# 3D-Construction of Vasculature of *Anaxagorea* and its Implications in the Integrated Axial-Foliar Origin of the Angiosperm Carpel

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Running Title: The Axial-Foliar Origin of Carpel

**Abstract**

How flowers have evolved is among the foremost topics in evolutionary science. However, previous studies can only explain in general how angiosperm flowers originated as a whole. The origin of flowers involves the evolution of key characters of floral organs. Therefore, investigating the origin of the particular component parts of the flower can bring us more clues to the origin of angiosperms. Carpel is the basic unit of the gynoecium in angiosperms, and it is also one of the most important morphological features of angiosperms different from gymnosperms. Therefore, the origin of carpels is of great significance to the phylogenetic origin of angiosperms. Conflicting theories with regard to flower origins have provided varied explanations. According to the traditional explanations, angiosperm carpels emerged from structures similar to macrosporophylls of pteridosperms or Bennettitales, which bear ovules on the surface of foliar organs. Conversely, other views based on the stachyosporous origin theory suggest that the carpel originates from foliar appendages enclosing the ovule-bearing axis in gymnosperms. Since it has been confirmed by morphological and molecular evidences that the carpel wall is certainly derived from foliar homologs, if the axial homologs in the carpel are determined, the carpel would more likely be derived from an ovule-bearing axis fused with the leaf-like structure than from the megasporophyll. The aim of this study is to trace the axial homologous in the carpel by analyzing the continuous changes of vascular diagrams in the receptacle, carpel and ovule in *Anaxagorea*. *Anaxagorea* is the most basal genus of primitive angiosperms Annonaceae. Each carpel in *Anaxagorea* has a notable long carpel stipe, which is one of the key characteristics of the genus. In this paper, floral organogenesis of *A. luzonensis* and *A. javanica* were described. The topological structure of vasculature in the receptacle and the carpel in different development stages were studied. And the 3D model of vascular diagrams of mature carpel of *A. javanica* was established. The results show that: (1) at flower stage, discrete vascular bundles (far more than three) entering carpel stipe of *Anaxagorea*, which were arranged as a ring, similar to the arrangement of vascular bundles in stems. All of these carpel bundles are originated from the central stele in the receptacle. (2) Below each of the placenta, the ring-arranged bundles at the base of carpel were reorganized into two groups of lateral bundle complexes, and in each group of lateral bundle complex, bundles are also arranged as a ring. (3) In each group of the lateral bundle complex, some non-adjacent bundles get close to each other through the topological secondary ring, and finally merged with each other into the ovule bundle. (4) Neither the dorsal bundle nor the ventral bundle is involved in formatting of the ovule bundles, and there is no connection between the ovule bundles and bundles in the ovary wall. These results support the stachyosporous origin theory that the carpel originates from the integration of the ovular axis and the foliar appendage, and provide a valuable trait for the molecular mechanism of carpel origin.

**Key words:** *Anaxagorea*; angiosperms; organogenesis; origin of the carpel; vascular anatomy; 3D reconstruction

## INTRODUCTION

Angiosperms, the flowering plants, account for a considerable proportion of the plants visible to modern human society. Both the beautiful flowers and the delicious fruits are intertwined with human life. The term "angiosperm" is derived from the Greek words angeion, meaning "container," and sperma, meaning "seed." Therefore, the carpel, a "seeds container," is the definitive characteristic in angiosperms. The carpel, which protectively surrounds the ovules by closure and sealing along their rims or flanks, is an angiosperm-specific female reproductive feature in flowers and is the basic unit of the gynoecium (Dunal, 1817; Robinson-Beers, 1992; Endress, 2015). The evolution of the carpel set angiosperms apart from other seed plants, which developed ovules that were exposed to the air. Since the time of Darwin, elucidating the origin of angiosperms and their evolutionary success has been a primary goal in plant science. Scientists have attempted to identify the potential ancestors of angiosperms through phylogenetic analyses based on fossil, morphological, and molecular data. In such efforts, particular emphasis has been placed on assessing which ovule-bearing structures of various seed plants could be transformed into carpels.

The history of exploring the origin of carpel has been tortuous. Conflicting theories with regard to flower origins have provided varied explanations and influenced hypotheses on the phylogenetic relationships among angiosperms and other seed plant lineages. The traditional phyllosporous origin hypothesis suggests that angiosperm carpels were derived from structures similar to macrosporophylls of Bennettitales, which bear ovules on the margins of foliar organs (Arber and Parkin, 1907; Eyde, 1975; Cronquist, 1988; Takhtajan, 1991). Conversely, according to the stachyosporous origin theory, the carpel originated from a compound shoot with the integration of the ovular axis and the foliar appendage. The placenta with ovules is homologous with a female short-shoot in gymnosperms with distally placed ovules, each with scales forming the outer integument (Stebbins, 1974; Retallack and Dilcher, 1981a; Crane, 1985; Doyle and Donoghue, 1986; Nixon *et al.*, 1994; Hickey and Taylor, 1996; Wang, 2010, 2018). In addition, according to the Caytonialean hypothesis, the cupule wall of seed ferns is homologous to the outer integument of angiosperm ovules and that expansion and folding of the cupule-bearing axis are precursors to the carpel (Doyle, 1978, 2006, 2008). Another theory posits that the carpel evolved via the ectopic expression of ovules on a phyllome (i.e., the Mostly Male theory [Frohlich and Parker, 2000]).

However, since no sister group of the angiosperms has been identified, the origin of the carpel remains a mystery. Progress has been made in morphological and functional genetics studies among angiosperms. In angiosperms, the placenta and ovary wall are served by distinct vascular bundles (Guo et al., 2013; Liu et al., 2014; Guo et al., 2017; Zhang et al., 2017) and are regulated by different genes associated with branch and leaf organs, respectively (Roe et al., 1997; Skinner et al., 2004; Mathews and Kramer, 2012). In distributed lines of angiosperms, the ovule primordium originates in the axillary position between the flower apex and carpel

wall (e.g., *Gyrostemon* [Hufford, 1996]; *Illicium* [Zhang et al., 2019]; *Phytolacca* [Zhang et al., 2018]). These are key clues with regard to whether the placenta and the carpel wall have different evolutionary origins; however, more solid evidence is required. According to the above-mentioned hypotheses, it is critical to find clear evidence for the existence of axial homologs in the carpel. Based on the premise that the carpel wall is certainly derived from foliar homologs, on condition that the axial homologs in the carpel are determined, the carpel would more likely be derived from an ovule-bearing axis fused with the leaf-like structure than from the megasporophyll.

To present more reliable evidence for the existence of axial homologs, the carpel of *Anaxagorea* (Annonaceae) was selected for organogenesis and vascular anatomic examination. Annonaceae represents one of the largest families in the Magnoliales, which is one of the most important lineages in the early radiation of angiosperms (Sauquet et al., 2003), while *Anaxagorea* is the most basal genus in Annonaceae (Doyle and le Thomas, 1996; Doyle et al., 2004; Chatrou et al., 2012; Chatrou et al., 2018), which are found in dimly-lit riparian zones in forest slope and understory habitats. *Anaxagorea* have simple leaves that are arranged alternately in two rows along the stems and flowers often have whorled phyllotaxis. Gynoecia are apocarpous (free carpels) throughout a life history (Deroin, 1988). Each carpel in *Anaxagorea* has a notable long stipe (Endress and Armstrong, 2011), which is one of the key characteristics of the genus. Morphological features in *Anaxagorea* are relatively primitive in the angiosperms and the presence of the notable carpel stipe makes it possible to determine whether there are “axial homologs” in the carpel through tissue sectioning.

