

**ANDROECIAL EVOLUTION IN CARYOPHYLLALES IN LIGHT OF A
 PARAPHYLETIC MOLLUGINACEAE¹**

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- *Premise of the study:* Caryophyllales are highly diverse in the structure of the perianth and androecium and show a mode of floral development unique in eudicots, reflecting the continuous interplay of gynoecium and perianth and their influence on position, number, and identity of the androecial whorls. The floral development of five species from four genera of a paraphyletic Molluginaceae (*Limeum*, *Hypertelis*, *Glinus*, *Corbichonia*), representing three distinct evolutionary lineages, was investigated to interpret the evolution of the androecium across Caryophyllales.
- *Methods:* Floral buds were dissected, critical-point dried and imaged with SEM. The genera studied are good representatives of the diversity of development of stamens and staminodial petaloids in Caryophyllales.
- *Key results:* Sepals show evidence of petaloid differentiation via marginal hyaline expansion. *Corbichonia*, *Glinus*, and *Limeum* also show perianth differentiation via sterilization of outer stamen tiers. In all four genera, stamens initiate with the carpels and develop centrifugally, but subsequently variation is significant. With the exception of *Limeum*, the upper whorl is complete and alternisepalous, while a second antesepalous whorl arises more or less sequentially, starting opposite the inner sepals. Loss or sterilization of antesepalous stamens occurs in *Glinus* and *Limeum* and is caused by altered carpel merism and inhibition by sepal pressures.
- *Conclusions:* Outer stamens of *Hypertelis* correspond with petaloids of Caryophyllaceae and suggest that staminodial petaloids and outer alternisepalous stamens are interchangeable in the Caryophyllales. We emphasize a switch in the position of first formed stamens from antesepalous to alternisepalous following the divergence of *Limeum*; thus stamen position is an important synapomorphy for the globular inclusion clade.

Key words: carpels; Caryophyllales; centrifugal stamens; floral development; globular inclusion clade; raphide clade; sepals; staminodial petaloids; stamen loss.

Spatial patterning in the angiosperm flower commonly proceeds in a proximal to distal direction, termed centripetal. However, a significant number of angiosperms deviate from this strict centripetal progression. Some floral organs, particularly within the gynoecium and androecium, develop in a distal-to-proximal sequence, in a pattern referred to as centrifugal (Payer, 1857; Sattler, 1972; Rudall, 2010, 2011). Taxa within the core eudicot clade Caryophyllales commonly exhibit centrifugal initiation of the androecium, which can in turn precipitate a number of further evolutionary consequences (Brockington et al., 2009; Ronse De Craene, 2010). One such consequence is the occurrence of pseudodiplostemony, in which the common eudicot pattern of two stamen whorls (diplostemony) is achieved, but via a completely different developmental sequence (hence the term “pseudo”). This can be clearly seen in the Caryophyllaceae, where initiation of antesepalous stamens is inverted and petals arise basipetally from antepetalous stamens, generally by division of common primordia (see Ronse De Craene and Smets, 1993; Hofmann, 1994; Ronse De Craene et al., 1998;

Smitsen and Garnock-Jones, 2002; Harris et al., 2012; Luo et al., 2012). A second consequence of the centrifugal initiation occurs through interaction with the perianth. Outer members of the centrifugal androecium may become flattened and showy and form the inner petaloid members of a differentiated perianth (e.g., Aizoaceae and *Corbichonia*). Furthermore, mutual repression between the centripetally advancing perianth and centrifugally developing androecium can cause organ loss in outer tiers of stamens. Thus, a centrifugally initiating androecium can contribute to both variation in perianth differentiation and to changes in merism within the androecium and perianth. In addition to a centrifugally developing androecium, there is a strong connection between the androecium and gynoecium in the development of a common platform not much different from an androgynophore. Changes in carpel merism seem to directly influence the upper stamens, as loss of carpels seems to be correlated with lower stamen numbers, at least in Caryophyllaceae (Ronse De Craene et al., 1998).

Given the evolutionary singularity of the androecium in the Caryophyllales, it is important to describe the occurrence and consequences of centrifugal androecium initiation across the order. Recent changes to our concept of the caryophyllid phylogeny also forces reconsideration of the evolution of many traits including androecial morphology. Here, the identification of Molluginaceae as paraphyletic is particularly important because there is considerable androecial variation in Molluginaceae *sensu lato*, and the now exiled lineages of Molluginaceae are widely scattered across the core Caryophyllales (Fig. 1). For example, *Corrigiola* and *Telephium*, previously members of the

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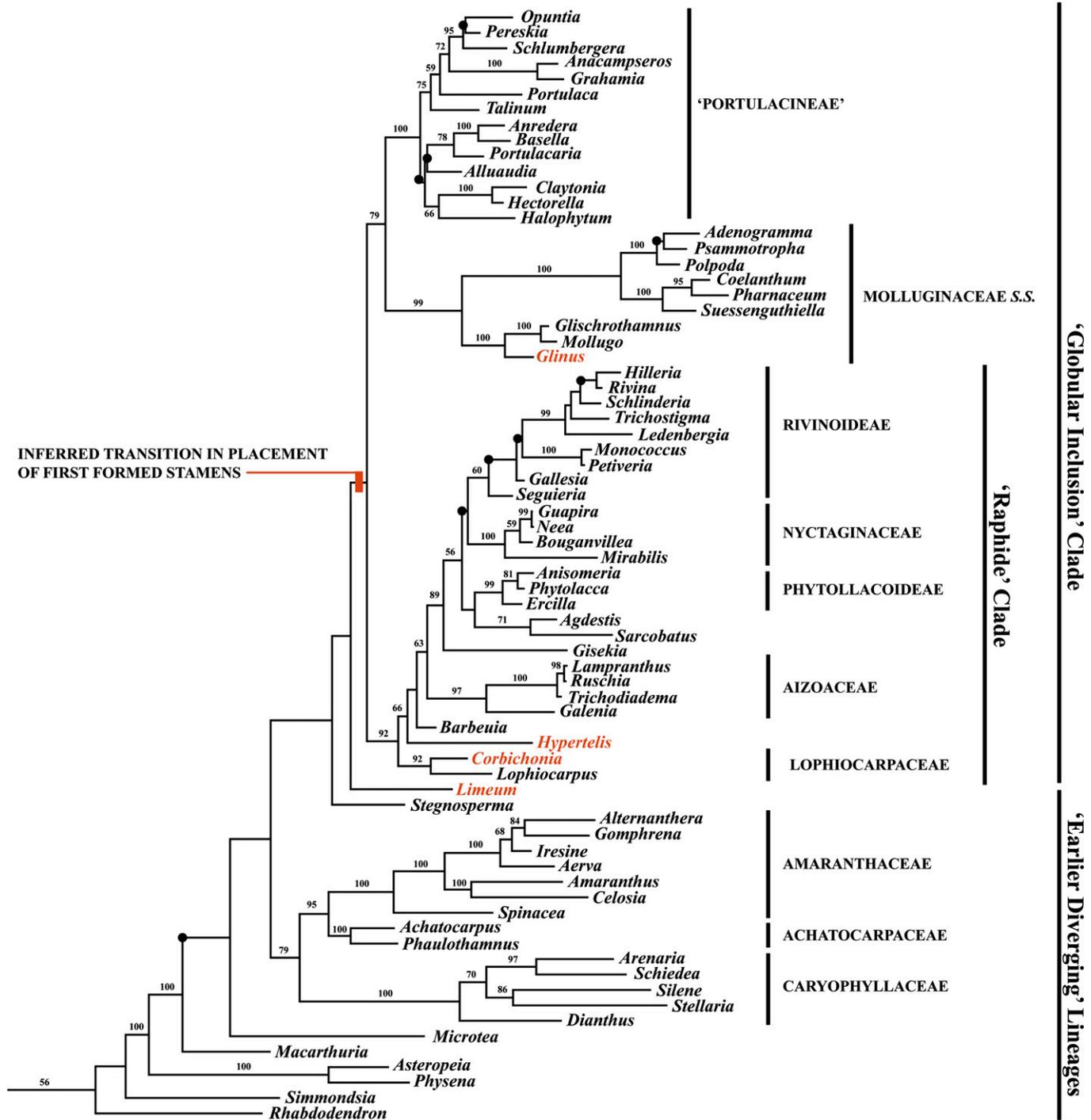


Fig. 1. Updated phylogeny of Caryophyllales, modified from Brockington et al. (2011). Genera whose floral development is described in this study are denoted in red. Major phylogenetic transition in androecium prior to the globular inclusion clade is annotated and marked with a red bar.

Molluginaceae have now been recognized as true members of Caryophyllaceae (Fior et al., 2006; Harbaugh et al., 2010). Cuénoud et al. (2002) found support for the removal of three other genera from Molluginaceae, *Corbichonia* as sister (with *Lophiocarpus*) to Phytolaccaceae, Nyctaginaceae, and Aizoaceae within the raphide clade, and *Limeum* as sister to the globular inclusion clade (a placement also recovered in Brockington et al., 2009). Schäfferhoff et al. (2009) found evidence for the exclusion

of *Hypertelis bowkeriana* from Molluginaceae and its placement within the raphide clade, as sister to Aizoaceae. Brockington et al. (2011) and Christin et al. (2011) also placed *Hypertelis salsoloides* within the raphide clade. However, Christin et al. (2011) also found *Hypertelis* to be a potentially paraphyletic genus with *Hypertelis spergulacea* still nested within *Mollugo*. Brockington et al. (2011) and Christin et al. (2011) both found *Macarthuria* to be placed as one of the early-diverging lineages

toward the base of the Caryophyllales, well outside the Molluginaceae. In summary therefore, Molluginaceae *sensu stricto* comprises the nine genera *Adenogramma*, *Polpoda*, *Psammotropha*, *Coelanthum*, *Pharnaceum*, *Suessenguthiella*, *Glinus*, *Glischrothamnus*, and *Mollugo* (Brockington et al., 2011; Christin et al., 2011). This narrower circumscription has in turn led to the recognition of the following lineages as distinct to the Molluginaceae: *Macarthuria*, *Limeum*, *Hypertelis*, Lophiocarpaceae (*Corbichonia*), *Corrigiola*, and *Telephium* (see Fig. 1).

The reconsideration of a paraphyletic Molluginaceae provides an ideal opportunity to reevaluate the floral morphology of a number of former members of the family. The floral development of *Hypertelis*, *Corbichonia*, *Limeum*, and *Glinus* is the focus of this study. The choice of these four genera is justified by the strategic position they occupy on the phylogenetic tree of Caryophyllales; *Limeum* is a member of the early-diverging lineages; the other genera are part of the “globular” inclusion” clade, with *Glinus* at the base of the “Portulacaceous alliance”, and *Hypertelis* and *Corbichonia* members of the “raphide” clade (Fig. 1). Given this broad distribution, observations on these taxa have bearing on floral evolution across the entire order.

Hypertelis is a small genus with 9–11 species distributed in southern Africa, with one species extending to Madagascar and tropical Africa and another endemic to St. Helena (Adamson, 1958a; Endress and Bittrich, 1993). The placement of *Hypertelis bowkeriana* and *Hypertelis salsoloides* within the raphide clade is still uncertain. Schäfferhoff et al. (2009) placed *Hypertelis bowkeriana* in the raphide clade as part of a polytomy with *Limeum*, Aizoaceae, *Gisekia*, and a Phytolaccaceae/Nyctaginaceae clade. Brockington et al. (2011) and Christin et al. (2011) placed *Hypertelis salsoloides* as sister to the remainder of the raphide clade following the divergence of *Corbichonia*. *Corbichonia* has two species, one endemic to southwestern Africa and the other widespread in Africa and southwestern Asia (Endress and Bittrich, 1993). *Corbichonia* is placed with *Lophiocarpus* in a small family Lophiocarpaceae (Cuénoud et al., 2002), and together they are placed at the base of the raphide clade by repeated molecular analyses (Schäfferhoff et al., 2009; Christin et al., 2011; Brockington et al., 2011). *Limeum* contains about 20 species, with a center of diversity in southern Africa, and is currently placed as sister to the globular inclusion clade with relatively weak support (e.g., Cuénoud et al., 2002; Brockington et al., 2009, Schäfferhoff et al., 2009; Christin et al., 2011). *Glinus* is a variable genus containing about six species. Christin et al. (2011) found that *Glinus* is monophyletic and represents a derived clade sister to *Glischrothamnus* and a number of species of *Mollugo*.

In this study we describe petals as “petaloids” as an indication of putative function, not as homology, which is variable in Caryophyllales. While a staminodial nature of the petals in e.g., Caryophyllaceae and Aizoaceae is undeniable, this is in contrast with petals derived from a calyx as is common in the Portulacaceae (Ronse De Craene and Brockington, 2013; Ronse De Craene, 2013). The former could be described as “staminodial petaloids”, while the latter as “calycinal petaloids”. The four genera studied here (*Hypertelis*, *Corbichonia*, *Glinus*, and *Limeum*) are diverse in their flower morphology, especially the number and arrangement of stamens and petaloids, and all are thought to exhibit centrifugal androecium formation (Hofmann, 1994). Although *Hypertelis* has been subject to limited morphological studies (e.g., Friedrich, 1956; Hofmann, 1973, 1994), no detailed floral ontogenetic analysis has been published. Batenburg et al.

(1984) described the morphology of the mature inflorescence of *Hypertelis salsoloides*. A short description of floral development in *Corbichonia rubriviolacea* is given by Hofmann (1994) and the floral development of *Corbichonia decumbens* has been partly investigated by Ronse De Craene (2007) to clarify the development of petaloids as part of the androecium. However, a more detailed investigation is needed. Hofmann (1994) shows some basic images of floral development in *Glinus lotoides* with the initiation of many stamens and petaloids, comparable to *Corbichonia*, but no detailed ontogenetic study has been performed. Moreover, *Glinus* is a variable genus, especially *G. lotoides*, with great variation in the number and arrangement of stamens, ranging from five alternisepalous stamens to several whorls with or without outer staminodes (Müller, 1908; Hofmann, 1973, 1994). Meanwhile, the floral development of *Limeum* is completely unknown, aside from basic observations on mature flowers (Hofmann, 1973). This is especially unfortunate given the pivotal position of *Limeum* as sister to the globular inclusion clade.

