral organ shape, and their repercussions on the interpretation of fragmentary floral fossils. Annals of the Missouri Botanic Garden 95: 101–120. http://dx.doi.org/10.3417/2006190

Endress P.K., Stumpf S. (1990) Non-tetrasporangiate stamens in the angiosperms: structure, systematic distribution and evolutionary aspects. Botanische Jahrbücher für Systematik 112: 193–240.

Flores-Olvera H., Smets E., Vrijdaghs A. (2008) Floral and inflorescence morphology and ontogeny in Beta vulgaris, with special emphasis on the ovary position. Annals of Botany 102: 643–651. http://dx.doi.org/10.1093/aob/mcn140

Flores-Olvera H., Vrijdaghs A., Ochoterena H., Smets E. (2011) The need to re-investigate the nature of homoplastic characters: an ontogenetic case study of the "bracteoles" in Atripliceae (Chenopodiaceae). Annals of Botany 108: 847–865. http://dx.doi.org/10.1093/aob/mcr203

Hagemann W. (1973) The organization of shoot development. Revista de Biologia 9: 43–67.

Hakki M.I. (1971) Blütenmorphologische und embryologische Untersuchungen an Chenopodium capitatum und Chenopodium foliosum sowie weiteren Chenopodiaceae. Botanische Jahrbücher für Systematik 92: 178–330.

Heywood V.H., Brummit R.K., Culham A., Seberg O. (2007) Flowering plant families of the world. London, Royal Botanic Gardens Kew.

Judd W.S., Campbell C.S., Kellogg E.A., Stevens P.F. (1999) Plant Systematics: A phylogenetic approach. Sunderland, Sinauer Associates Inc.

Kadereit G., Borsch T., Weising K., Freitag H. (2003) Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C_4 photosynthesis. International Journal of Plant Sciences 164: 959–986. http://dx.doi.org/10.1086/378649

Leins P., Erbar C. (2010) Flower and fruit. Morphology, ontogeny, phylogeny, function and ecology. Stuttgart, Schweizerbart Science Publishers.

Matthews M.L., Endress P.K. (2002) Comparative floral structure and systematics in Oxalidales (Oxalidaceae, Connaraceae, Cephalotaceae, Brunelliaceae, Cunoniaceae, Elaeocarpaceae, Tremandraceae). Botanical Journal of the Linnean Society 140: 321–381. http://dx.doi.org/10.1046/j.1095-8339.2002.00105.x

Matthews M.L., Endress P.K. (2011) Comparative floral structure and systematics in Rhizophoraceae, Erythroxylaceae, and the potentially related Ctenolophonaceae, Linaceae, Irvingiaceae, and Caryocaraceae. Botanical Journal of the Linnean Society 166: 331–416. http://dx.doi.org/10.1111/j.1095-8339.2011.01162.x

Moquin-Tandon C.H.B.A. (1849) Amaranthaceae. In: de Candolle A.P. (ed.) Prodromus Systematis Naturalis Regni Vegetabilis Vol. 13: 231–424. Paris, Masson.

Müller K., Borsch T. (2005) Phylogenetics of Amaranthaceae using matK/trnK sequence data – evidence from parsimony, likelihood and Bayesian approaches. Annals of the Missouri Botanical Garden 92: 66–102.

Payer J.B. (1857) Traité d'organogénie comparée de la fleur. Paris, Masson.

Reynders M., Vrijdaghs A., Larridon I., Huygh W., Leroux O., Muasya A.M., Smets E., Goetghebeur P. (2012) Gynoecial anatomy and development in Cyperoideae (Cyperaceae, Poales): con-

genital fusion of carpels facilitates evolutionary modifications in pistil structure. Plant Ecology and Evolution 145: 96–125. http://dx.doi.org/10.5091/plecevo.2012.675

Rohweder O. (1967) Centrospermen-Studien 3. Blütenentwicklung und Blütenbau bei Silenoideen (Caryophyllaceae). Botanische Jahrbücher für Systematik 86: 130–185.