## MATERIALS AND METHODS

### Scanning Electron Microscopy and Paraffin Sectioning

Flower samples of *Anaxagorea luzonensis* (July 2017 from Diaoluo Mountain, Hainan, China) and *Anaxagorea javanica* (May 2017 from Xishuangbanna Tropical Botanical Garden, Yunnan, China) were collected at different floral stages (from early bud to young fruit). Gynoecia were isolated from the other parts of the flower and preserved in formalin–acetic acid–70% alcohol (5:5:90, v/v). The fixed materials were dehydrated through an alcohol series (50% to 100%). To delineate the structure and development of the carpel, some carpels were removed from gynoecia and passed through an iso-pentanol acetate series (SCR, Shanghai, China), critically point-dried, sputter-coated with gold, and observed and photographed under a scanning electron microscope (Tescan VEGA-3-LMU, Brno, Czech Republic). To illustrate the vasculature, flower and carpels were embedded in paraffin, sectioned serially into 10–12-μm thick sections, and then stained with Safranin O and Fast Green. Complete transverse and longitudinal sections were examined and photographed under bright-field microscopy (Olympus BX-43-U, Tokyo, Japan). In addition, longitudinal hand sections were made as a rough check for a better understanding of vascular diagram.

### Topological Analysis of Carpel Vasculature

12-μm consecutive paraffin slices of *A. javanica* were stained with Aniline Blue,

examined and photographed after excitation at 365 nm using the epifluorescence microscope (Olympus BX-43-U, Tokyo, Japan) and Semiconductor refrigeration CCD (RisingCam MTR3CMOS). 45 images were selected equidistant from 423 slices for 3D reconstruction. The figures are organized using Adobe Photoshop CC 2017 and Illustrator CC 2017 according to the outline of vascular bundles of the sections. The contour of xylem and phloem was drawn manually, extracted as path by pen tool, and exported as DWG format. the DWG files were imported into 3Ds max 2016 and sorted according to distance and order of the slices. The path was Convert to Editable Spline into a curve to generate the basic contour of modeling. Loft command of Compound Objects was used to Get Shape of Editable Spline, and a complete 3D model of carpel vasculature generated.

## RESULTS

### Gynoecium Structure and Carpel Organogenesis

In the two study species, the flowers are trimerous with a whorl of sepals and two morphologically distinct whorls of petals. The number of stamens (and inner staminodes) is large (**Figures 1A–D**).

*A. luzonensis* usually has two to four carpels, which are completely free from each other (**Figures 1A, G**). Carpel primordia are almost initiated hemispherically and larger than stamen primordium (**Figure 1F**). Each carpel consists of a plicate zone, a very short ascidiate zone (**Figures 3H, 4I, J**), and a conspicuous long stipe (**Figure 2F**). Carpel stipe originates at the basal part in the early stages of carpel development (**Figure 2B**), remains elongate, accounts for roughly a quarter of the length of the carpel at anthesis, and continues to elongate during the fruit-stage. Continuous growth of the flanks on the ventral side of the young carpel triggers the early closure. The closure does not extend to the bottom of the carpel (**Figure 2C**). Subsequently, the dorsal part of each carpel thickens markedly and the stigma forms (**Figures 2D, E**). At anthesis, carpels are widest at the basal part with an arch on the abaxial side (**Figure 1F**). Each carpel has two lateral ovules with the placentae at the base of the ovary (**Figures 3I, J**).

*A. Javanica* has a multicarpellate gynoecium (**Figures 1B, J**). Carpels are completely free from each other, and appearing whorled at initiation (**Figure 1I**), while as the carpel volume increases, the whorled structure becomes less obvious as space at floral apex becomes limited (**Figure 1K**). Each carpel consists of a plicate zone and a conspicuous long stipe, whereas lacking the short ascidiate zone. Carpel stipe originates at the basal part in the early stages of carpel development (**Figure 2H**), remains elongate during flower stage and fruit stage (**Figures 2I–J, 6E**). Each carpel has two lateral ovules.

### Vasculature from Receptacle to the Carpel

In cross-sections of *A. luzonensis*, the base of receptacle presents a hexagon of 18 bundles from the stele of the pedicel, build up the pseudosteles (**Figure 3A**). Among which 6 breaks of the central stele bundles built up a crown of the cortical vascular

system (CVS), 3 of them serve the sepals. At a slightly upper level, 6 bundles emerge and separated from the central stele, joint together with the CVS to supply the two whorls of petals and the stamens (**Figures 3B, C**). The central stelar system is always composed of 18 bundles, finally break into 2 groups, each made of 9 bundles, all run into the 2 carpels gynoecium. At the base of each carpel, those 9 bundles assembled as a ring around the parenchyma. At anthesis, there are some sclereids scattered throughout the parenchyma cells (**Figures 3D–F**). Slightly on the upper part of each carpel, several bundles emerge at lateral side and the ring-formed discrete bundles break into 3 groups, which are a dorsal bundle and 2 groups of lateral bundle complexes. In each group, bundles tend to joint adjacent ones and finally assembled into an amphicribral pattern (xylem surrounded by the phloem, **Figure 3G**). Below each placenta, each of the amphicribral bundle complex transformed into a set of "C"-shaped lateral bundle complex, from which the ovule bundles separated, while other bundles into the carpel wall (**Figures 3H–I**). In the cross-section of the ovary locule, lateral bundles in the carpel wall seems poorly differentiated strands. There are no horizontal connections between the dorsal bundle and the ovule bundles, nor the lateral ones (**Figure 3J**).

Pseudosteles at the base of receptacle of *A. Javanica* is triangular in shape, with *ca.* 45 bundles all together. Among which the outer 6 cortical traces were cylindric, and used up to serve the sepals and petals (**Figures 4A–C**). At a slightly upper level, androecial bundles emerge and serve stamens by the manner of repeated branching (**Figure 4D)**. Staminodes and carpels are fed from the enlarged central stele at same level (**Figures 4E, F**). Before entering the gynoecium, the central stele breaks up into *ca.* 70 bundles to feed 9 carpels. Each carpel is served by 7-10 bundles (**Figures 4G, H**). The arrangement of vascular bundles is similar to *A. luzonensis* in the ascending sections of each carpel (**Figures 4I, J**).

**Vasculature Topological of Mature Carpel**

At the base of a mature carpel of *A. luzonensis*, 15 discrete bundles formed a ring around the central parenchyma, though 4 of them has not distinct xylem (**Figures 5A**). Slightly on the upper part, the ring-arranged bundles curving inward at the ventral side, and breaks from the invagination (**Figures 5B, C**). Bundles other than the dorsal one divided into two groups, each has bundles arranged as a secondary ring. These cross-sections correspond to the above-mentioned sections of amphicribral bundle complex at flower stage (**Figures 5D–F**). Below each placenta, bundles of each secondary ring breaks at dorsal side and transformed into a "C"-shaped lateral bundle complex (**Figures 5G, H**). From which bundles at the inner side of each bundle complex gather together and gets into each ovule, while other bundles into the carpel wall (**Figures 5I, J**). Ventral bundles separated from the ventral concave of each "C"-shaped lateral bundle complex at the level of the base of ovule locule and connected with lateral bundles by phloem in the carpel wall (**Figures 5K, L**).

Consecutive cross-sections of *A. Javanica* are similar with *A. luzonensis* in vascular

diagram (**Figures 6A–D**). At the base of the carpel, *A. Javanica* shows 16 distinct bundles formed as a ring, which are lacking of obvious dorsal ventral in arrangement (**Figure 6A**). The 3D model show that the ovule bundles are fed directly by bundles from the base of carpel (**Figures 6E, F)**. Each ovule bundle is formed of several non-adjacent lateral bundles, which distributes both relatively dorsally and laterally positioned. Two of these bundles supplied to each ovule are connected with each other (**Figure 6G**). The dorsal bundle and the ventral bundles remained independent all along, they did not participate in the lateral bundle network in formation of the ovule bundles (**Figure 6G–I,** for details, refer to the 3ds file in the supplemental data).

## DISCUSSION

The present study investigated the organogenesis and the changes in the vascular bundle at different development stages of the carpel in *Anaxagorea*. The unique arrangement of vascular bundles in the carpel suggests that in *Anaxagorea*, (1) Carpel bundles are only originated from the central stele, or axial system, which were larger than three in each carpel. (2) The arrangement of vascular bundles at the base of carpel is discrete and ring-formed. (3) The ovule bundles are fed directly by bundles from a ring-formed lateral bundle complex in the carpel stipe, rather than from the dorsal bundle or through a vascular network in the ovary wall.