As part of ongoing studies of floral development in the Caryophyllales, we describe the floral ontogeny of three genera formerly placed in Molluginaceae (*Hypertelis salsoloides*, *Limeum africanum*, and *Corbichonia decumbens*) and two species of *Glinus*, *G. lotoides*, and *G. dahomensis*, which belong in the newly circumscribed Molluginaceae (Fig. 1). We primarily focus on describing patterns of androecium development in these lineages in the context of their recent phylogenetic placement, and with a view to patterns of floral evolution across Caryophyllales.

MATERIALS AND METHODS

Hypertelis salsoloides (Burch.) Adamson was cultivated from seed by S.F.B. in the greenhouses at the Department of Plant Sciences obtained from Silverhill Seeds (Cape Town, South Africa). *Glinus dahomensis* A.Chev. (considered as a synonym of *Glinus lotoides* var. *virens* Fenzl.) was cultivated from seed (from RBG Seed Bank, Wakehurst) by S.F.B. in the greenhouses at the Department of Plant Sciences, Cambridge. *Limeum africanum* L. was collected wild in South Africa in FAA (90 mL ethanol 70%, 5 mL acetic acid, 5 mL formaldehyde 40%) by S.F.B. in Namaqualand, South Africa (*sine numero*). Floral material was fixed in FAA. Floral buds of *Corbichonia decumbens* Scop. (nr 5007 - Tanganyika, coll. Mrs H. Faulkner 1589, 16 April 1955) and *Glinus lotoides* L. (nr 3282 - N. Rhodesia Petanke Distr., col. P. J. Greenway 8045, 5 Sept 1947) were sampled in the spirit collections in Kew by L.R.D.C. Floral buds and mature flowers were transferred to 70% ethanol and prepared using a Wild MZ8 stereomicroscope (Leica, Wetzlar, Germany). Dissected material was dehydrated in an ethanol-acetone series and critical-point dried with a K850 Critical Point Dryer (Emitech, Ashford, Kent, UK). The dried material was mounted on aluminum stubs and coated with approximately 180 nm of gold using an Emitech K575 sputter coater (Emitech) and examined with a Supra 55VP scanning electron microscope (LEO Electron Microscopy, Cambridge, UK) at RBG Edinburgh and a FEI Philips XL30 FEGSEM electron microscope at Cambridge.

RESULTS

Hypertelis salsoloides (Figs. 2A, B, 3–5, 16A)—Mature flowers of *Hypertelis* are relatively small, approximately 1 cm across by 1 cm high: Fig. 2A, B). While they are globular in bud, with sepals and pedicels covered with red trichomes, opened flowers have a reflexed perianth with distinctive outer and inner sepals (Fig. 2A, B). Outer sepals are green with narrow, white or pink margins, while margins of inner sepals are expanded and pink or white. Sepal three shows a combination of outer and inner sepals because one margin only is well



Fig. 2. Mature flowers of investigated species. A, B. *Hypertelis salsoloides*. (A) Lateral view of three flowers at different stages of development. (B) Apical view of mature flower; note the morphological difference between outer and inner sepals (numbered), and the intermediate shape of sepal 3. (For photograph of flower, see <http://www.flickr.com/photos/taiwanicus/2907299590/>). (C) *Corbichonia decumbens*. Partial view of inflorescence with flowers at different stages of development. The pink petaloids represent staminodes. For photograph of flower, see http://www.zimbabweflora.co.zw/speciesdata/image-display.php?species_id=122960&image_id=4. D, E. *Limeum africanum*. (D) Apical view of flower showing small spatulate petaloids. (E) Flower from below. Note the five sepals with white margins and larger inner sepals. (F) *Glinus lotoides*. Partial view of inflorescence with flowers at different developmental stages. José Quiles Hoyo, <http://www.florasilvestre.es/mediterranea/index.htm>. Bars: A, C–F = 10 mm; B = 5 mm.

developed (Fig. 2B). *Hypertelis salsoloides* always has 15 stamens arranged in three alternating whorls. The inner stamens are longest and alternate with sepals; those of the intermediate whorl are opposite sepals, and those in the outer whorl alternate with sepals (Figs. 4F, 5A, 5B). The stamens are basally fused in an inflated ring that is glandular on the inside, and the outer whorl is attached at the bottom of this ring (Fig. 5C). The 15 stamens are erect with yellow, basifixed anthers and pink filaments. The green globular ovary bears five yellow, terminal stigmatic extensions (Figs. 2A, 2B, 5B).

Inflorescences are thyrsoïd with a variable number of flowers. Each inflorescence develops into a terminal flower after initiating three lateral branches in the axil of pherophylls (bracts) (Fig. 3A, B). Each lateral branch terminates in a flower enclosed by two rapidly growing prophylls (bracteoles) (Figs. 3A–C, 4B). A smaller flower bud often develops in the axil of the first formed prophyll (Fig. 3B). In the terminal flower, the transition of bracts to sepals is continuous, with the first sepal occupying the position to be expected by a pherophyll on a normal racemose inflorescence (Figs. 3A, 3B, 4B). In lateral

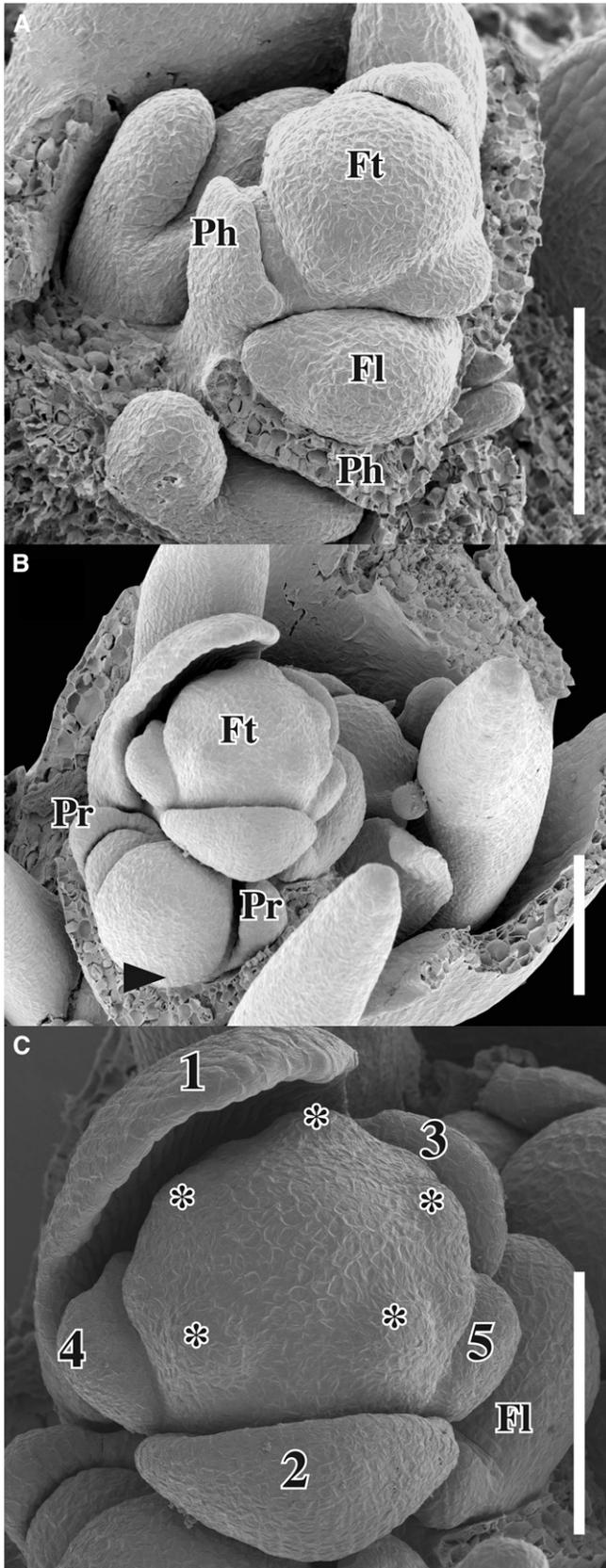


Fig. 3. *Hypertelis salsoloides*. SEM micrographs of early floral development. (A) Young lateral inflorescence showing terminal flower

flowers, the first sepal arises on the side opposite to the first formed prophyll (Fig. 3B, arrowhead), and the second sepal is placed adaxially. Pherophylls and prophylls have two large basally attached stipules (Fig. 3A, B). The five sepals are always initiated in a 2/5 phyllotaxis (Figs. 3C, 4A–F). Sepals one and two grow rapidly (Figs. 3C, 4A, 4C, 4D, 4F); growth of the remaining sepals is slower after initiation (Figs. 3C, 4A–F). Aestivation of sepals in bud is imbricate. Inner sepals increase considerably in size only in preanthetic buds (Fig. 5B). Glandular red trichomes are scattered over the abaxial side of sepals, bracts and pedicels (Fig. 2A).

The androecium develops on a hemispherical floral apex (Fig. 3C). Five upper alternisepalous stamens arise simultaneously and are followed by five stamens opposite the sepals (Figs. 3C, 4A–C). Initiation of antesepalous stamens appears to be in a basipetal sequence with those opposite sepals 3–5 developing more rapidly than those opposite sepals 1–2 (Fig. 4A–C). A third whorl of stamens arises below the first whorl in a centrifugal direction in close connection with the upper stamens alternating with the sepals (Fig. 4A–C). Stamen growth exceeds the development of sepals in younger stages, and the ovary occupies a prominent terminal position (Fig. 4C–F). It is only at anther and locule differentiation that the outer sepal lobes cover the floral bud completely (Fig. 4F). Globular anther lobes develop and subsequently split into two thecae that become again divided in two parallel symmetrical lobes; anthers are well developed with dorsal pollen sacs almost equal to ventral pollen sacs (Figs. 4F, 5A, 5B). In preanthetic buds stamens are more or less arranged in three whorls (Fig. 5A–C). Filaments only elongate just before anthesis. Stamens are lifted up by a common stamen tube that develops as a bulging rim (Fig. 5C). At anthesis, the stamens push their way through the closed perianth prior to it becoming reflexed. While the inner stamens are inserted on top of the rim, the middle whorl occupies a position halfway the rim, and the outer stamens are inserted just below the outer margin of the rim. The inside of the stamen tube develops as a nectary.

The gynoecium starts development as a relatively flat-topped pentagon on the floral apex at the initiation of the third stamen whorl (Fig. 4B). While the central area bulges slightly out, five prominent lobes are differentiated opposite the sepals and half moon-shaped depressions become visible in the middle of each carpel primordium (Fig. 4C–E). Five septal branches converge in the center of the flower, while the abaxial side of each carpel grows more extensively as to cover the locular space (Figs. 4F, 5A, 5B). The dorsal tips extend into five terminal stigmatic appendages before closing of the ovary is complete (Fig. 5B). The mature gynoecium grows as a globular ovary topped by five long stigmatic branches as no style is formed (Fig. 2B). Within each carpel, ovules develop in two rows on free-central placentae (not shown). A floral diagram shows the position of organs in the flower (Fig. 16A).

surrounded by smaller flowers arising sequentially. (B) Older lateral inflorescence showing the differentiation of the terminal flower and basipetal development of younger flowers. (C) Detail of terminal flower in B with sequential sepal initiation and simultaneous initiation of five alternisepalous primordia (asterisks) on the androecial plug. Bars = 100 μ m. *Abbreviations*: Aa, alternisepalous stamen; Ao, antesepalous stamen; Fl, lateral flower; Ft, terminal flower; Ph, pherophyll; Pr, prophyll.

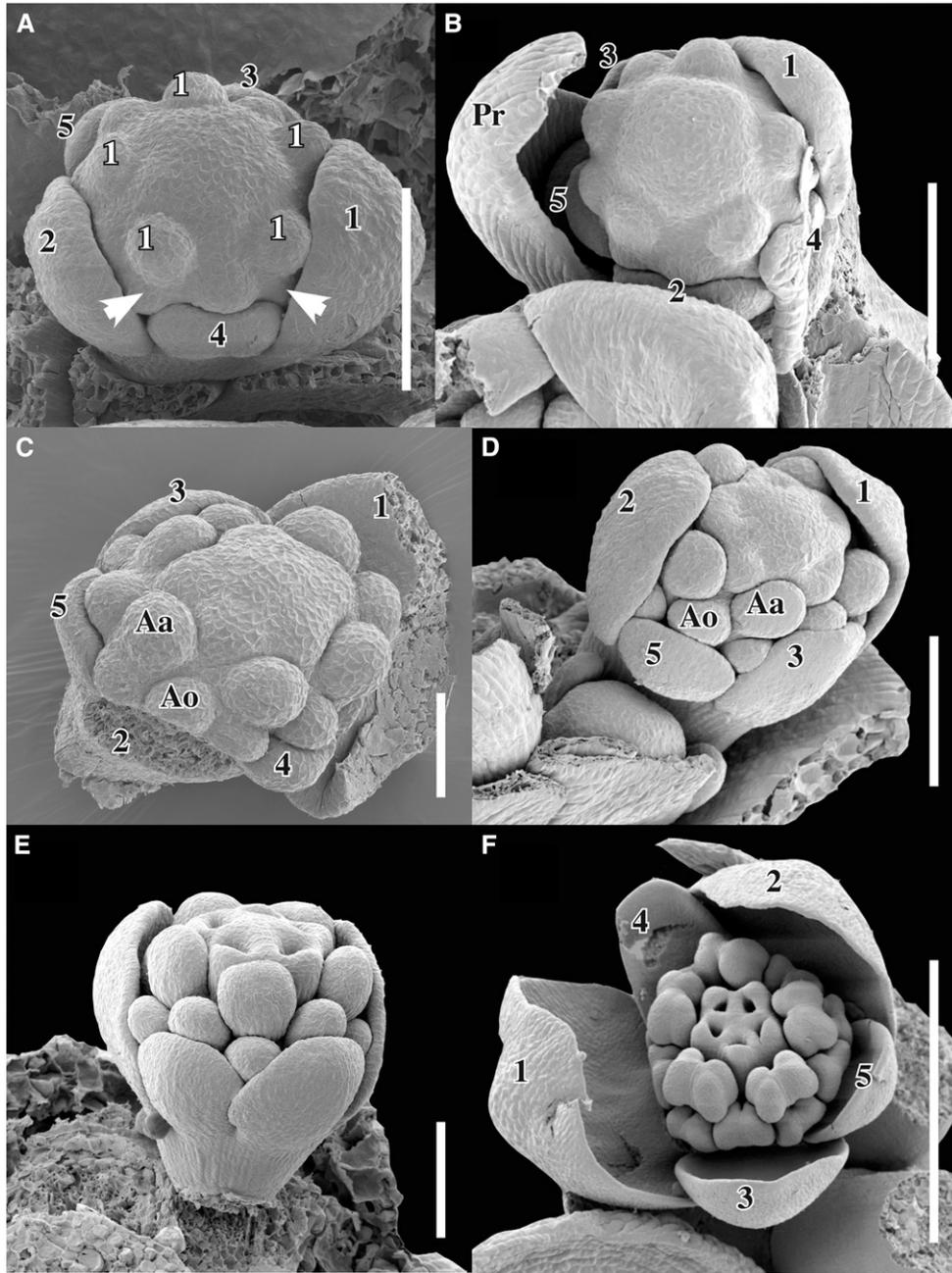


Fig. 4. *Hypertelis salsoloides*. SEM micrographs of early floral development. (A) Centrifugal initiation of three stamen whorls. Arrows point to outer alternisepalous stamen primordia. (B) Differentiation of pentagonal gynoecial dome. Note unequal size of antesealous stamen primordia. (C) Development of carpel primordia and separation of lower alternisepalous primordia from upper stamens; sepals one and two removed. (D, E) Deepening of carpelary lobes and differentiation of stamens. (F) Development of septal branches and differentiation of anthers. Bars = 100 μ m; C = 50 μ m, F = 500 μ m. Abbreviations: Aa, alternisepalous stamen; Ao, antesealous stamen; Pr, prophyll.