Rohweder O. (1970) Centrospermen-Studien 4. Morphologie und Anatomie der Blüten, Früchte und Samen bei Alsinoideen und Paronychioideen s. lat. (Caryophyllaceae). Botanische Jahrbücher für Systematik 90: 201–271.

Rohweder O., Huber K. (1974) Centrospermen-Studien 7. Beobachtungen und Anmerkungen zur Morphologie und Entwicklungsgeschichte einiger Nyctaginaceen. Botanische Jahrbücher für Systematik 94: 327–359.

Ronse Decraene L.P., Smets E. (1991) The floral nectaries of Polygonum s.l. and related genera (Persicarieae and Polygoneae): position, morphological nature and semophylesis. Flora 185: 165–185.

Ronse Decraene L.P., Smets E. (2001) Staminodes: Their morphological and evolutionary significance. The Botanical Review 67: 351–402. http://dx.doi.org/10.1007/BF02858099

Sánchez-del Pino I., Borsch T., Motley T.J. (2009) TrnL-F and rpl16 sequence data and dense taxon sampling reveal monophyly of unilocular anthered Gomphrenoideae (Amaranthaceae) and improved picture of their relationships. Systematic Botany 34: 57–67. http://dx.doi.org/10.1600/036364409787602401

Sánchez-del Pino I., Motley T.J. (2010) Evolution of Tidestromia (Amaranthaceae) in the deserts of the southwestern United States and Mexico. Taxon 59: 38–48.

Schinz H. (1893) Amaranthaceae. In: Engler A., Prantl K. (eds) Die Natürlichen Pflanzenfamilien Vol. 3: 91–118. Leipzig, Engelmann.

Smets E. (1986) Localization and systematic importance of the floral nectaries in the Magnoliatae (Dicotyledons). Bulletin du Jardin botanique national de Belgique /Bulletin van de Nationale Plantentuin van België 56: 51–76. http://dx.doi.org/10.2307/3667757

Smets E., Cresens E. (1988) Types of floral nectaries and the concepts 'character' and 'character-state' - a reconsideration. Acta Botanica Neerlandica 37: 121–128.

Stevens P.F. (2001 onwards) Angiosperm Phylogeny Website. Version 12, July 2012 [online]. Available from http://www.mobot.org/MOBOT/research/APweb/ [accessed 17 Oct. 2013].

Townsend C.C. (1993) Amaranthaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants, Vol. II, Flowering plants: dicotyledons: 70–91. Berlin, Springer-Verlag.

Urmi-König K. (1981) Blütentragende Spross-Systeme einiger Chenopodiaceae. Dissertationes Botanicae 63: 1–150.

Vandenbussche M., Zethof J., Royaert S., Weterings K., Gerats T. (2004) The duplicated B-class heterodimer model: whorl- specific effects and complex genetic interactions in Petunia hybrida flower development. Plant Cell 16: 741–754. http://dx.doi.org/10.1105/tpc.019166

Vanvinckenroye P., Smets E. (1996) Floral ontogeny of five species of Talinum and of related taxa (Portulacaceae). Journal of Plant Research 109: 387–402. http://dx.doi.org/10.1007/BF02344554

Viaene T., Vekemans D., Irish V.F., Geeraerts A., Huysmans S., Janssens S., Smets E., Geuten K. (2009) Pistillata – Duplications as a mode for floral diversification in (basal) Asterids. Molecular Biology and Evolution 26: 2627–2645. http://dx.doi.org/10.1093/molbev/msp181

Vrijdaghs A., Muasya A.M.M., Goetghebeur P., Caris P., Nagels A., Smets E. (2009) A floral ontogenetic approach to homology questions within the Cyperoideae (Cyperaceae). The Botanical Review 75: 30–51. http://dx.doi.org/10.1007/s12229-008-9021-9

Zumaya S., Flores-Olvera H., Borsch T. (2013) Two new Mexican endemic species of Iresine (Amaranthaceae). Systematic Botany 38: 434–443. http://dx.doi.org/10.1600/036364413X666633

Communicating Editor: Catarina Rydin.