Peltate carpels have been suggested to be plesiomorphic in Annonaceae (Deroin, 1988; Igersheim and Endress, 1997; Surveswaran et al., 2010; Couvreur et al., 2011). *Anaxagorea* carpels have been reported to have ascidiate base in some studies (Deroin, 1988), while they have been described as completely plicate in others (Endress and Armstrong, 2011). In the present study, organogenesis reveals that the carpel stipe of *A. luzonensis* and *A. javanica* carpel emerges at the early stages of carpel development and continues to elongate with the development of the carpel, In the flower stage, the ventral slit of *A. luzonensis* terminate closely upon the base of the ovule locule, so there is a very short ascidiate zone. While in *A. javanica*, the ventral slit of *A. luzonensis* may continue for a distance below the ovule locule. Such variants may suggest transformation from peltate carpels to plicate carpels in this genus.–The developed base in *Anaxagorea* carpel provides a buffer space for the drastic changes of vascular diagrams at the base of the carpel, whereas in most angiosperms with apocarpy, the base of the carpel is very close to the placenta and the vascular bundle in this part tends to be simplified (e.g., *Michelia* [Tucker, 1961]; *Sagittaria* [Kaul, 1967]; *Drimys* [Tucker, 1975]; *Illicium* [Robertson and Tucker, 1979]; *Brasenia* [Endress, 2005]).

In previous studies, gynoecium of Annonaceae is feed by the enlarged central stele, and each carpel is usually feed by three bundles, a median bundle, and two lateral bundles (Decraene, 1993; Deroin, 1989; Deroin and Norman, 2016; Deroin and Bidault, 2017). However, in *A. luzonensis* and *A. javanica*, the number of vascular bundles fed the carpel during anthesis is far more than three no matter how many carpels there are. Bundles entering the carpel are arranged in a radiosymmetric pattern, with no obvious dorsal ventral in arrangement, and would reorganize as two sets of secondary ring-arranged bundle complex in the carpel. Radiosymmetric vasculature is

a universal feature in the stems of vascular plants (Metcalfe and Chalk, 1979; Evert, 2006; Beck, 2010; McKown and Dengler, 2010; Evert and Eichhorn, 2011). In stem cross-sections in most angiosperms other than monocots, discrete collateral bundles form single rings or continuous hollow cylinders around the pith (e.g., Caprifoliaceae; Leguminosae; Tiliaceae; Ulmaceae). Conversely, in leaves, vascular bundles are laterally organized in most seed plants, arranged in an arc in the petiole or base, and the largest collateral bundles often extend along the long axis of the leaf as a midvein from which smaller veins diverge laterally (e.g., Pinaceae; Gnetaceae; Magnoliaceae; Gramineae; Brassicaceae). In addition, despite it has been reported that in some *Anaxagorea* species, the petiole possessing an adaxial plate of xylem in the midrib, however, the adaxial plate is absent in Asian clade (*A. borneensis*, *A. javanica*, and *A. luzonensis*). They possess the simple vascular arc only (Scharaschkin and Doyle, 2006).

In *Anaxagorea*, ovules have been described to be served by the lateral bundle complex from the base of the carpel and there are horizontal connections between the dorsal and lateral ones (e.g. *A. luzonensis* [Deroin, 1997]; *A. crassipetala* [Endress, 2011]). Our study shows the details of vascular diagram of the lateral bundle complex and how the ovules served by them. The topological structure of the secondary ring-formed discrete bundles plays a key role in the formation of ovule bundles that it makes the non-adjacent bundles from the relatively dorsally and ventrally sides get close to each other. The dorsal bundle remained independent all along, and there are no horizontal connections between it and the ring-formed lateral bundle complexes. The ventral bundles take part in forming the ring-formed lateral bundle complexes. however, it did not connect with other bundles. This differs from other genera, in which the ovules are served from separate vascular bundles departing directly from the dorsal bundles (e.g. *Cananga* [Deroin and Le Thomas, 1989]; *Deeringothamnus* [Deroin and Norman, 2016]; and *Pseudartabotrys* [Deroin and Bidault, 2017]), or from relatively dorsally positioned bundles of the lateral network of bundles (e.g. *Meiocarpidium* [Deroin, 1987]; and *Ambavia* [Deroin and Le Thomas, 1989]). In these genera, the decrease of carpel bundles to 3 may be the result of increasing the number of organs in the evolutionary process (de Craene, 1993).

Development of amphicribral bundle complexes into ring-formed lateral bundle complexes was observed in *A. luzonensis* carpels at different stages. Amphicribral bundles are frequently observed in small branches of early land plants, in monocots, or in young branches of dicots as simplification and combination of stem bundles (Fahn, 1990). In *Magnolia* (Liu et al., 2014) and *Michelia* carpels (Zhang et al., 2017), amphicribral bundles were observed supplying the ovules and emerged from cortical amphicribral bundles. In *Actinidia* (Guo et al., 2013) and *Dianthus* pistils (Guo et al., 2017), amphicribral bundles are reported in the placenta and are distinct from the collateral bundles in the carpel wall. Nevertheless, amphicribral bundles seem widespread in placenta and funiculus of angiosperms (e.g., *Papaver* [Kapoor, 1973], *Psoraleae* [Lersten and Don, 1966], *Drimys* [Tucker, 1975], *Nicotiana* [Dave et al., 1981], *Whytockia* [Wang and Pan, 1998], *Pachysandra* [Von Balthazar and Endress, 2002]). However, in our study, each ovule bundle is composed of several adjacent vascular bundles, does not necessarily organized as an amphicribral bundle. Amphicribral bundles also appears in the receptacle, it is formed of several bundles

which were separated from the central stele. Each of these amphicribral bundle supplies multiple perianths and stamens by further branching especially in the case of large number of organs. The amphicribral bundles could be discrete inversely collateral bundles near the place of fusion because their xylem portions need to approach each other before they become concentric (Endress, 2019).

Interestingly, these results support the Unifying Theory (Wang, 2010, 2018) that angiosperm carpels are derived from the sterile bracts (formed carpel wall) attached to the ovule-bearing secondary shoot (formed ovule/placenta). Firstly, the distribution of vascular bundles at the base of carpel of *Anaxagorea* is not obvious dorsal ventral, the ring-formed discrete bundles at the basal part act in a manner consistent with the typical form of the stem vascular anatomy of most angiosperms. The secondary ring-formed discrete bundles in the carpel seems difficult to be explained by the leaf origin theory because the foliar homologs cannot form such a topology by coiling transformation since the topological structure of the lateral organized bundles is equivalent to a curve, and the curved surface can only form a one-way tunnel (like macaroni pasta) by coiling. However, the secondary ring-formed discrete bundles in the carpel of *Anaxagorea* are analogous to three-way tunnels. Secondly, the pattern that bundles serve the placenta in *Anaxagorea* is different from other genera of Annonaceae, even the Magnoliales. The secondary ring-formed bundle complex below each placenta plays an important role to forming each ovule bundle. These bundles are directly connected with the central stele, instead of the vascular network in the ovary wall. This result may indicate that ovule and ovary wall are relatively independent in the organization of vascular bundles. The study of floral meristem and cells of chromosomal chimeras indicate that the placental tissue is developing independently of the septum and of the carpel wall (Satina and Blakeslee 1941, 1943; Satina 1944, 1945). And the ovule/placenta and ovary wall are proved to control by two distinct, exclusive sets of genes (Roe et al. 1997; Wynn et al. 2014; Angenent et al. 1995).

Our results provide a new evidence that although carpel and ovule became tightly synorganized so that they appear as a unified organ in angiosperms, they were not a morphological unit from their evolutionary beginnings. However, the investigations in the present study were limited to a single genus. We hypothesize that the axial homologs are a regular feature in the angiosperm carpel. However, such homologs may have diverse characteristics such as the ring-formed lateral bundle complexes and the amphicribral bundles because the vascular bundle in the carpel is often simplified. Future studies should conduct more extensive studies, with a focus on early-diverging angiosperm groups with carpel stipe, such as Cabomboideae, Illicieae, and Schisandreae, to test the validity of the hypothesis. More robust insights would be obtained from more integrative models that consider multiple traits in both extant plants and fossil records, high-quality taxonomic data, molecular mechanisms of character evolution, and information in databases cataloguing georeferenced occurrences.