Corbichonia decumbens (Figs. 2C, 6–8, 16B)—Flowers are initiated in condensed cymes. Most terminal flowers are generally accompanied by two smaller flowers arising sequentially in the axil of prophylls. This process is repeated in lateral flowers (Fig. 6A–F). The inflorescence is a mixture of dichasial and monochasial branches, as often only one lateral flower reaches maturity. Each flower is subtended by a large pherophyll and two prophylls. Prophylls develop rapidly and are mucronate, forming a cap-like protuberance (Fig. 6A, B). Initiation of sepals

is continuous in a 2/5 sequence, and the two outer sepals rapidly cover the bud, while the inner sepals are still small (Figs. 6A–F, 7B, 7C); sepal two is strictly positioned in a median position (Figs. 6B, 6E, 6F, 7B). Outer sepals develop a mucronate tip similar to prophylls (Figs. 6E, 7B). The inner sepals remain small during most of the floral development, and the extensive pressure of the outer sepals molds the flower bud in a distinctive dissymmetric shape (Figs. 6E, 7B, 7C). The inner sepals reach considerable size only at anther differentiation. Immediately after

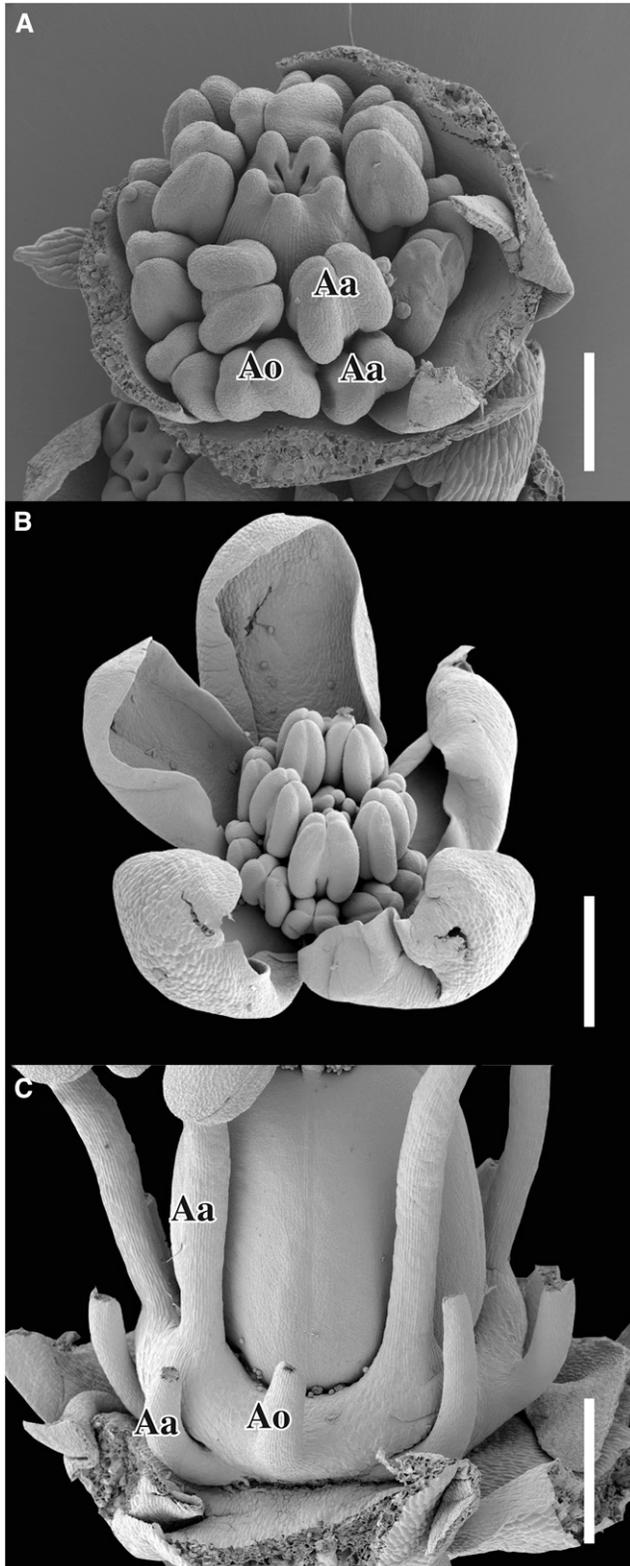


Fig. 5. *Hypertelis salsoloides*. SEM micrographs of late floral development. (A) Lateral view at style differentiation; the stamens are crowded within the calyx. (B) Nearly mature flower at closure of the carpels; the sepals have been moved apart. (C) Detail of mature androecium. Note the staminal ring with three tiers of stamens. Bars: A = 200 μm ; B, C = 500 μm . Abbreviations: Aa, alternisepalous stamen; Ao, antesepalous stamen.

sepal initiation, the floral apex takes a flattened convex shape and stamens are rapidly initiated on the periphery (Fig. 6B–D). The earliest stages available show the simultaneous initiation of five alternisepalous stamen primordia (Fig. 6B–D). Immediately after (or at the same time as) the alternisepalous stamens, antesepalous stamen primordia appear at a slightly lower level (Fig. 6B–D). Initiation of antesepalous stamens is sequential, with stamens opposite sepal four, five, and three arising first (Fig. 6D, F). The next stamens arise opposite sepal one and finally sepal two, where the space remains empty for some time (Fig. 6D–F).

While a central pentagonal area is delimited as the gynoecium, stamen development extends centrifugally with a pair of additional stamens arising in close connection with the antesepalous (Fig. 7B, C) or more rarely the alternisepalous (Fig. 7A) stamens. As these three stamens are more closely connected, they appear as complex primordia (Fig. 7A,B). The clustered arrangement becomes more clear when three additional primordia are initiated externally, followed by two more (Figs. 7C, 8B).

The three upper tiers of stamens extend upward and develop anther tissue starting with the alternisepalous stamens (Figs. 7C, 7D, 8A–C). Anthers are positioned at different levels in the flower, with alternisepalous stamens at the top, an intermediate level opposite the sepals, and a third level with paired anthers next to the antesepalous stamens (Fig. 8A–E). Anthers are slightly displaced in bud by pressure of the closed perianth, but they retain their respective sizes (Fig. 8C, D). While the H-shaped anthers differentiate pollen sacs, filament growth lifts the stamens up, concomitant with the growth of the styles (Fig. 8C, D, F).

While the three inner series of the androecium develop as normal stamens, the fate of the two outer series is highly different. Primordia remain globular at first, and contrary to the fertile stamens, they become flattened by marginal growth (Fig. 8B, E). The flattened staminodes remain small at first, but grow progressively upward, covering each other in a haphazard way (Fig. 8F, G). Prior to anthesis, they reach beyond the stamens and form the main attraction of the flower (Figs. 8H, 2C). In total, 45 stamen primordia are initiated in the flower, and the number of fertile stamens and staminodial petaloids is 20 and 25, respectively, and remains highly constant (Fig. 16B).

Simultaneously with stamen initiation, a pentagonal gynoecial dome is delineated (Fig. 6E, F), and rounded carpillary lobes develop on the five angles. A triangular depression becomes differentiated midway between the margin and apex, and five carpillary lobes emerge, separated by broad septal branches converging in the center (Fig. 7B–D). The dorsal carpillary lobes do not initially grow much, and ovule primordia start developing in an axillary position (Fig. 7D). Later, more extensive abaxial growth lifts the carpel wall up, while the ovary deepens by extensive growth below the lobes (Fig. 8A, B). Finally, the carpillary lobes converge in the center and close the ovary, while they grow up as five stylar branches (Fig. 8C, D, F, G). The stylar branches are covered with papillae on their adaxial side and lie horizontally on top of the mature ovary. A floral diagram shows the position of organs in the flower (Fig. 16B).

Limeum africanum (Figs. 2D, E, 9–11, 16C)—Mature flowers are clustered at the end of branches in dense pseudoheads with flowers arranged in a repetitive cymose pattern. Partial cymes consist of a top flower accompanied by two smaller lateral flowers of different age (Figs. 9C–F). Prophylls are generally compressed toward the axis by the pressure of the pherophyll (Fig. 9D, F). Mature flowers have sepals arranged in an imbricate pattern enclosing small spatulate petaloids and erect stamens

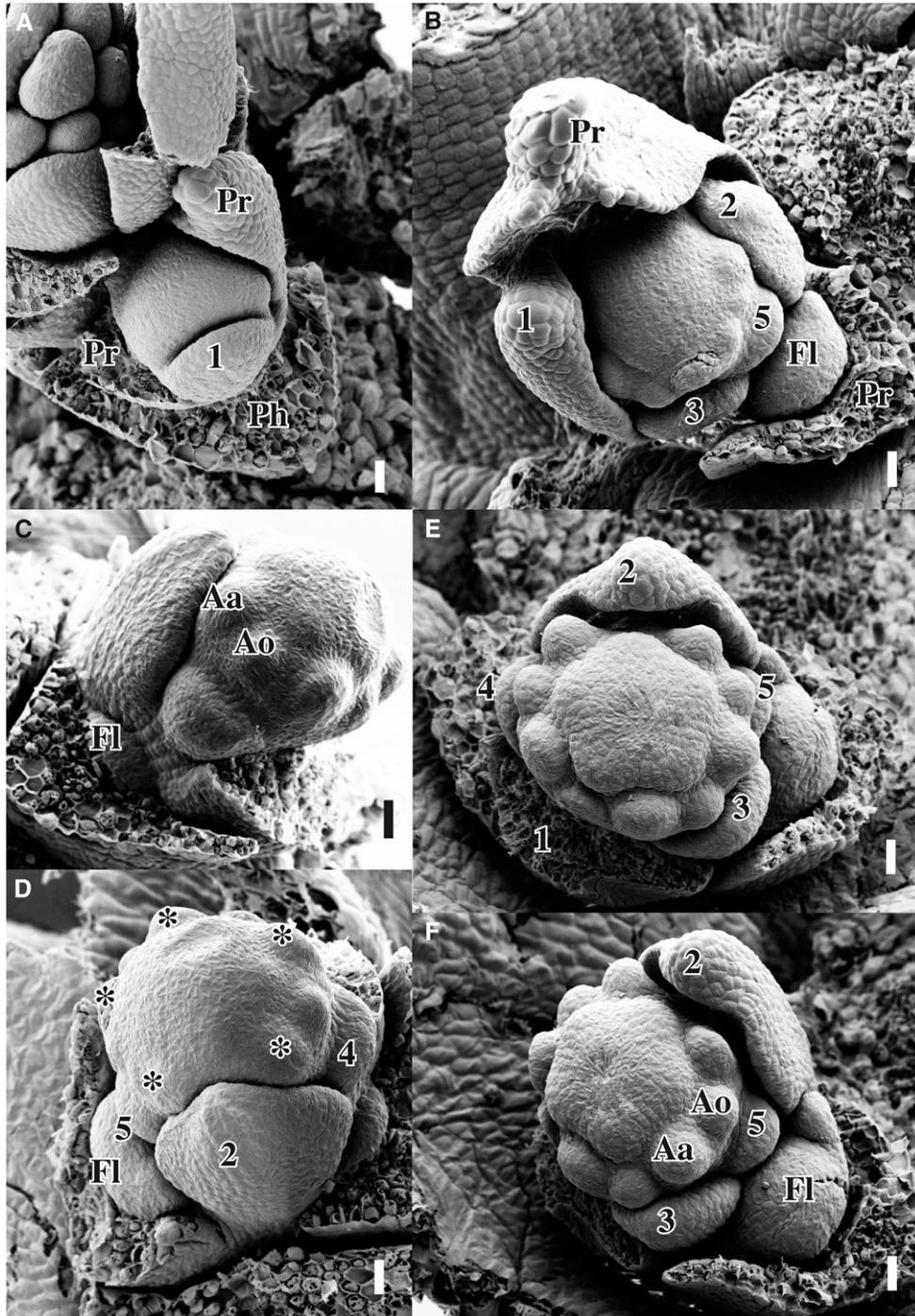


Fig. 6. *Corbichonia decumbens*. SEM micrographs of early floral development. (A) Lateral view of young lateral flower showing initiation of the first sepal. (B) Apical view of older flower at initiation of the androecium. (C) Side view of young flower toward sepal four: initiation of two whorls of stamens at different levels and first traces of carpels. (D) Lateral view of similar stage—alternisepalous stamens shown by asterisk; note the absence of a stamen opposite sepal two. (E, F) Two different views at the differentiation of a gynoecial pentagon. Note the unequal size of antesealous stamens; no stamen has been formed opposite sepal two. Bars = 20 μ m. Abbreviations: Aa, alternisepalous stamen; Ao, antesealous stamen; Fl, lateral flower; Ft, terminal flower; Pr, prophyll.

(Fig. 2D). The sepals develop in a 2/5 sequence, and the first sepal is rapidly followed by sepal two situated against the axis in median position (Fig. 9A–D). Both outer sepals develop a distinctive mucronate apex (Figs. 9A, 9C, 9D, 10A). The three inner sepals remain initially smaller and only expand in size at

maturity as to exceed the outer sepals in size (Fig. 2D, E). The mature sepals develop a broad central zone of chlorophyllous tissue around the midrib with narrow white margins. As soon as sepals have been initiated, stamen primordia differentiate on a hemispherical platform (Fig. 9B–E). Stamens are positioned in

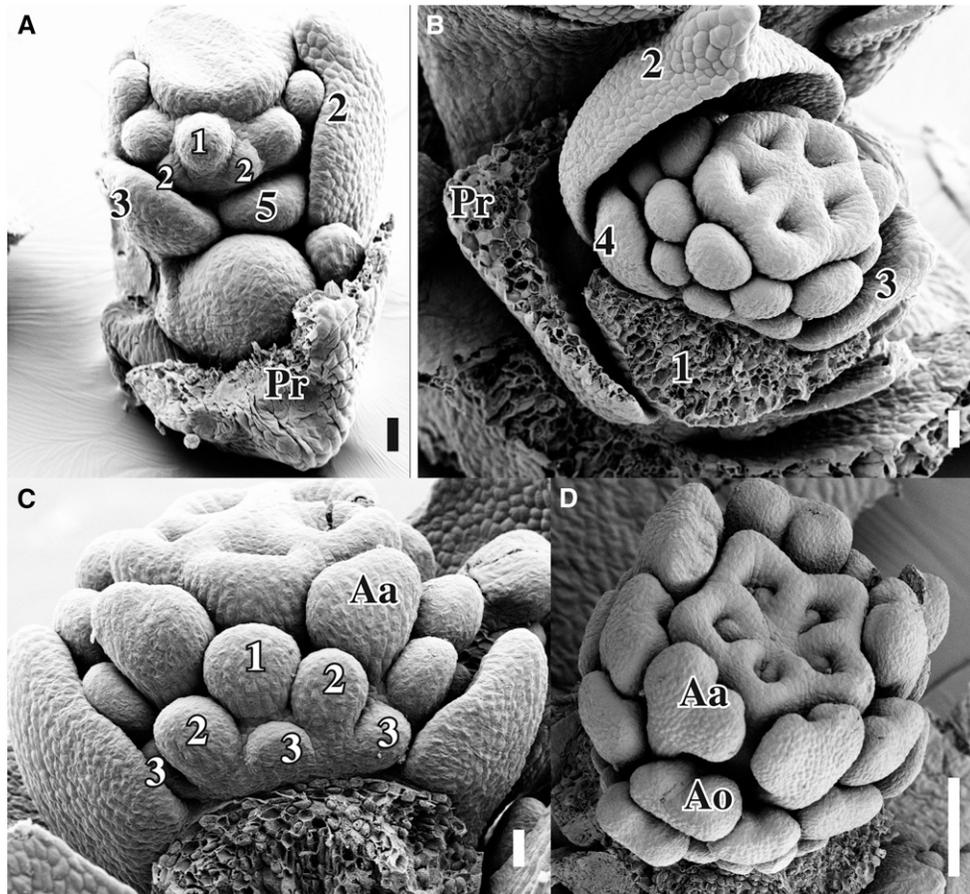


Fig. 7. *Corbichonia decumbens*. SEM micrographs of early floral development. (A) Lateral view showing centrifugal development of the androecium (white numbers) linked with the alternisepalous stamens. (B) Abaxial view of flower at differentiation of the septa. (C) Detail of stamen initiation. Numbers show centrifugal development of antesepalous stamen groups. (D) View of flower at anther differentiation and ovule development. Bars: A–C = 20 μ m; D = 100 μ m. Abbreviations: Aa, alternisepalous stamen; Ao, antesepalous stamen; Pr, prophyll.

a single whorl. The number of stamens initiated varies between seven (Figs. 9C–F, 10A, 10F, 11B, 11C) and six (Figs. 10B–D, 11A). Stamens are variously inserted opposite sepals and petaloids. With seven stamens, a stamen is always absent opposite the petaloid between sepals three and five and opposite sepals one and two (Figs. 9F, 10A, 10D, 10F, 16C). With six stamens, another stamen is absent between sepals two and five (Fig. 10B–D). Initiation is rapid with stamens at different levels and with a different size (Fig. 9C–E). While the initiation sequence of the stamens is difficult to observe, subsequent growth of stamens demonstrates a regular sequence approximating a $3/8$ pattern that can be traced from stamen differentiation until maturity (Figs. 9F, 10A, 10D, 10F, 11A–C). The development of stamens runs in an inversed spiral sequence starting either with the stamens opposite sepal five and sepal four although it was difficult to discern which was the first to arise (Figs. 9F, 10A, 10C, 10D, 10F, 11B, 11C). Stamens differentiate globular anthers on filaments of different length reflecting the sequential growth (Figs. 10D, 10F, 11A–F). The two first-formed stamens remain the longest in bud (Figs. 10, 10F, 11C, 11D, 11F). The base of the filaments inflates and becomes covered with trichomes on its margins and on the adaxial side (Fig. 11F, G). Filaments are connected by a short tube that is covered with nectarostomata on the adaxial side below the level of the trichomes. Primordia of the petaloids arise immediately after (or simultaneously with)

stamen initiation (Fig. 9C–E) and are delayed in growth (Figs. 9F, 10C–F, 11C). Initiation of petaloids is independent of upper stamens (Fig. 10C), but they appear closely connected to the alternisepalous stamens. The petaloid alternating with sepals three and five has no opposite stamen (Fig. 16C). A petaloid is rarely absent (Fig. 10B, asterisk). Petaloids become dorsiventrally flattened and grow as short, strongly spatulate appendages at the base of the stamen tube (Figs. 2D, 11F, 11G). In a few buds, petaloids develop as short erect filament-like appendages (Fig. 11D).

The ovary initiates as a globular protuberance on top of the staminal platform (Fig. 9F). A central depression is delineated by a low peripheral rim initially growing on one side (Fig. 10B–D). The ovary extends toward sepal two, forming a second protuberance that will eventually develop into a second style (Fig. 10F, 11A, 11C). The second rim is delayed relative to the first, as the ovary is pseudomonomerous. Although two ovules are initiated (Fig. 11E), only the adaxial one reaches maturity, while both styles are equal in size. Carpels are always oriented in a median, though slightly oblique position as carpel one lies on the radius of sepal one. A floral diagram shows the position of organs in the flower (Fig. 16C).

Glinus lotoides (Figs. 2F, 12, 13, 16D)—Mature flowers of *Glinus lotoides* are greenish-white with five sepals and a

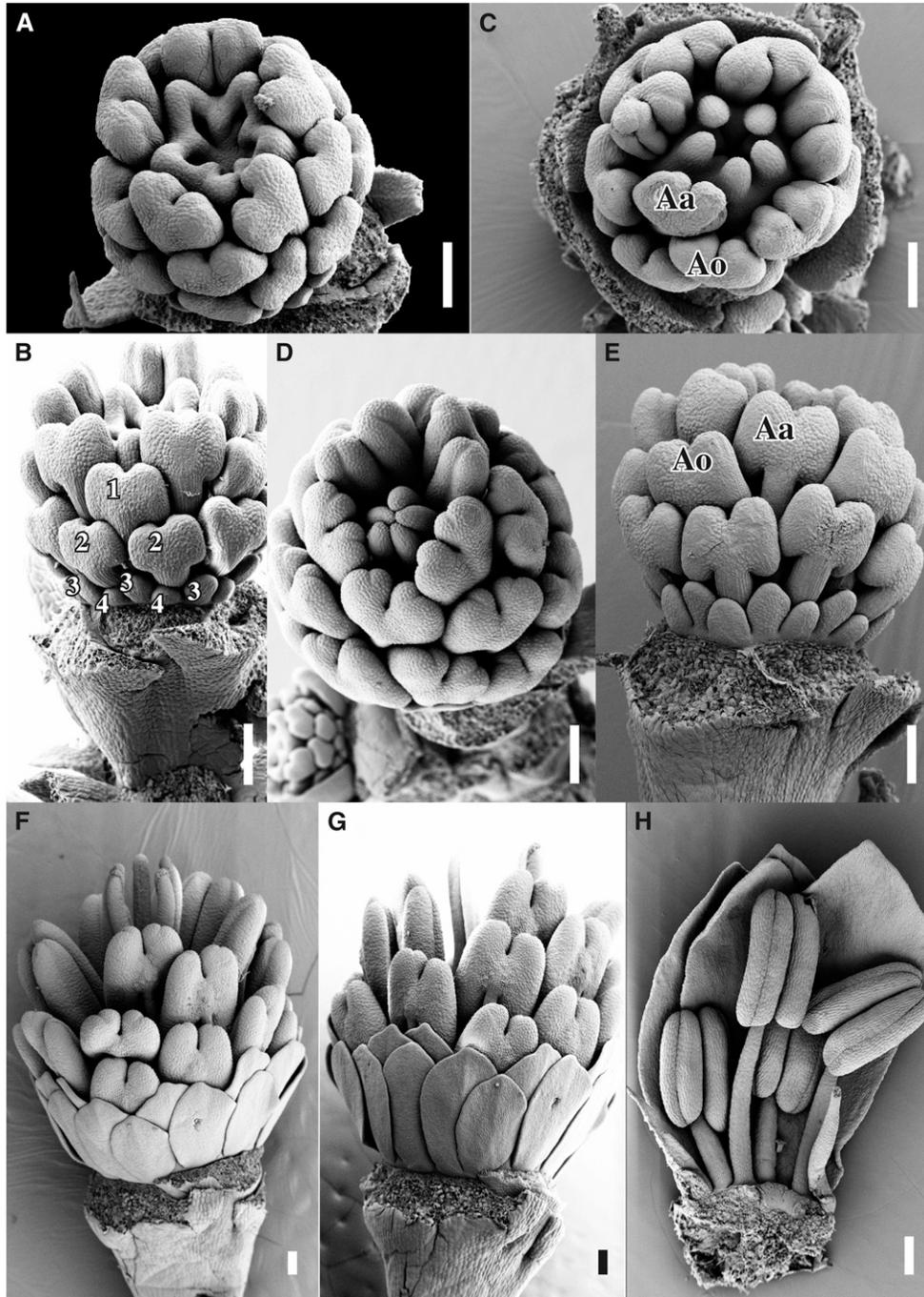


Fig. 8. *Corbichonia decumbens*. SEM micrographs of late floral development. (A) Apical extension of carpellary lobes. (B) Lateral view of the same. Note the centrifugal differentiation of four whorls of antesepalous stamens. (C) Apical view showing differentiation of stigmatic lobes. (D) Apical view of older stage. Note the crowding of the stamens in several series. (E) Lateral view of the same. Note the arrangement of staminodes in two irregular tiers. (F, G) Successive stages in the expansion of the staminodes. (H) Adaxial view of part of the androecium in a preanthetic bud. Note the staminodial petaloids overtopping the stamens. Bars = 100 μ m; H = 200 μ m. *Abbreviations:* Aa, alternisepalous stamen; Ao, antesepalous stamen.

variable number of stamens, staminodes, and petaloids. As for *Hypertelis*, the inner sepals have broader white margins than the two outer sepals, and sepal three is intermediate (Figs. 2F, 16D). Flowers arise on compound cymose inflorescences containing several lateral branches (Fig. 12A). Lateral branches are monochoasial, with a terminal flower and a smaller axillary flower (Fig. 12A). The number of lateral branches is variable

but ranges from three to five. Each partial inflorescence is subtended by a pherophyll and two prophylls (Fig. 12A). Within each partial inflorescence, the top flower differentiates five sepals in a 2/5 sequence with sepal one positioned toward the bract and sepal two against the axis (Fig. 12A–C). The two outer sepals expand above the developing flower bud and become slightly hooded (Fig. 12B, C). While the inner sepals are