## AUTHOR CONTRIBUTIONS

YL planned and designed the research, performed experiments, collected images and drawn the illustration, and wrote the article; YC made the 3D model; WD performed

experiments and complemented the writing; SW complemented the writing; X-FW supervised the experiments and complemented the writing.

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## REFERENCES


Arber, E. A. N., and Parkin, J. (1907). On the origin of angiosperms. *Journal of the Linnean Society of London* 38, 29–80. doi: 10.1111/j.1095-8339.1907.tb01074.x

Beck, C. B. (2010). *An Introduction to Plant Structure and Development: Plant Anatomy for the Twenty-first Century*, 2nd edn. Cambridge, New York: Cambridge University Press.

Chatrou, L. W., Pirie, M. D., Erkens, R. H. J. , Couvreur, T. L. P., Neubig, K.M., Abbott, J. R., et al. (2012). A new subfamilial and tribal classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society* 169, 5–40. doi:10.1111/j.1095-8339.2012.01235.x

Chatrou, L. W., Turner, I. M., Klitgaard, B. B, Maas, P. J. M., and Utteridge, T. M. A. (2018). A linear sequence to facilitate curation of herbarium specimens of Annonaceae. *Kew Bulletin* 73:39. doi: 10.1007/S12225-018-9764-3

Couvreur, T. L. P., Pirie, M. D., Chatrou, L. W., Saunders, R. M. K., Su, Y. C. F., Richardson, J. E., et al. (2011). Early evolutionary history of the flowering plant family Annonaceae: steady diversification and boreotropical geodispersal. *Journal of Biogeography* 38, 664–680. doi: 10.1111/j.1365-2699.2010.02434.x

Crane, P. R. (1985). Phylogenetic analysis of seed plants and the origin of angiosperms. *Annals of the Missouri Botanical Garden* 72, 716–793. doi: 10.2307/2399221

Cronquist, A. (1988). *The evolution and classification of flowering plants*, 2nd edn. New York Botanical Garden: Bronx.

Dave, Y. S., Patel, N. D., and Rao, K. S. (1981). Structural design of the developing fruit of *Nicotiana tabacum*. *Phyton* 21, 63–71.

Decraene, L. P. R., and Smets, E. F. (1993). The distribution and systematic relevance of the androecial character polymery. *Botanical journal of the Linnean Society* 113, 285–350. doi: 10.1111/j.1095-8339.1993.tb00341.x

Deroin, T. (1988). *Aspects anatomiques et biologiques de la fleur des Annonacées*. PhD Thesis. Centre d'Orsay: Université de Paris-Sud, France.

Deroin, T. (1989). Définition et signification phylogénique des systèmes corticaux floraux: l'exemple des Annonacées. *Comptes Rendus de l'Académie des Sciences, Paris* 308, Série III, 71–75.

Deroin, T, and Norman, É. M. (2016). Notes on the floral anatomy of *Deeringothamnus* Small (Annonaceae): cortical vascular systems in a chaotic pattern. *Modern Phytomorphology* 9, 3–12.

Deroin, T., and Bidault, E. (2017). Floral anatomy of *Pseudartabotrys* Pellegrin (Annonaceae), a monospecific genus endemic to Gabon. *Adansonia* 39, 111–123. doi: 10.5252/a2017n2a2

Doyle, J. A., and Donoghue, M.J. (1986). Seed plant phylogeny and the origin of angiosperms: an experimental cladistic approach. *The Botanical Review* 52, 321–431. doi: 10.1007/BF02861082

Doyle, J. A., and Le Thomas, A. (1996). Phylogenetic analysis and character evolution in Annonaceae. *Adansonia* 18, 279–334.

Doyle, J. A., Sauquet, H., Scharaschkin, T., and Le Thomas, A. (2004). Phylogeny, molecular and fossil dating, and biogeographic history of Annonaceae and Myristicaceae (Magnoliales). *International Journal of Plant Sciences* 165, S55–S67. doi: 10.1086/421068

Doyle, J. A. (1978). Origin of angiosperms. *Annual Review of Ecology and Systematics* 9, 365–392. doi: 10.1146/annurev.es.09.110178.002053

Doyle, J.A. (2006). Seed ferns and the origin of angiosperms. *Journal of the Torrey Botanical Society* 133, 169–209. doi: 10.3159/1095-5674(2006)133%5B169:SFATOO%5D2.0.CO;2

Doyle, J. A. (2008). Integrating molecular phylogenetic and paleobotanical evidence on origin of the flower. *International Journal of Plant Sciences* 169, 816–843. doi: 10.1086/589887

Dunal, M. F. (1817). Monographie de la famille des Anonacées. Paris: Treuttel & Würtz.

Endress, P. K., Armstrong, J. E. (2011). Floral development and floral phyllotaxis in *Anaxagorea* (Annonaceae). *Annals of Botany* 108, 835–845. doi: 10.1093/aob/mcr201

Endress, P. K. (2005). Carpels in *Brasenia* (Cabombaceae) are Completely Ascidiate Despite a Long Stigmatic Crest. *Annals of Botany* 96, 209–215. doi: 10.1093/aob/mci174

Endress, P. K. (2015). Patterns of angiospermy development before carpel sealing across living angiosperms: diversity, and morphological and systematic aspects. *Botanical Journal of the Linnean Society* 178, 556–591. doi: 10.1111/boj.12294

Endress, P. K. (2019). The morphological relationship between carpels and ovules in angiosperms: pitfalls of morphological interpretation. *Botanical Journal of the Linnean Society* 189, 201–227. doi: 10.1093/botlinnean/boy083

Evert, R. F., and Eichhorn, S. E. (2013). *Raven Biology of Plants,* 8th edn. Palgrave Macmillan: W.H. Freeman Press.

Evert, R. F. (2006). *Esau's Plant Anatomy. Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development,* 3rd edn. Hoboken, NJ: John Wiley & Sons, Inc.

Eyde, R. H. (1975). The foliar theory of the flower. *American Scientist* 63, 430–437.

Fahn, A. (1990). *Plant anatomy,* 4th edn. Oxford: Pergamon Press.

Frohlich, M. W., and Parker, D. S. (2000). The mostly male theory of flower evolutionary origins: from genes to fossils. *Systematic Botany* 25, 155–170. doi: 10.2307/2666635

Frohlich, M. W. (2006). Recent developments regarding the evolutionary origin of flowers. *Advances in Botanical Research* 44, 64–116. doi: 10.1016/S0065-2296(06)44002-7

Guo, X. M., Xiao, X., Wang, G. X., and Gao, R. F. (2013). Vascular anatomy of kiwi fruit and its implications for the origin of carpels. *Frontiers in Plant Science* 4, 391. doi: 10.3389/fpls.2013.00391

Guo, X. M., Yu, Y. Y., Bai, L., and Gao, R. F. (2017). *Dianthus chinensis* L.: the structural difference between vascular bundles in the placenta and ovary wall suggests their different origin. *Frontiers in Plant Science* 8, 1986. doi: 10.3389/fpls.2017.01986

Hickey, L. J., and Taylor, D. W. (1996). "Origin of the angiosperm flower," In: *Flowering plant origin, evolution & phylogeny*. ed. D. W. Taylor and L. J. Hickey (New York: International Thomson publishing) 176–231.

Hufford, L. (1996). Developmental morphology of female flowers of *Gyrostemon* and *Tersonia* and floral evolution among Gyrostemonaceae. *American Journal of Botany* 83, 1471–1487. doi:10.1002/j.1537-2197.1996.tb13941.x

Igersheim, A., and Endress, P. K. (1997). Gynoecium diversity and systematics of the Magnoliales and Winteroids. *Botanical Journal of the Linnean Society* 124, 213–271. doi: 10.1111/j.1095-8339.1997.tb01792.x

Kapoor, L. D. (1973). Constitution of amphicribral vascular bundles in capsule of *Papaver somniferum*. Linn. *Botanical Gazette* 134, 161–165. doi: 10.1086/336698

Kaul, R. B. (1967). Development and vasculature of the flowers of *Lophotocarpus calycinus* and *Sagittaria latifolia* (Alismaceae). *American Journal of Botany* 54, 914–920. doi: 10.1002/j.1537-2197.1967.tb10715.x

Lersten, N. R., and Don, K. W. (1966). The discontinuity plate, a definitive floral characteristic of the *Psoraleae* (Leguminosae). *American Journal of Botany* 53, 548–555. doi: 10.2307/2440004

Liu, W. Z., Hilu, K., and Wang, Y. L. (2014). From leaf and branch into a flower: *Magnolia* tells the story. *Botanical Studies* 55, 28. doi: 10.1186/1999-3110-55-28 doi: 10.1186/1999-3110-55-28

Mathews, S. and Kramer, E. M. (2012). The Evolution of Reproductive Structures in Seed Plants: A Re-Examination Based on Insights from Developmental Genetics. *New phytologist* 194, 910–923. doi: 10.1111/j.1469-8137.2012.04091.x

McKown, A. D., and Dengler, N. G. (2010). Vein patterning and evolution in C4 plants. *Botany* 88, 775–786. doi: 10.1139/B10-055

Metcalfe, C. R., and Chalk, L. (1979). *Anatomy of the Dicotyledons, vol. 1, Systematic Anatomy of Leaf and Stem, with a Brief History of the Subject*, 2nd edn. Oxford: Clarendon Press.