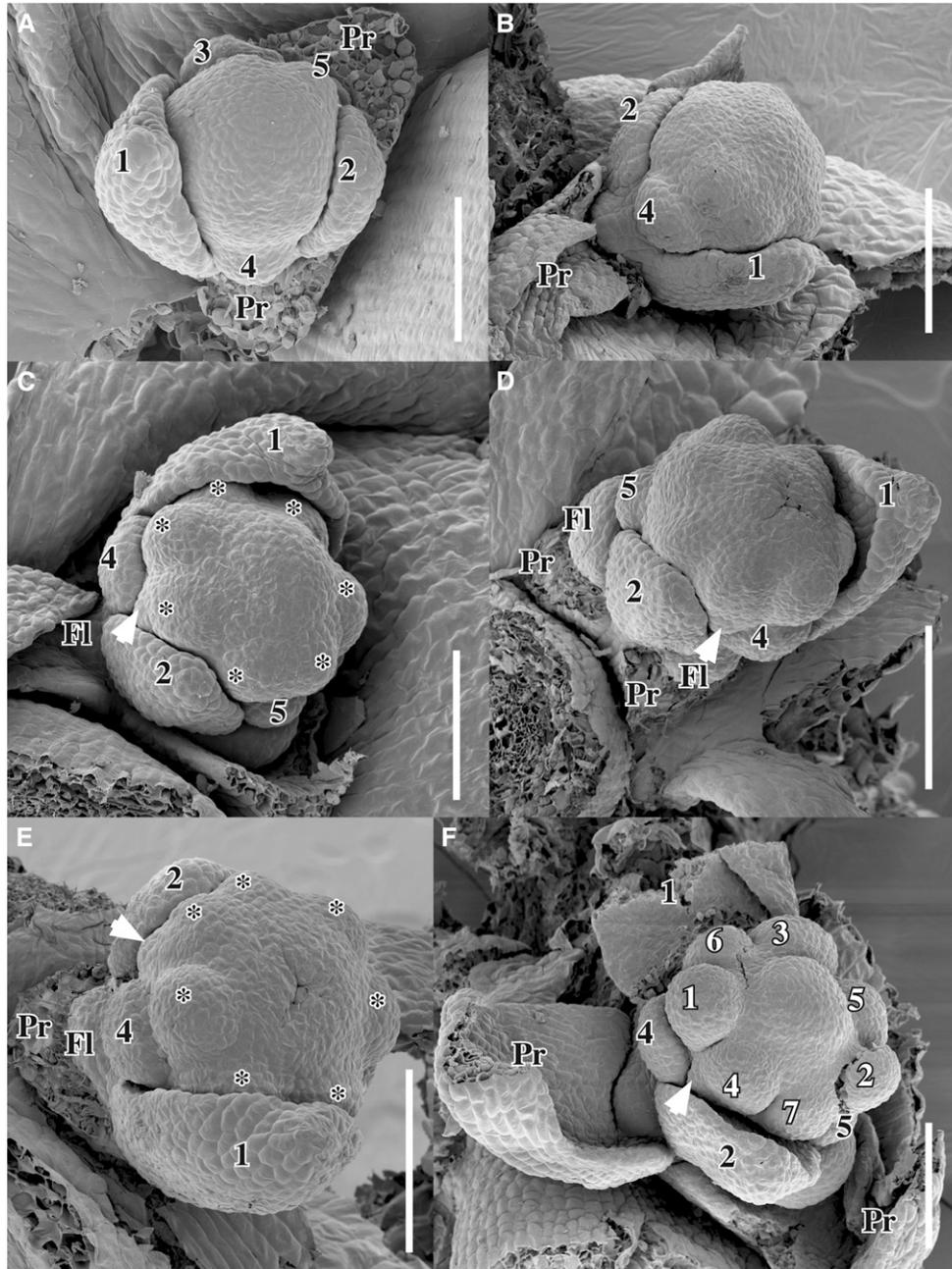


Fig. 9. *Limeum africanum*. SEM micrographs of early floral development. (A) Apical view of early initiation of the sepals. (B) Lateral view with early differentiation of androecial plug. (C–E) Rapid sequential initiation of seven androecial primordia (asterisks) and first traces of petaloid primordia (arrows). (F) Sequential differentiation of stamen primordia (white numbers) and formation of gynoecial dome. Bars = 100 μ m. Abbreviations: Fl, lateral flower; Pr, prophyll.

still small, the floral apex has become pentangular, and five alternisepalous stamen primordia are initiated almost simultaneously on its margins (Fig. 12B, C). The stamens next to sepal one are slightly advanced compared to the other stamens (Fig. 12B). Primordia are inserted in alternation with the sepals but tend to converge in two pairs (opposite sepals one and two) and a single stamen between sepals three and five (Fig. 12D, E). There is more space between the stamens in the areas opposite sepals three, four, and five (Fig. 12E). After the differentiation of the gynoecium, a number of extra stamens can be initiated opposite

the sepals (Figs. 12E, 12F, 13A). The extra stamens are generally sterile and develop either as small, two-lobed, petaloid appendages (Fig. 13A–C) or as small stubs (Figs. 12F, 13A, 13B); they rarely develop as fully fertile stamens and may have half of the anther sterile (Fig. 13C). While the spaces opposite sepals three, four, and five are generally occupied by sterile stamens, stamens are more rarely formed opposite sepals one and two, and the extent of development of the stamens is inversely correlated with the sequence of sepal initiation (Fig. 16D). When fully developed, petaloid staminodes have a long bifid

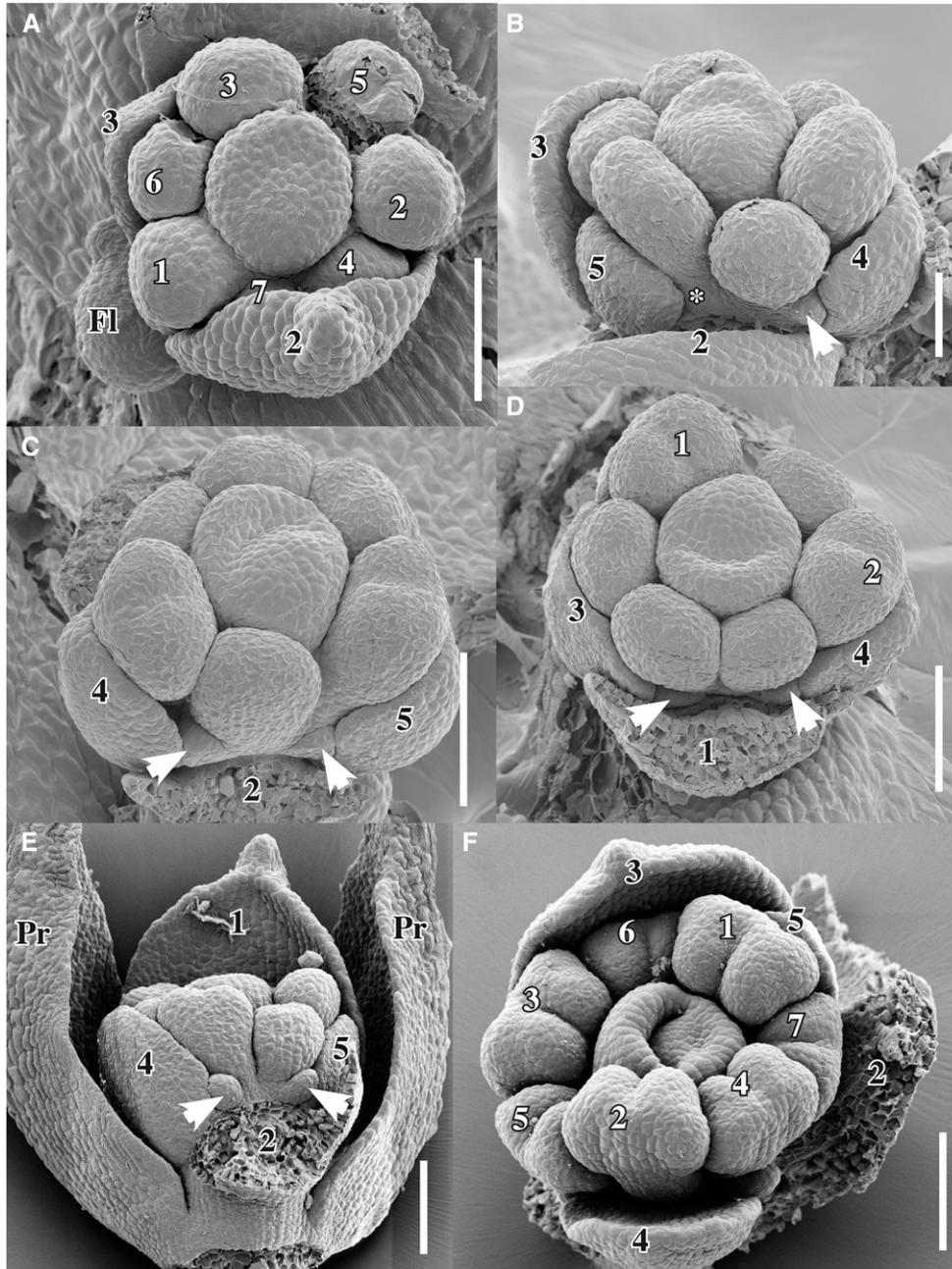


Fig. 10. *Limeum africanum*. SEM micrographs of early floral development. (A) Apical view showing sequence of differentiation of stamens and development of a central depression on the gynoecium. (B) Adaxial view of flower with six stamens. Note the empty position opposite sepal 2 (asterisk) and single petaloid (arrow). (C) View of young bud from the adaxial side. Note two petaloids (arrows), single stamen opposite sepals 2, and initiation of carpellary rim. (D) Similar stage seen from the abaxial side. Note two petaloids (arrows) and unequal stamen pair opposite sepal 1. (E) Adaxial view at differentiation of the filaments; note the small petaloids (arrows). (F) Lateral view at differentiation of the anthers and carpels. Note the initiation of a second carpellary rim and the different length of the filaments, reflecting the sequence of differentiation of the stamens (white numbers). Bars: A, C–F = 100 μ m; B = 50 μ m. Abbreviations: Fl, lateral flower; Pr, prophyll.

apex (Fig. 13C). Fertile stamens differentiate as a broad rectangular organ; further development leads to H-shaped anthers containing two extrorse thecae on a narrow connective and a stout filament (Fig. 13A, C). The ovary develops as a triangular apical primordium with the angles facing sepals one, two, and three (Fig. 12D). Three deep slits are formed midway on the angles delimiting a central meristematic triangle (Fig. 12E). The slits deepen

progressively by the upward growth of the underlying gynoecial tissue (Fig. 12F). As a result, the gynoecium develops as a tall cylinder, retaining apical slits (Fig. 13A). Within each slit, a locule develops with several ovules arranged in four rows. The areas abaxial to the apical slits extend as overarching tissue and develop as short styles only before anthesis (Fig. 13C). A floral diagram shows the position of organs in the flower (Fig. 16D).

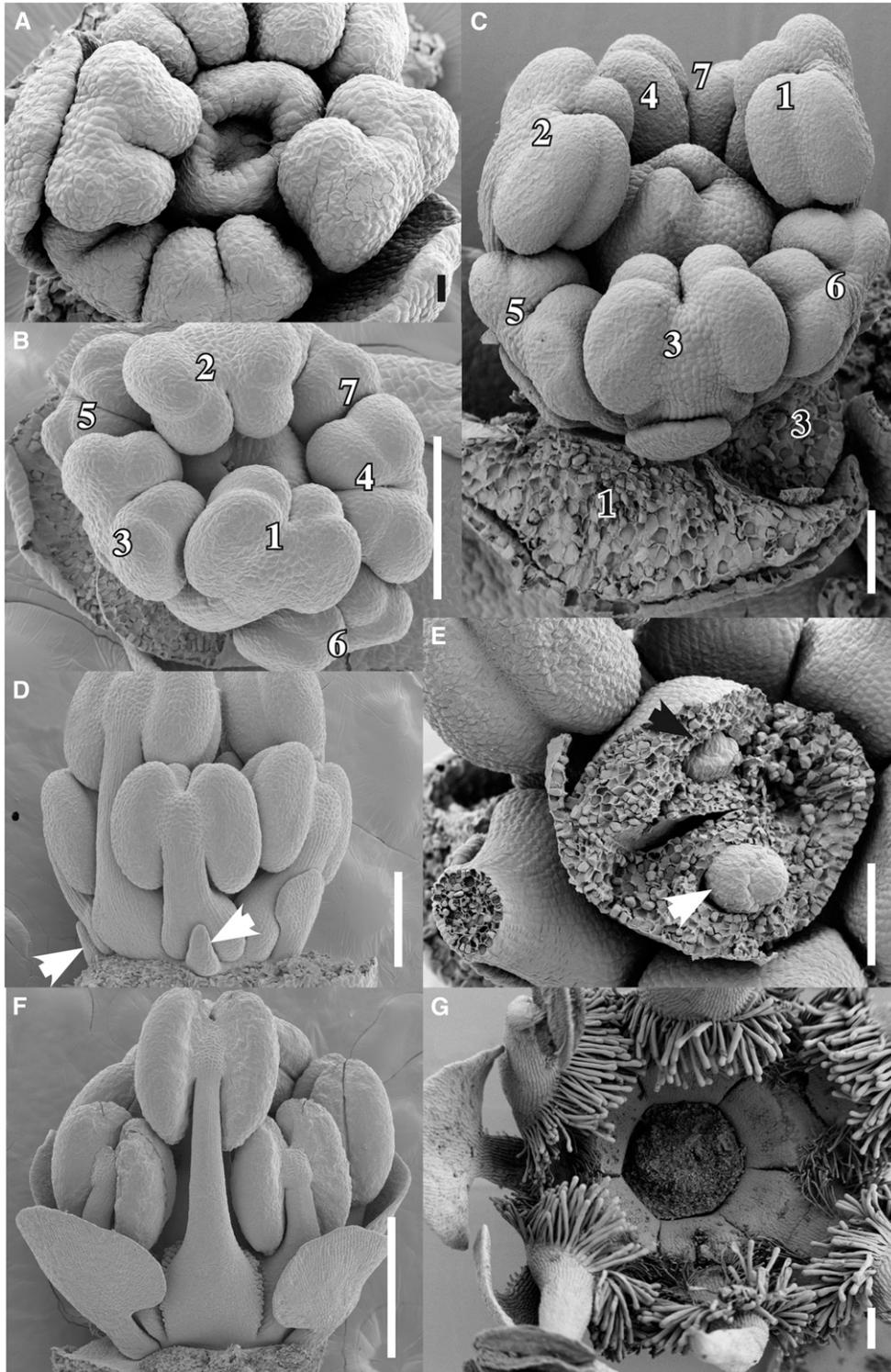


Fig. 11. *Limeum africanum*. SEM micrographs of late floral development. (A) Apical view with upward growth of carpellary lobes. (B) Apical view at anther differentiation and filament growth reflecting the sequence of initiation (white numbers). (C) Lateral view of flower at closure of the carpels. White numbers indicate the sequence of stamen initiation. (D) Lateral view of flower at anther and petaloid differentiation. Note that two petaloids are staminoid-like (arrows). (E) Cross section of young ovary showing two ovules of different size (arrowheads); the upper ovule will eventually abort. (F) Preanthetic bud showing small spatulate petaloids and inflated filament bases with trichomes developing on the adaxial side. (G) Apical view of mature flower; ovary removed. Note the long trichomes on the base of the filaments and nectariferous area below. Bars: A = 20 μ m; B, C, E = 100 μ m; D, F, G = 200 μ m.