Nixon, K. C., Crepet, W. L., Stevenson, D., and Friis, E. M. (1994). A reevaluation of seed plant phylogeny. *Annals of the Missouri Botanical Garden* 81, 484–533. doi: 10.2307/2399901

Retallack, G. and Dilcher, D. L. (1981a). "A coastal hypothesis for the dispersal and rise to dominance of flowering plants," in *Paleobotany, Paleoecology and Evolution*, ed. K. J. Niklas (New York: Praeger Publishers), 27–77.

Roe, J. L., Nemhauser, J. L., and Zambrysk, P. C. (1997). Tousled Participates in Apical Tissue Formation During Gynoecium Development in Arabidopsis. *Plant Cell* 9: 335–353. doi: 10.2307/3870486

Robertson, R. E., and Tucker, S. C. (1979). Floral ontogeny of *Illicium floridanum*, with emphasis on stamen and carpel development. *American Journal of Botany* 66, 605–617. doi: 10.1002/j.1537-2197.1979.tb06264.x

Robinson-Beers, K., Pruitt, R. E., and Gasser, C. S. (1992). Ovule development in wild-type Arabidopsis and two female-sterile mutants. *Plant Cell* 4, 1237–1249. doi: 10.1105/tpc.4.10.1237

Sauquet, H., Doyle, J. A., Scharaschkin, T., Borsch, T., Hilu, K. W., Chatrou, L. W., et al. (2003). Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Botanical Journal of the Linnean Society* 142, 125–186. doi: 10.1046/j.1095-8339.2003.00171.x

Skinner, D. J., Hill, T. A., Gasser, C. S. (2004). Regulation of Ovule Development. *Plant Cell* 16, S32–S45. doi: 10.1105/tpc.015933

Stebbins, G. L. (1974). *Flowering Plants: Evolution above the Species Level*. Cambridge, Massachusetts: The Belknap Press of Harvard University Press.

Surveswaran, S., Wang, R. J., Su, Y. C. F., and Saunders, R. M. K. (2010). Generic delimitation and historical biogeography in the early-divergent 'ambavioid' lineage of Annonaceae: *Cananga*, *Cyathocalyx* and *Drepananthus*. *Taxon* 59, 1721–1734. doi: 10.1002/tax.596007

Takhtajan, A. L. (1991). *Evolutionary trends in flowering plants*. New York: Columbia University Press.

Tucker, S. C. (1975). Carpellary vasculature and the ovular vascular supply in *Drimys*.

*American Journal of Botany* 62, 191–197. doi: 10.1002/j.1537-2197.1975.tb14052.x

Tucker, S. C. (1961). Phyllotaxis and vascular organization of the carpels in *Michelia fuscata*. *American Journal of Botany* 48, 60–71. doi: 10.1002/j.1537-2197.1961.tb11605.x

Von Balthazar, M., and Endress, P. K. (2002). Reproductive structures and systematics of Buxaceae. *Botanical Journal of the Linnean Society* 140, 193–228. doi: 10.1046/j.1095-8339.2002.00107.x

Wang, X. (2010). *The dawn angiosperms*. Heidelberg: Springer.

Wang, X. (2018). *The dawn angiosperms*, 2nd edn. Heidelberg: Springer.

Wang, Y. Z., and Pan, K. Y. (1998). "Comparative floral anatomy of *Whytockia* (Gesneriaceae) endemic to China," in *Floristic Characteristics and Diversity of East Asian Plants*, ed. A. L. Zhang, and S. G. Wu (Beijing: China Higher Education Press), 352–366.

Zhang, Q., Huang, L-J., Wang, X-F. (2018). The unusual gynoecium structure and extragynoecial pollen-tube pathway in *Phytolacca* (Phytolaccaceae). *Plant Systematics and Evolution* 304: 885–894. doi: 10.1007/s00606-018-1518-z

Zhang, X., Liu, W., and Wang, X. (2017). How the ovules get enclosed in magnoliaceous carpels. *PLoS One* 12, e0174955. doi:10.1371/journal.pone.0174955

Zhang, X., zhang, Z. X. and Zhao, Z. (2019). Floral ontogeny of *Illicium Lanceolatum* (Schisandraceae) and its implications on carpel homology. *phytotaxa* 416, 200–210. doi: 10.11646/phytotaxa.416.3.1

**FIGURE 1.** Floral morphology and gynoecium development of two *Anaxagorea* species. **(A)** A flower of *Anaxagorea luzonensis*. **(B)** A flower of *Anaxagorea javanica*. **(C)** Young fruit of *A. luzonensis*. **(D)** Mature fruit of *A. javanica*. **(E–G)** Floral development of *A. luzonensis*. **(H–J)** Gynoecium development of *A. javanica*. Abbreviations: p, petal; c, carpel; st, stamen; si, staminode. Scale bars=200μm.

**FIGURE 2.** Carpel organogenesis of two *Anaxagorea* species. **(A–F)** *A. luzonensis*. **(A)** Carpel primordia. **(B–C)** Emergence of the carpel stipe. **(D–E)** Carpel thickening and stigma formation, with the carpel stipe elongating. **(F)** Mature carpels. **(G–J)** *A. javanica* shows similar carpel developmental features correlated with changes depicted in **(A–E, F)**. The end of ventral slit is marked by arrows. Scale bars=200μm.

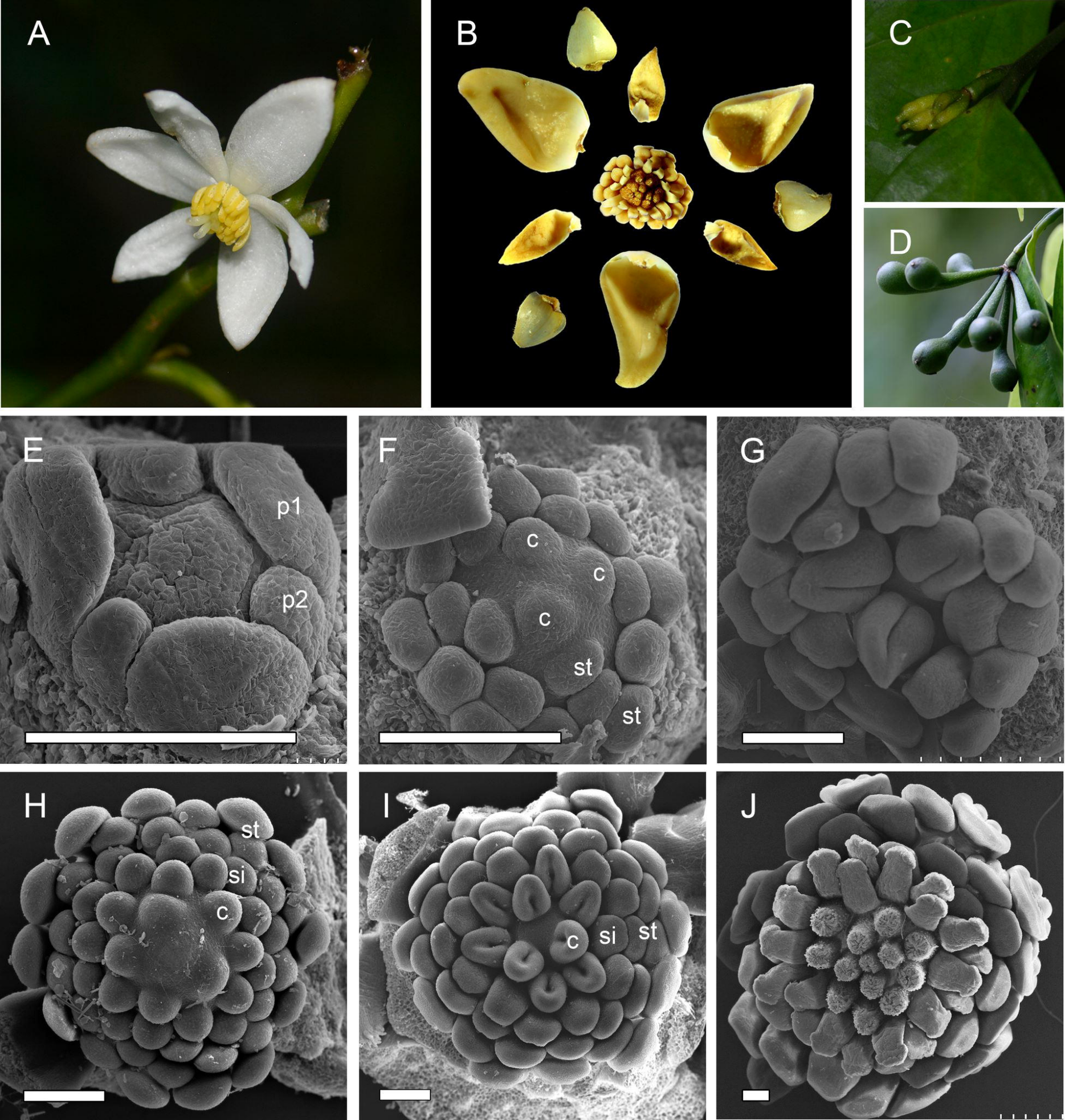
**FIGURE 3.** Paraffin transections of the flower of *A. luzonensis* from the base upward, stained with Safranin O and Fast Green. **(A)** Base of the receptacle. **(B)** The central stele and the cortical vascular system (CVS). **(C)** The stamen bundles. **(D)** The central stele was divided into two groups, with 9 bundles in each group. **(E)** Bundles from the central stele feed the carpel. **(F)** The discrete vascular bundles are arranged as a ring at the base of each carpel. **(G)** The amphicribral lateral bundle complexes. **(H)** The "C"-shaped lateral bundle complexes. **(I)** The base of the ovule locule. **(J)** The ovule locule and lateral bundle complexes in the ovary wall. Abbreviations: pe, outer petal; pi, inner petal; st, stamen; db, dorsal bundle; vb, ventral bundle; ob, ovule bundle; lbc, lateral bundle complex. Scale bars =500 μm.

**FIGURE 4.** Paraffin transections of the flower of *A. javanica* from the base upward, stained with Safranin O and Fast Green. **(A)** The cortical vascular system (CVS) is separated from the central stele at the base of the receptacle. **(B)** Perianth bundles. **(C–D)** Androecial bundles serve stamens by repeated branching. **(E–F)** Staminode bundles and carpel bundles emerge from the enlarged central stele. **(G)** The central stele breaks, and bundles were distributed into the carpels. **(H)** The base of the gynoecium. **(H1)** Detailed view of the two carpels in the center of the gynoecium in **(H)**. **(I–J)** Arrangement of vasculatures at different levels of transections in carpels. **(I1)** The ring- arranged discrete bundles at the base of carpel, detailed view of **(I)**. Abbreviations: s, sepal; pe, outer petal; pi, inner petal; st, stamen; si, staminode; gb, gynoecium bundles; db, dorsal bundle. Scale bars = 500 μm.

**FIGURE 5.** Paraffin transections of a mature *A. luzonensis* carpel from the base upward, stained with Safranin O and Fast Green. **(A)** The ring-arranged discrete bundles at the base of the carpel. **(B–C)** The ring-arranged bundles breaks at the ventral side. **(D–F)** The lateral bundles were reconstituted to two sets of secondary ring-arranged lateral bundle complexes. **(G)** Each secondary ring-arranged lateral bundle complex were broken at the dorsal side to form a "C"-shaped lateral bundle complex. **(H–I)** Bundles are get close to each other to form the ovule bundle below each placenta. **(J–K)** Formation of the ovule bundles. **(L)** The base of the ovule locule.

Abbreviations: db, dorsal bundle; lb, lateral bundle; vb, ventral bundle; ob, ovule bundle. Scale bars = 500 μm.