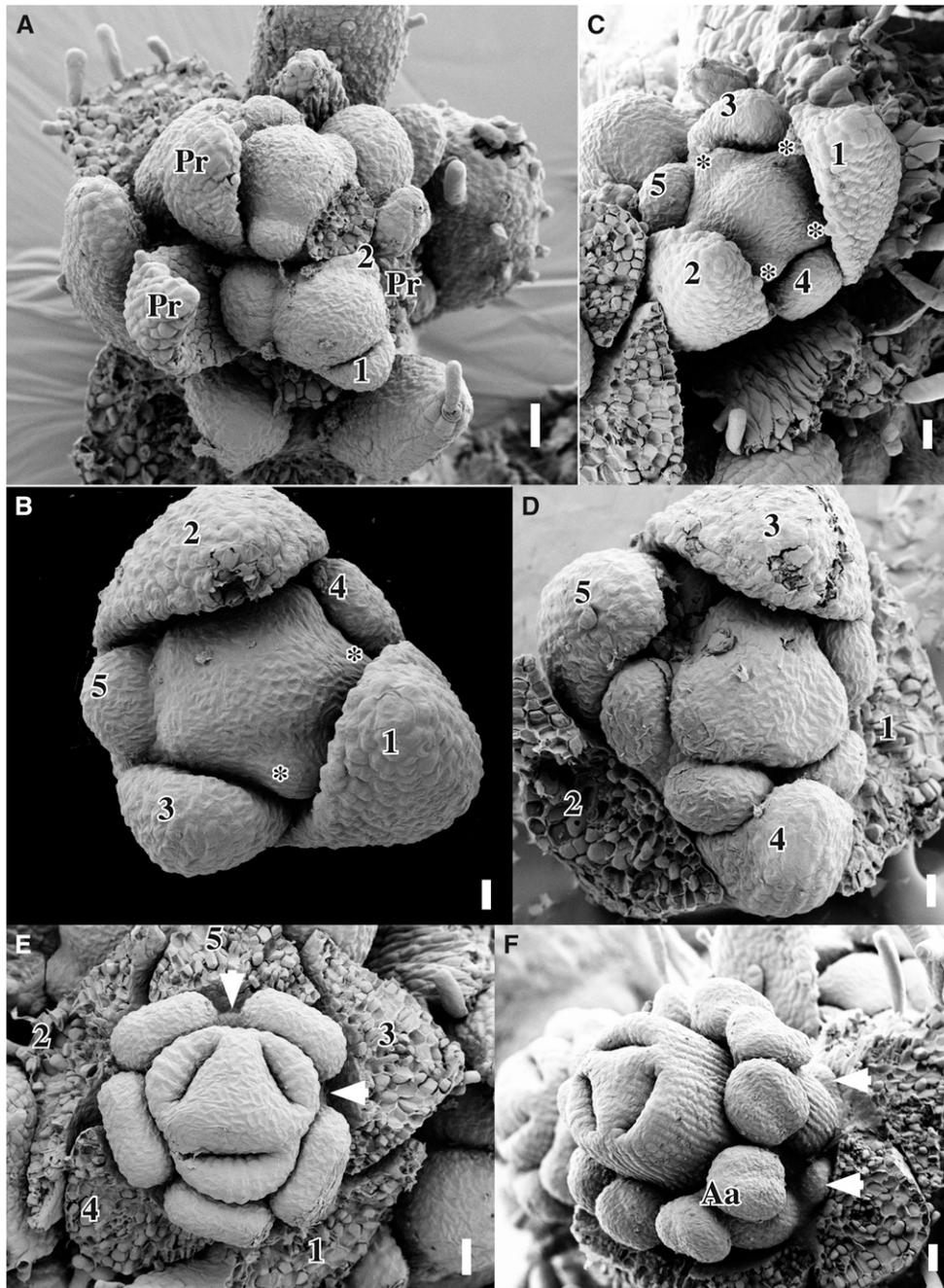
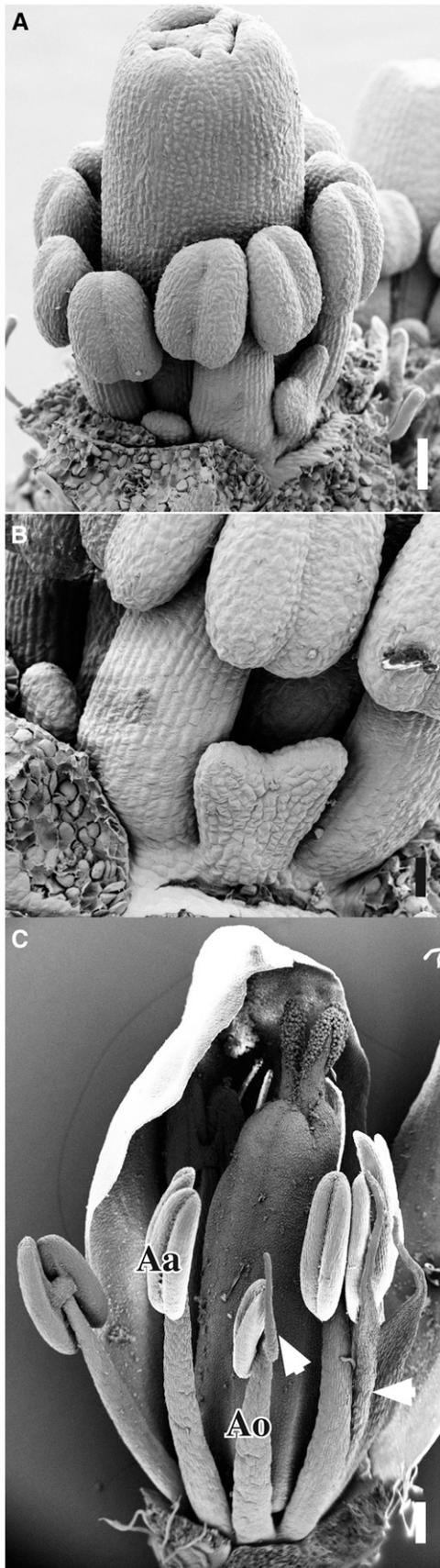


Fig. 12. *Glinus lotoides*. SEM micrographs of early floral development. (A) View of young inflorescence showing initiation of younger flowers around a terminal flower. Initiation of first sepals on lateral flower (numbers). (B) Formation of pentangular apex after sepal initiation; note that the adaxial side is advanced with two stamens being initiated (asterisks). (C) Slightly older bud showing the differentiation of five alternisepalous stamen primordia; four are visible (asterisks). (D) Development of three carpel primordia and expansion of the alternisepalous stamens. (E) Apical view of flower at carpel initiation. Arrows point to spaces between stamens where further stamens initiate. (F) Lateral view of flower at differentiation of anther tissue. Arrows point to small antesealous primordia. Bars = 20 μ m. Abbreviations: Aa, alternisepalous stamen; Pr, prophyll.

Glinus dahomensis (Figs. 14, 15)—The species shows a pattern of early floral development similar to *G. lotoides* with cymose inflorescences containing several lateral branches (Fig. 14A). Lateral flowers arise between a pherophyll and two prophylls, and an extra flower usually develops only in the axil of the first formed prophyll (Fig. 14B, C). Sepals are initiated in a regular 2/5 sequence, and the outer sepals rapidly cover the inner sepals

(Fig. 14A–E), although differences in size are not as strong as in other genera. Sepals develop a mucronate apex (Fig. 14D, E). Five alternisepalous stamens are initiated more or less on the same level but in a rapid sequence starting with a stamen between sepals three and five and ending with a stamen between sepals one and four (Fig. 14C, E). Antesealous stamens initiate at a lower level and their development is also sequential



(Fig. 14C, E, F). Stamens opposite sepals one, two, and three are visible before those opposite sepals four and five (Fig. 14C, D), and stamens generally differ in size (Fig. 14E, F). Alternisepalous stamens become subequal in size, and differentiated anther tissue develops extrorsely (Figs. 14F, 15A). The antesepalous whorl is generally fertile but with shorter filaments (Fig. 15B). Occasionally stamens are misshapen with a sterile half-anther (Fig. 15B,E), or stamens are sterile and petaloid with a bifid apex (Fig. 15C, F). Petaloids develop opposite the alternisepalous stamens after differentiation of the anthers (Fig. 15D). They develop into bifid, heart-shaped appendages (Fig. 15B, E) and eventually grow into narrow forked structures (Fig. 15C). The gynoecium is initiated in the same way as in *Glinus lotoides* (Figs. 14E, 14F, 15A–C).

DISCUSSION

The floral morphology of (core) Caryophyllales is unique among eudicots, with a basic absence of petals, and a strong convergent evolution with other petal-bearing angiosperms through the evolution of staminodial petaloids. In addition, the androecium development and evolution is highly divergent. These four genera (*Corbichonia*, *Limeum*, *Glinus*, *Hypertelis*) exhibit a number of floral developmental features indicative of evolutionary processes across the Caryophyllales. These processes include perianth differentiation within the calyx whorl or via stamen sterilization, centrifugal stamen initiation on an androecial platform surrounding the gynoecium, reversed initiation of antesepalous stamens, starting opposite sepals four and five, variable merism of the gynoecium linked with a predictable loss of antesepalous stamens, and finally phylogenetically informative alterations in patterns of androecium development. These processes will be explored further with reference to evolutionary patterns of floral development across the Caryophyllales.

Essential patterns of centrifugal stamen initiation within the androecium—As demonstrated by the four genera studied here, centrifugal initiation of the androecium appears to be extremely common in the Caryophyllales (Payer, 1857; Hofmann, 1994; Leins and Erbar, 1994; Ronse De Craene, 2010). It is at present unclear what regulates the centrifugal initiation of organs in flowers, as it may reflect a tendency for an increase in organ number (as in complex polyandry: Ronse De Craene and Smets, 1992) as frequently observed in several Caryophyllales (e.g., *Corbichonia*, Aizoaceae, Portulacaceae, Cactaceae), or a regression of organs and their loss (delayed organ initiation and growth: Ronse De Craene et al., 1993), also found in Caryophyllales (e.g., Caryophyllaceae, *Limeum*, *Glinus*: Ronse De Craene, 2010; Ronse De Craene et al., 1998; Wagner and Harris, 2000; Harris et al., 2012). The common underlying genetic factors for centrifugal stamen initiation are currently unknown, but they

Fig. 13. *Glinus lotoides*. SEM micrographs of late floral development. (A) Lateral view at closure of carpels and differentiation of anthers. Note fully developed alternisepalous stamens and small antesepalous appendages. (B) Detail of A showing bilobed staminodial petaloid and small underdeveloped structure. (C) Lateral view of nearly mature flower at differentiation of stigmatic lobes. Note bifid staminodial petaloid (right arrow) and abnormal antesepalous stamen, with one anther half sterile (left arrow). Bars: A = 100 μ m; B = 20 μ m; C = 200 μ m. Abbreviations: Aa, alternisepalous stamen; Ao, antesepalous stamen.

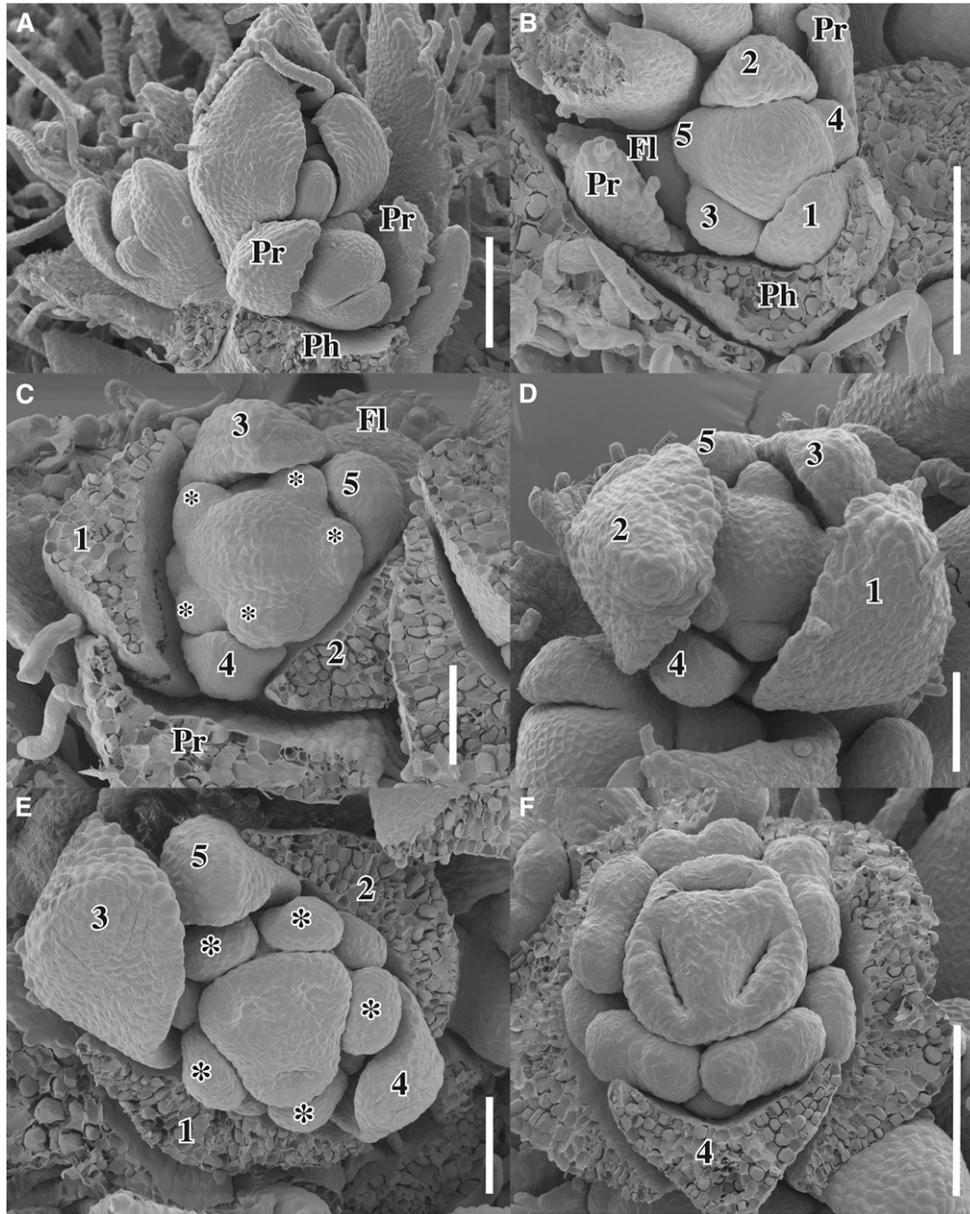


Fig. 14. *Glinus dahomensis*. SEM micrographs of early floral development. (A) Lateral view of young inflorescence. Note younger flowers developing around more advanced terminal flower. (B) Lateral flower showing the initiation of five sepals. Note smaller flower in the axil of the left prophyll. (C) Apical view at early initiation of alternisepalous stamens (asterisks). Note smaller antesepalous stamen opposite sepals one and three. (D) Older bud showing continuous growth of sepals and larger alternisepalous stamens. (E) Apical view at differentiation of three carpels. Note additional stamens opposite sepals one-four. (F) Apical view at differentiation of septa and anther formation. Note antesepalous stamens of different size. Bars: A, B, F = 100 μ m; C–E = 50 μ m. *Abbreviations*: Fl, lateral flower; Ph, pherophyll; Pr, prophyll.

are probably diverse. Centrifugal stamen initiation may represent a synapomorphy for the order, or substantial part thereof; however, early-diverging lineages have not yet been studied for their androecium development. In a centrifugally initiating androecium, it is the gynoecium that dictates the phyllotaxis of the first-formed stamen primordia, thus an understanding of gynoecium development in Caryophyllales is especially important for understanding patterns of centrifugal androecium development (see below). In Caryophyllales, carpels within the gynoecium always occupy an antesepalous position when isomerous, with very few exceptions (Ronse De Craene, 2013), and thus in an

isomerous flower, the first-formed stamens are expected to be alternisepalous, the second centrifugal tier is then antesepalous, where there is less pressure from the carpels, the third alternisepalous, and so on. However, as we discuss subsequently, the first-formed stamens are not always alternisepalous, and there are some important deviations to this pattern. Further complexity emerges in the patterns of initiation within each stamen tier. For example, although the first alternisepalous stamen primordia arise almost simultaneously, subsequent antesepalous stamens in these four genera arise sequentially. The sequential initiation of antesepalous stamens runs mostly in an inverted

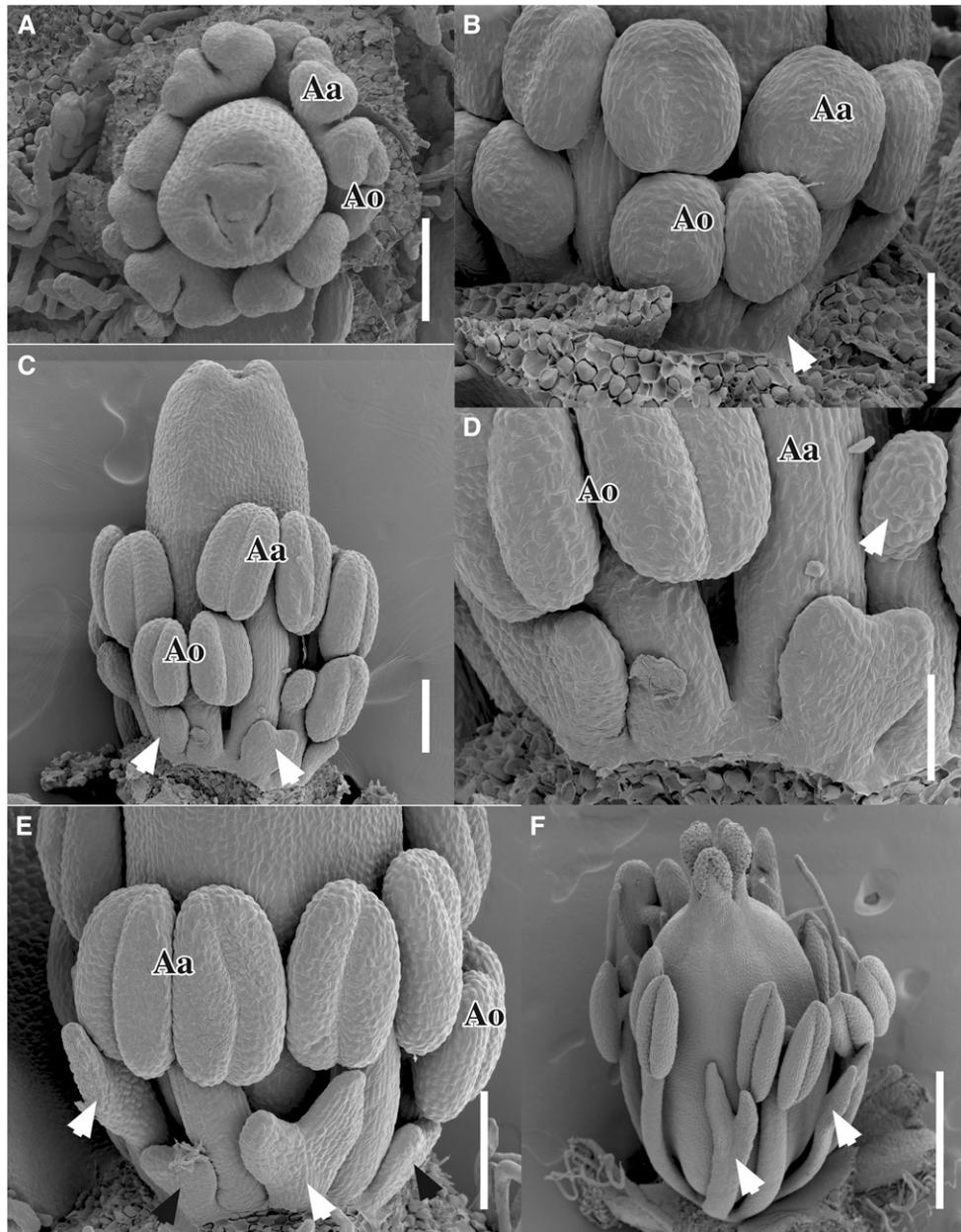


Fig. 15. *Glinus dahomensis*. SEM micrographs of late floral development. (A) Apical view of flower showing differentiation of upper extrorse anthers. (B) Detail of equivalent developmental stage. Note longer antesealous stamens and initiation of a petaloid (arrow). (C) Lateral view of slightly older stage at style formation. Note the initiation of petaloids (arrows). (D) Detail of C; note petaloid differentiation opposite alternisepalous stamen. Arrow points to undeveloped theca of antesealous stamen. (E) Flower with variable development of petaloids (black arrows) and antesealous staminodes (white arrows). (F) Nearly mature flower bud at stigma differentiation. Note longer alternisepalous stamens and variable development of antesealous staminodes (arrows). (D) Lateral view at initiation of alternisepalous petaloid (arrow). Bars: B, D = 50 μ m; A, C = 100 μ m; E = 200 μ m; F = 500 μ m. Abbreviations: Aa, alternisepalous stamen; Ao, antesealous stamen.

sequence relative to the sepals, and the first stamens initiate opposite sepals four and five. This pattern has been reported in a number of families, such as Caryophyllaceae, Phytolaccaceae, and Nyctaginaceae (Hofmann, 1994; Ronse De Craene et al., 1997, 1998; Ronse De Craene, 2010). Inversed sequence initiation is subsequently reflected in length differences within the antesealous stamens, with the first-formed stamens being longer. Furthermore, as discussed in the next section, this inversed sequence initiation is reflected in patterns of stamen loss, with

later initiating stamens opposite sepals one and two being the most likely to be lost.

Genus-specific variation in patterns of stamen initiation and loss—*Limeum*, *Hypertelis*, and *Glinus* exhibit patterns of stamen loss. Stamen number is unstable among different species of *Glinus*, ranging from five to several series of stamens including outer staminodes (Hofmann, 1973, 1994). Androecia with higher numbers look similar to *Corbichonia* (see Hofmann,

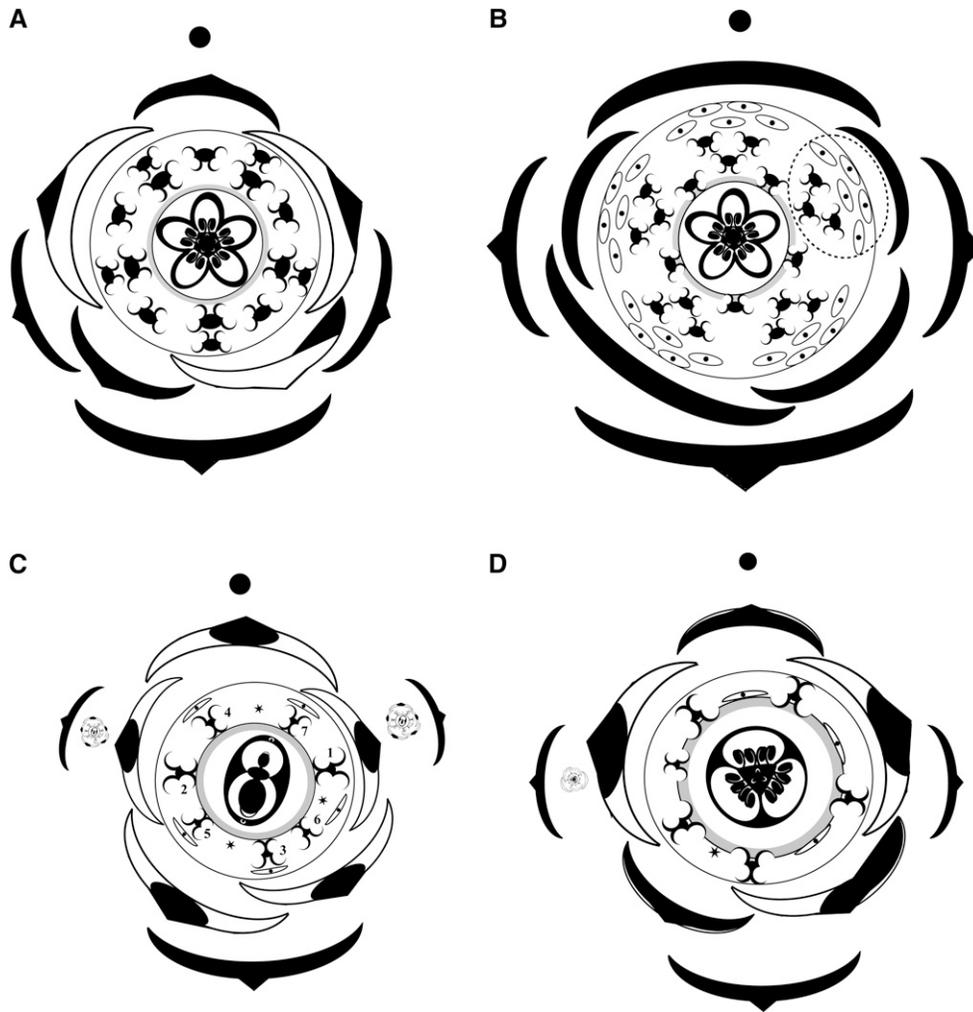


Fig. 16. Floral diagrams of investigated species. (A) *Hypertelis salsoloides*; (B) *Corbichonia decumbens*; (C) *Limeum africanum*; (D) *Glinus lotoides*. Petaloid calyx (parts) shown in white, sepaloid calyx (parts), pherophyll and prophylls shown in black; ovals and white arcs with central dots: staminodial petaloids; full circles: confines of androecial ring; gray ring: intrastaminal nectary. Position of styler tissue shown by small arcs on the carpels; asterisks, missing stamens; in B, dotted circle shows stamen group; in C, numbers show sequence of stamen initiation.

1994: fig. 17), suggesting an increase of stamens. Alternatively, Sharma (1963) described *Glinus oppositifolius* as having 10–13 stamens in three whorls, suggesting a tendency for reduction with loss of the outer stamen whorl. However, the species investigated here show mostly 10 stamens in *Glinus dahomensis*, and rarely more than five in *Glinus lotoides*. The fluctuation in stamen number in *Glinus* is not random. The five upper alternisepalous stamens are always present in all species, but the number of antesealous stamens fluctuates strongly, and this is generally linked with an inversed initiation sequence. However, in *Glinus dahomensis* antesealous stamens are exceptionally not inversed, with those opposite sepals one and two arising before those opposite sepals three, four, and five. This sequence may be linked with the rapid initiation of alternisepalous stamens seen in this taxon. However, in all cases, stamens opposite sepals one and two are the first to disappear. Other Molluginaceae also invariably have five alternisepalous stamens, probably reflecting the loss of all antesealous stamens.

Within families of the raphide clade and even among genera, stamen number can also fluctuate strongly, as exemplified by *Hypertelis*. The androecium of *Hypertelis* varies between three

and many stamens (Endress and Bittrich, 1993), encompassing a high variability. Hofmann (1973) studied mature herbarium material of *Hypertelis*, and her description of the androecium based on unpublished material of *Hypertelis salsoloides* (Hofmann, 1994) corroborates our data. The flower of *Hypertelis* is distinctive in the presence of 15 stamens arranged in three alternating whorls. Flowers with three alternating stamen whorls are rare, if not completely absent from other core eudicots. *Hypertelis bowkeriana* has only five alternisepalous stamens, probably corresponding to the first-formed stamens of *H. salsoloides*.

When comparing the floral development of *Corbichonia* with *Hypertelis*, there is a concordance in development up to the second whorl of stamens (Ronse De Craene, 2008, 2010). *Corbichonia* and *Hypertelis* share an inversed sequence of antesealous stamen initiation with the other genera, but the antesealous whorl is always fully developed. Friedrich (1956) interpreted the androecium of *Corbichonia rubriviolacea* (Friedr.) as consisting of five alternisepalous stamens and five antesealous groups of five stamens each plus outer petaloids. Hofmann (1973) rejected this interpretation as implausible, although our floral developmental data tend to corroborate the interpretation

of Friedrich. The sister genus of *Corbichonia*, *Lophiocarpus*, has only four stamens: three alternating with sepals and one opposite a sepal (Eckardt, 1974; Stannard, 1988). The unusual presence of one antesepalous stamen in *Lophiocarpus* could indicate that this stamen was derived from an original condition with five alternisepalous stamens by a fusion of two stamens under influence of a reduced tetramerous ovary, similar to a process occurring in *Mollugo* (Batenburg and Moeliono, 1982). Meanwhile, *Corbichonia* resembles Aizoaceae more closely in the development of several outer whorls of staminodes. However, contrary to Aizoaceae, initiation is less clearly confined to the alternisepalous sectors of the flower, and stamen increase often starts in the antesepalous sector of the flower.