**FIGURE 6.** 3D-construction of vasculature of *A. javanica.* **(A–D)** Aniline blue staining slices of *A. javanica* for modeling. **(E)** The dotted lines on the longitudinal section indicate the position of vascular diagrams in the carpel as shown in the 3D-model. **(F)** Perspective for the base of carpel vasculature. **(G–I)** 3D-construction of xylem only. **(G)** Perspective for the base of carpel vasculature. the arrow shows a site of connection. **(H–I)** Spatial relationship of xylem bundles from different perspectives. The phloem is marked green, xylem red, and the bundles involved in the formation of ovule bundles are marked as purple. Abbreviations: db, dorsal bundle; vb, ventral bundle; ob, ovule bundle, lb, lateral bundle. Scale bars = 500 μm.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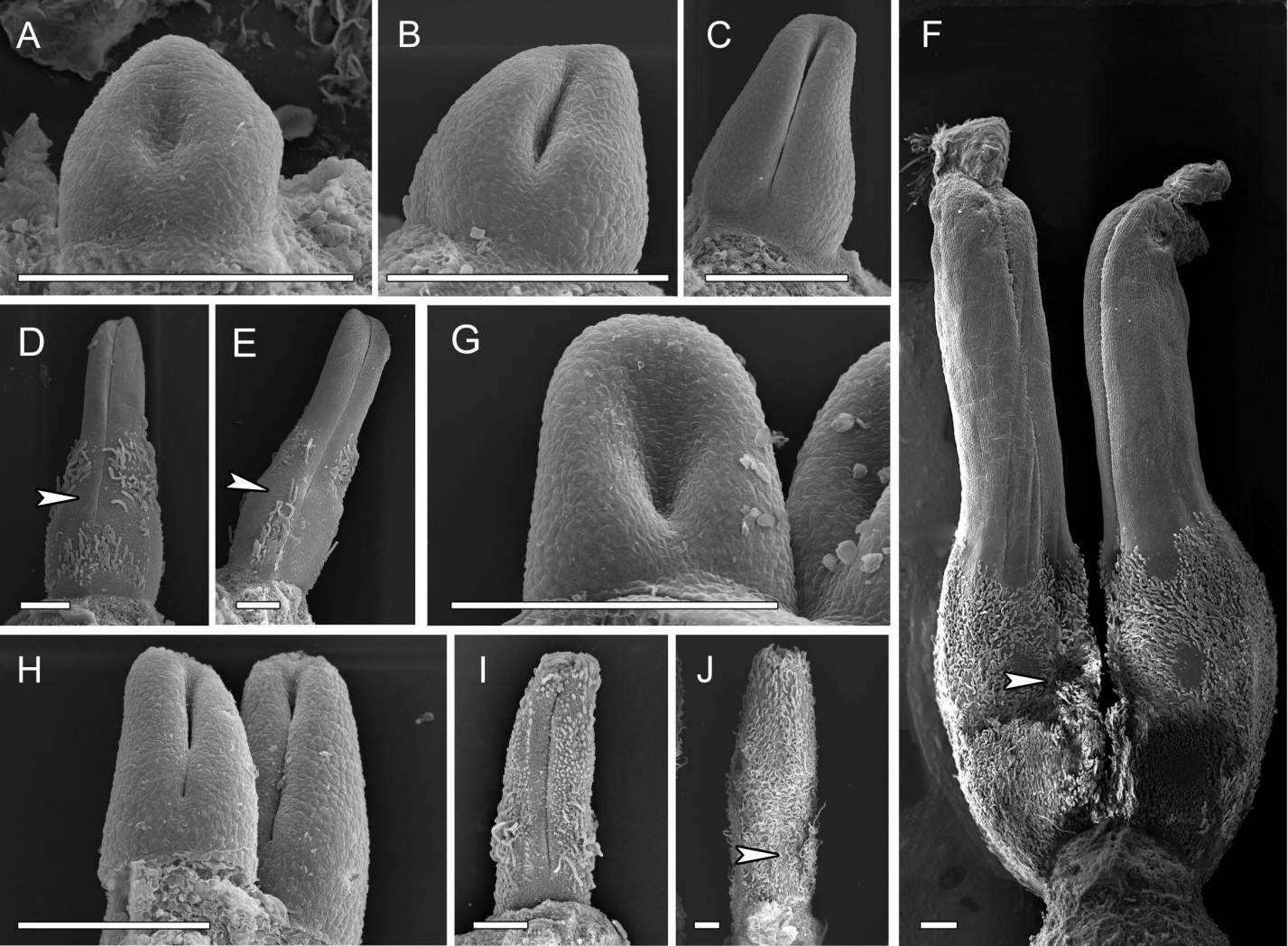
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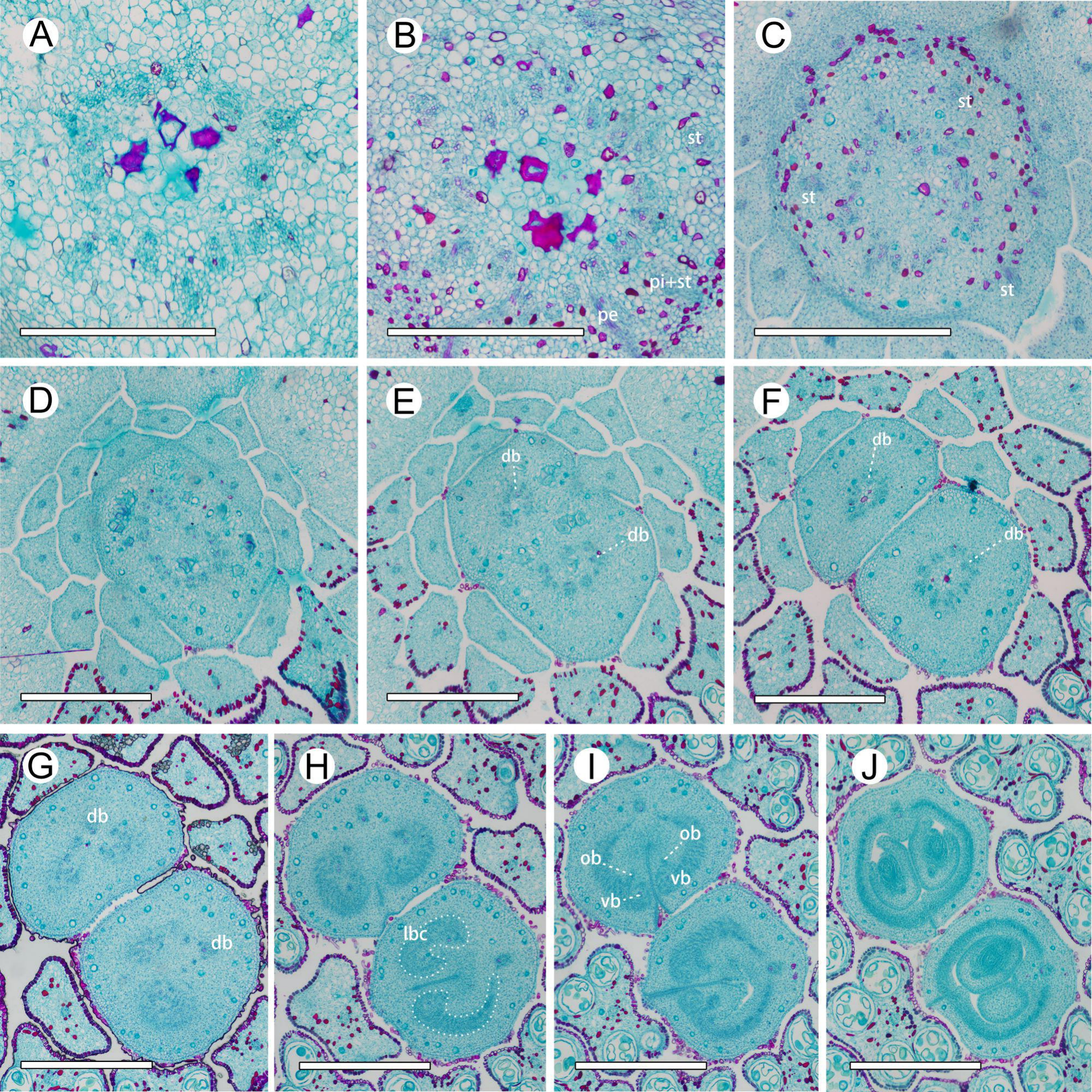
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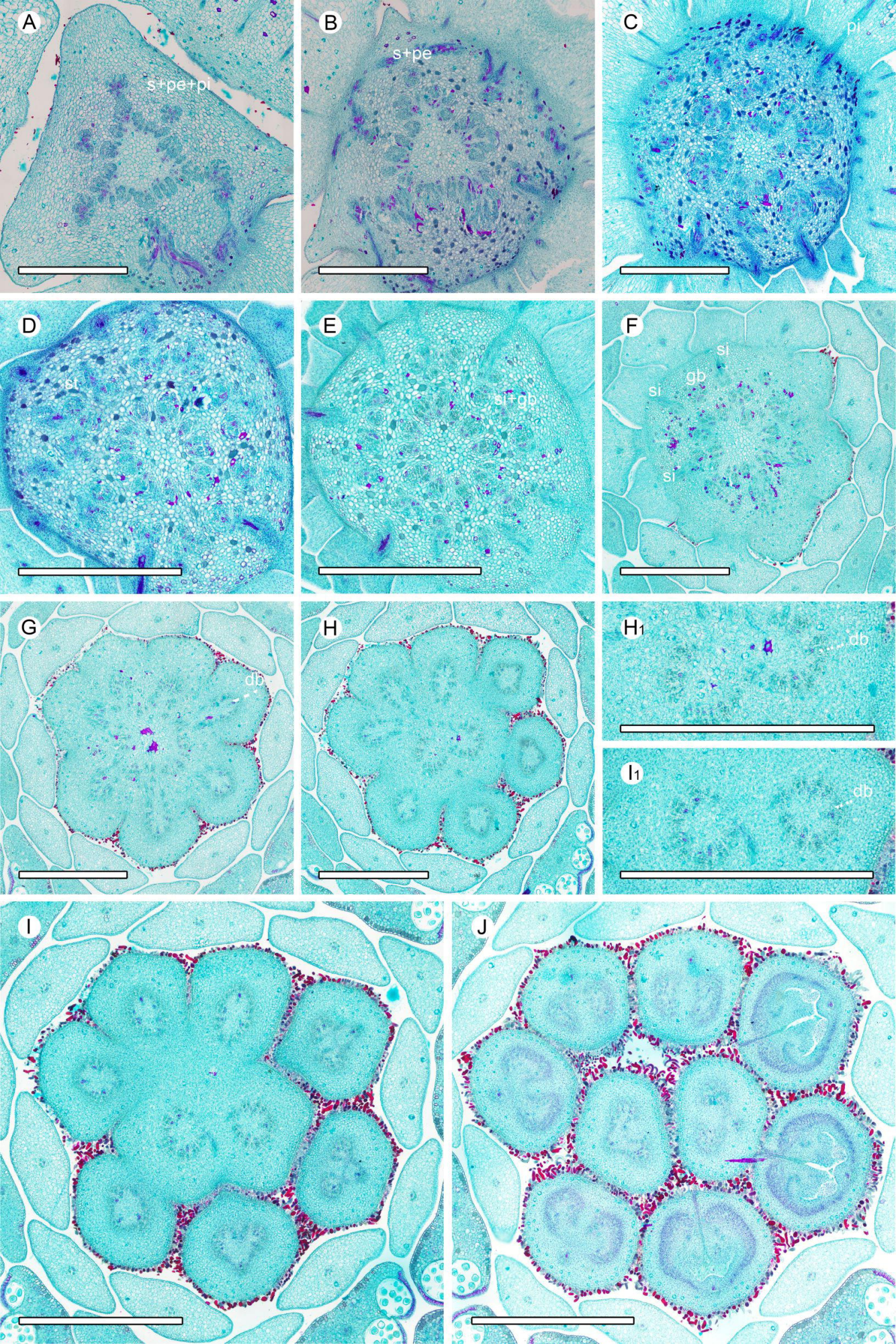
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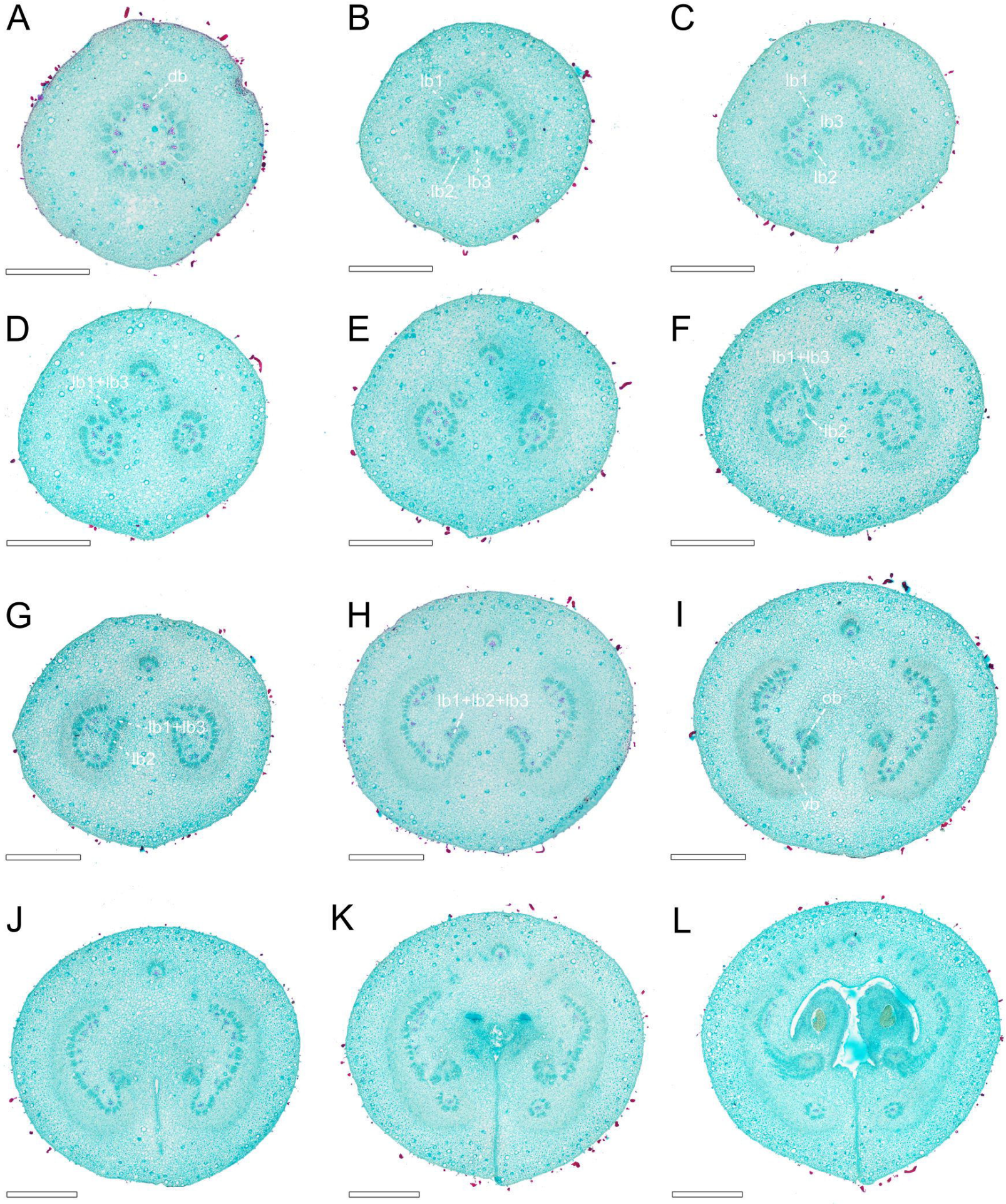
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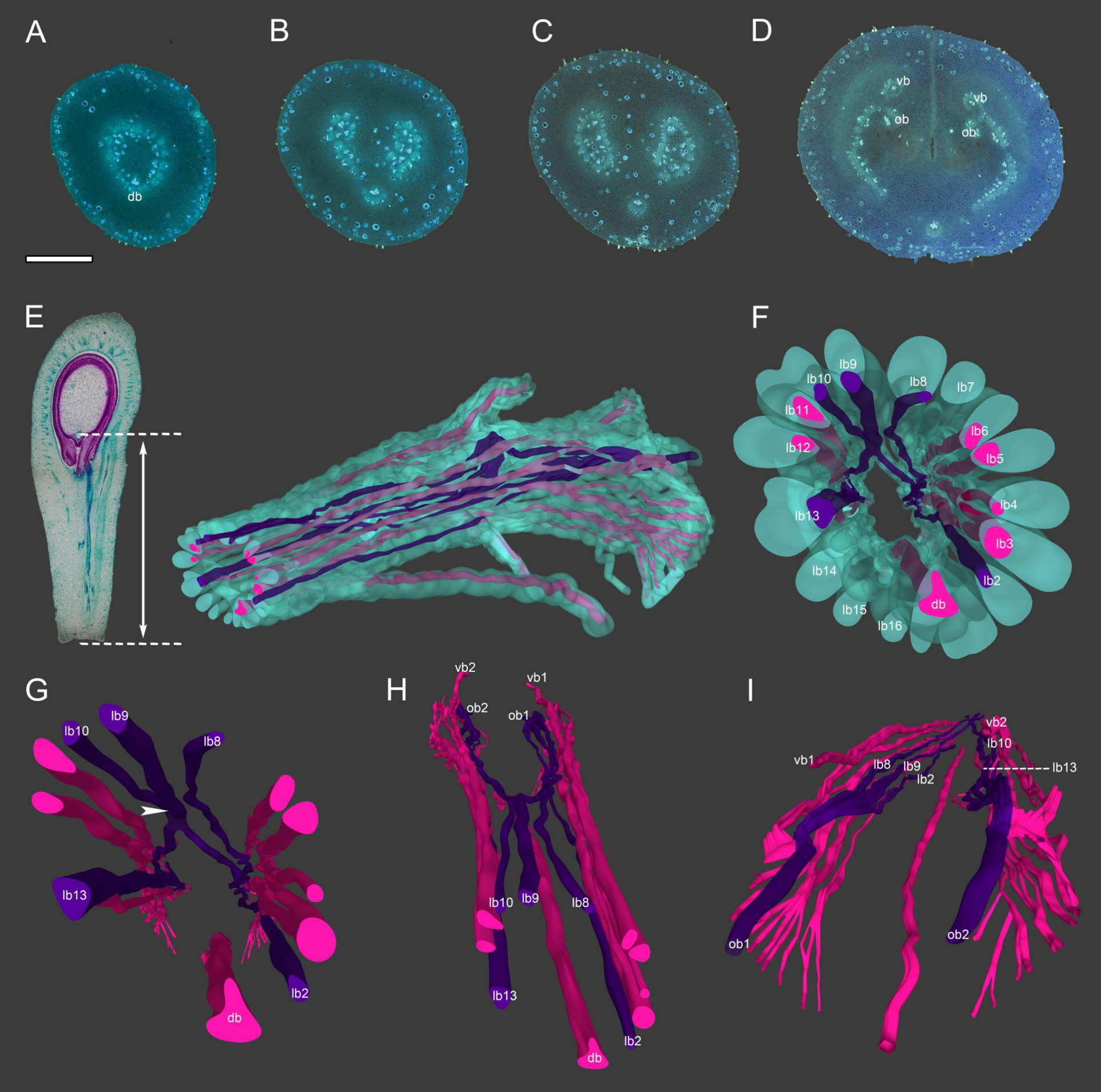
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