The inversed sequence of stamen initiation is more complex in *Limeum* and includes alternisepalous stamens. Descriptions of stamen positions in *Limeum* are often contradictory. Friedrich (1956) and Hofmann (1973, 1994) concluded that flowers with seven stamens have five shorter alternisepalous stamens and two longer stamens opposite sepals four and five (three stamens are assumed to be lost). However, our investigation shows a progressive decrease in stamen sizes with three stamens in antesepalous positions and a pair of unequal size situated opposite sepals one and two. One alternisepalous stamen position is also empty. This pattern of initiation is comparable to *Bougainvillea* and some other Nyctaginaceae (see Vanvinckenroye et al., 1993; Ronse De Craene, 2013), where stamen position and initiation is apparently disconnected from the perianth. Several species, including *L. africanum* possess outer staminodial petaloids. *Limeum* corresponds to Caryophyllaceae and other members of the basal grade of Caryophyllales in its larger upper antesepalous stamens opposite sepals four and five. In several members of Caryophyllaceae, stamens are lost in an inversed sequence to the sepals (Thomson, 1942; Ronse De Craene et al., 1998).

Processes underlying stamen loss—In general, we can propose two possible causes for the stamen reduction and loss seen in these four genera and in the Caryophyllales as a whole. In essence, the gynoecium and centripetally advancing sepals act as two separate forces on the intervening androecium to cause stamen loss (Fig. 17). First, stamen loss is linked with a reduction of carpels from five to three or two (Fig. 17A). There is a strong correlation between numbers of stamens in the upper tier and numbers of carpels. Upper stamens always alternate with carpels, and if carpels are increased or decreased in number, this invariably affects the upper stamen whorl. In taxa with lower carpel numbers, such as *Limeum*, the longer antesepalous stamens alternate with the remaining carpels, while stamens tend to be reduced or lost in the radius of the carpels lobes, presumably because the stamens are then forced into a cramped position between carpel and sepal that then engenders stamen loss (Fig. 17A). Meanwhile, the best-developed antesepalous stamens are those that alternate with carpels. However, in *Glinus* with three carpels, the alternisepalous stamens tend to converge in pairs against the flanks of the carpels and are thus protected to an extent from pressure by the sepals, but they leave less space for the initiation of the antesepalous stamens.

Second, the stamens to be lost are those that undergo the highest pressure from sepals during development. Given the predictable pattern of stamen loss whereby the later initiating stamens are preferentially lost, a plausible explanation for the inversed initiation and loss of stamens is inhibitory pressure of the larger outer sepals against the developing flower buds. For example, sepal two, which is in a median adaxial position,

greatly limits space for the initiation of a stamen, as this is often the last position to be filled. Consequently, stamens opposite sepal two are frequently missing or at least delayed, as we observed in *Corbichonia* and *Hypertelis*. With five carpels, the antesepalous stamens are naturally those undergoing the strongest pressure from carpels and perianth, leading to their reduction or loss in Aizoaceae and Phytolaccaceae. The alternisepalous stamens have sufficient space to increase stamen numbers by division of initially formed primordia (Fig. 17B).

Disjunct patterns of androecium development across Caryophyllales—Our analysis of these four genera emphasizes an unusual disjunction in patterns of androecial development across the Caryophyllales that merits further investigation. The first-formed and upper stamens are invariably in alternisepalous position in *Glinus*, *Corbichonia*, and *Hypertelis*; however, they are in an antesepalous positions in *Limeum*. *Limeum* also differs from the other genera in the inversed spiral sequence of stamen initiation affecting both antesepalous and alternisepalous stamens. The globular inclusion clade containing Portulacineae, Molluginaceae, and the raphide clade appear to share a common pattern of androecial development, while the paraphyletic alliance of lineages, which diverge earlier than the globular inclusion clade (e.g., Caryophyllaceae, *Stegnosperma*, *Macarthuria*, and *Limeum*) share a different pattern of androecial development (Fig. 1).

This phylogenetic disjunction is revealed by the following two observations. The first observation is that the upper longer stamens are positioned in an *alternisepalous position* in the globular inclusion clade, and in an *antesepalous position* in the early-diverging lineages. The alternisepalous position links *Hypertelis* with both the Portulacineae and raphide clade, where stamens are often in alternisepalous position or have been

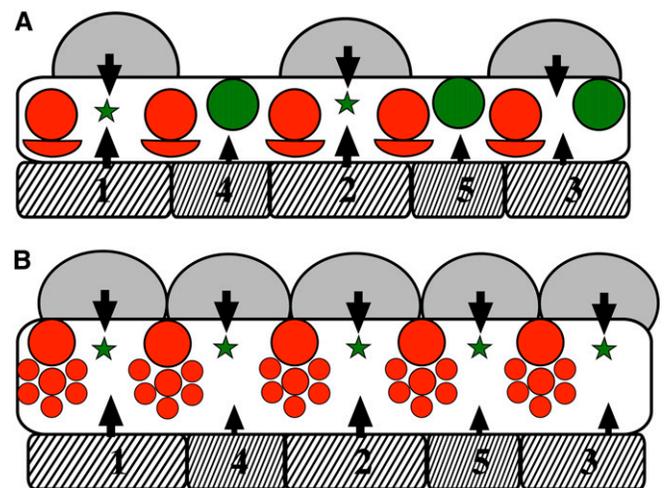


Fig. 17. Illustrations of spatial patterning in the development of the flower of Caryophyllales; longitudinal overviews. (A) Upper position of antesepalous stamens with trimerous ovary and loss of stamens opposite sepals one and two. Development of outer staminodial petaloids (e.g., Limeaceae, *Macarthuria*, Caryophyllaceae). (B) Upper position of alternisepalous stamens with loss of antesepalous stamens. Centrifugal multiplication of alternisepalous stamens (e.g., Aizoaceae, Phytolaccaceae). Arrows highlight the pressure from the gynoecium and perianth. Gray area: gynoecium; hatched area: calyx; white area: androecium; red dots, alternisepalous stamens; green dots, antesepalous stamens; stars, lost stamens; half moons, staminodial petaloids.

increased by secondary expansion of a ring primordium or by division of primary stamens. In some Portulacaceae, there is apparently a reversal to an upper antesealous position (some Didiereaceae, Montiniaceae; Ronse De Craene, 2013). Also in the variable Phytolaccaceae, upper or first-formed stamens tend to be in alternisealous position or are shifted in pairs opposite sepals (e.g., *Phytolacca dodecandra*: Ronse De Craene et al., 1997; *Ercilla volubilis*: Ronse De Craene, 2010). A second observation is the tendency for loss of some of the upper stamens in the earlier-diverging lineages; these are always those situated opposite sepals one and two (and sometimes three) (e.g., *Macarthuria* with eight stamens; *Limeum sulcatum*, *L. pterocarpum*, and *L. viscosum* with seven stamens; Friedrich, 1956; Sharma, 1963; Hofmann, 1973; several Caryophyllaceae with an incomplete antesealous whorl, e.g., *Stellaria*, *Scleranthus*, *Spergularia*: Ronse De Craene et al., 1998; Hofmann, 1994; Smissen and Garnock-Jones, 2002). The lost stamens are incidentally those that appear latest in the sequence of antesealous stamen initiation, similar to the sequence observed in *Hypertelis*. Alternisealous stamens on the contrary do not suffer similar pressures, and, especially for the raphide clade, these alternate with five carpels. Although it is not clear how these mechanical causes became fixed within families, they can be translated in a phylogenetic context. All taxa with upper antesealous stamens branch off before the globular inclusion clade, indicating a profound change in androecium morphology.

Perianth “differentiation” within the calyx—The perianth of the four studied genera adhere to the general concept of the Caryophyllid perianth in the following characteristics: (1) the perianth is pentamerous with a clear 2/5 arrangement and a strong differentiation between outer and inner sepals; (2) the sepal shape is rounded and often dorsally mucronate, and (3) the sepal margins are petaloid, while the area around the vascular bundle is green (Fig. 2). In *Corbichonia*, the calyx is greenish; however, in *Glinus*, *Limeum*, and *Hypertelis*, the calyx becomes partially petaloid. Several Caryophyllales develop a comparable calyx, and the sepals are differentiated along the proximal–distal axis, with a lower flattened petaloid zone and an upper subapical pointed mucro (e.g., *Sesuvium*, *Trianthema*, and *Galenia* in Aizoaceae, Paronychioideae of Caryophyllaceae: Hofmann, 1973; Brockington et al., 2012; Ronse De Craene, 2010). Sepals correspond to transformed leaves in the possession of these proximal and distal zones; the petaloid lateral zone represents the hyaline margin of a leaf sheath and the upper tip representing a reduced unifacial leaf blade (*Vorläufer-spitze*: Kaplan, 1975). The hyaline “leaf-sheath” margin can be co-opted in the development of petal-like tissue (Brockington et al., 2012), as is clear by the extensive petaloid tissue developing in the inner sepals of *Hypertelis*. Outer sepals have a small, lateral, colored rim, while sepal three is asymmetric with a one-sided extension of a colored flap (Fig. 2B). This observation likely indicates that petaloidy in *Hypertelis* is caused by extensive marginal growth of the narrow rim of tissue present in other Caryophyllales. The differentiation of petaloid tissue on the inner sepals is possibly related to exposure to light; the outer sepals and half of the third sepal are chlorophyllous, while the covered floral parts develop pink hues. This differentiation is similar to that of sepal tissues observed in several Polygonaceae (L. P. Ronse De Craene, unpublished data) and is reminiscent of experimental observations of the differentiation of perianth in Nymphaeaceae by Warner et al. (2009). While initially larger and enclosing the inner sepals in bud, the outer sepals are narrower

and smaller at maturity in *Glinus*, *Hypertelis*, and *Limeum*. The calyx of *Corbichonia* retains its larger outer sepals, and attraction is mainly through the whorls of staminodial petaloids. Adamson (1958b) mentions that the perianth of *Corbichonia decumbens* is green outside and often colored adaxially.

Perianth differentiation by stamen sterilization—Staminodial petaloids are variously present in the investigated species, reflecting the condition found in other Caryophyllales, where the second perianth whorl is invariably of staminodial origin (Ronse De Craene et al., 1998; Brockington et al., 2009; Ronse De Craene, 2010, 2013). The petaloid organs of staminodial origin are the latest to be initiated and lag behind in their development, only exceeding stamen size in a few instances at later stages of development (e.g., *Corbichonia*, *Limeum*). With the exception of *Corbichonia*, where numerous outer staminodes are regularly arranged as petaloid appendages, a single-whorled corolla tends to be infrequent and variable. In *Glinus*, outer petaloids are present depending on the vigor of the flower and space for their initiation. Petaloid number in *Glinus* can fluctuate enormously and can either be in antesealous position (replacing a fertile stamen) or in alternipetalous position as an appendage of the upper stamen. In *Limeum*, spatulate petaloids are generally present in the flower. *Hypertelis* is unusual as it lacks petaloids altogether, but stamens occupy the position of petaloids in other Caryophyllales. A similar arrangement was observed in *Trichostigma* and *Ledenbergia* of Phytolaccaceae-Rivinoideae, but with a tetramerous merism (Ronse De Craene and Smets, 1991). The close connection of the petaloids with the androecium, their shape and development, and occasional replacement by stamens points to a clear staminodial origin.

A number of Caryophyllales with comparable androecium morphologies to *Hypertelis* have the outer stamens replaced by petaloid structures (e.g., *Macarthuria*, *Limeum*, *Glinus*, *Stegnosperma*, possibly *Asteropeia*, and most Caryophyllaceae). In all observed cases, a narrow petaloid appendage develops on the outside of the staminal rim, or below it where the staminal rim is protruding. The petaloid structure is often indistinguishable from a staminode or is occasionally replaced by a stamen (e.g., *Stellaria*, *Sagina*, and *Scleranthus* in Caryophyllaceae: Ronse De Craene et al., 1998). Thus, there seems little difficulty in recognizing the outermost stamen whorl of *Hypertelis* as homologous with the staminodial petaloids of other Caryophyllales on the basis of position. Other Caryophyllales with staminodial petaloids have more stamens arising centrifugally in girdles as part of a complex multistaminate androecium. (e.g., *Corbichonia*, *Mesembryanthemoideae*, and *Ruschoideae* in the Aizoaceae).

Development of the gynoecium—Gynoecial development is strikingly similar among a range of Caryophyllales, which in the data presented here is best exemplified by *Hypertelis*. In *Hypertelis*, the gynoecium develops as a central protuberance surrounded by the androecial platform. Stamens and carpels thus form a distinct association (Fig. 17). Development starts with a hemispherical dome, and locules arise as half-moon-shaped slits separated by prominent septa. In genera of the raphide clade, more active growth of the dorsal carpel parts leads to overtopping of the locules, and terminal stigmatic lobes are formed while the ovary develops as a globular structure with axile placentation. *Corbichonia* closely resembles *Hypertelis* in carpel development. In *Glinus*, growth of dorsal appendages is less prominent. The development of the ovary is generally

more pronounced than the stigmatic lobes, leading to a cylindrical structure with apical slits resembling a salt-shaker. A comparable pattern of development is visible in members of the Portulacaceae (L. P. Ronse De Craene, personal observations). *Limeum* is highly unusual for Caryophyllales in becoming pseudomonomerous, developing from two carpels. However, reductions to three, two, or a single carpel are not uncommon within the different clades of Caryophyllales, while secondary increases of carpels are more limited (e.g., in Cactaceae, Phytolaccaceae).

Conclusions—The data generated here fill some significant phylogenetic gaps in our understanding of androecial development across Caryophyllales, most notably with the inclusion of *Limeum*. We reveal two important patterns of stamen development including (1) the preferential loss of antesealous stamens starting opposite sepals one and two and (2) a switch in the position of the first formed stamens from antesealous to alternisealous following the divergence of *Limeum* (Fig. 1). This switch provides important morphological data in further support of the globular inclusion clade. The data also provide insight into the link between centrifugal development and meristic variation in the androecium. The study emphasizes the importance of developmental morphology in providing cryptic morphological synapomorphies and in providing insight into the developmental basis of morphological variation. Future studies should explore the evolution of Caryophyllid floral development in early-diverging lineages and the impact of centrifugal development on the developmental genetics of organ identity.

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