Organogenesis and Vasculature of *Anaxagorea* and its Implications for the Integrated Axial-Foliar Origin of Angiosperm Carpel

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Running Title: The Axial-Foliar Origin of Carpel

Abstract

The carpel is the definitive structure of angiosperms, the origin of carpel is of great significance to the phylogenetic origin of angiosperms. Traditional view was that angiosperm carpels were derived from structures similar to macrosporophylls of pteridosperms or Bennettitales, which bear ovules on the surfaces of foliar organs. In contrast, other views indicate that carpels are originated from the foliar appendage enclosing the ovule-bearing axis. One of the key differences between these two conflicting ideas lies in whether the ovular axis is involved in the evolution of carpel. The aim of this paper is to investigate whether the axial homologs remain in the carpel of extant angiosperms, and thereby to prove the carpel is integrated axial-foliar originated. Two *Anaxagorea* species was used for organogenesis and comparative anatomical study due to its outstanding and unusually long carpel stipe. The continuous change of vascular bundles at carpel stipe in different developmental stages was described in detail. Organogenesis study shows that the carpel stipe of *Anaxagorea* occurs at the early stage of carpel development. Vascular bundles at the base of *Anaxagorea* carpel are a set of discrete ring-arranged collateral bundles (RACBs), which branch out at the upper portion into two sets of RACBs below each ovule. The ring-arranged collateral bundles indicates a clear morphological evidence for the existence of the axial homologs in the carpel and thus support the idea that carpels originated from the integration of the ovular axis and foliar parts. This finding may also promote reconsiderations of the phylogenetic relationship between angiosperms, gnetophytes and Cordaitales.

Key words: *Anaxagorea*; axial homologs; carpel; organogenesis; origin of angiosperms; vascular anatomy.

INTRODUCTION

Angiosperms—the flowering plants— make up much of the visible world of modern plants. Both the beautiful flowers and the delicious fruits are closely related to human life. The name “angiosperm” is derived from the Greek words angeion, meaning “container,” and sperma, meaning “seed.” Therefore, the carpel, a “seeds container”, is the definitive structure of angiosperms. The carpel is an angiosperm-specific female reproductive feature in flowers and is the basic unit of the gynoecium, which protectively surrounds the ovules by closure and sealing along their rims or flanks (Dunal, 1817; Robinson-Beers, 1992; Endress, 2015). The evolution of the carpel set angiosperms apart from other seed plants, which developed ovules that are exposed to the air. Since the time of Darwin, elucidating the origin of angiosperms and their evolutionary success represents a primary goal in plant science. Scientists have attempted to search for possible ancestors of angiosperms through phylogenetic analysis based on fossil, morphological, and molecular data. In these efforts, particular emphasis has been placed on assessing which the ovule-bearing structures of various seed plants could be transformed into a carpel.

The history of exploring the origin of carpel has been tortuous. Competing theories of flower origins provide differing explanations and shaped different 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). Alternatively, the stachyosporous origin theories suggest that the carpel is originated from a compound shoot with the integration of the ovular axis and foliar appendage, the placenta with ovules in the carpel is homologous with a female short-shoot of gymnosperms with distally placed ovules, each with scales forming the outer integument (Stebbins, 1974; Retallack and Dilcher, 1981; Crane, 1985; Doyle and Donoghue, 1986; Nixon *et al.*, 1994; Hickey and Taylor, 1996; Wang, 2010, 2018). The Caytonialean hypothesis holds that the cupule wall of seed ferns provides a homolog for the outer integument of angiosperm ovule and that expansion and folding of the cupule-bearing axis became the progenitor of the carpel (Doyle, 1978, 2006, 2008). And in still another fashion, the carpel has evolved by ectopic expression of ovules on a phyllome (e.g., the Mostly Male theory [Frohlich and Parker, 2000]).

However, since the sister group of the angiosperms is unknown, the origin of carpel is still enigmatic. Progress has been made in the study of morphology and functional genes among angiosperms. The placenta and ovary wall of angiosperms were respectively served by independent vascular bundles (Guo et al., 2013; Liu et al., 2014; Guo et al., 2017; Zhang et al., 2017), and were controlled by different genes that the corresponding to a branch organ and a leaf organ respectively (Roe et al.,1997; Skinner et al.,2004; Mathews and Kramer, 2012). These are necessary conditions for the carpel if the placenta and the carpel wall have different evolutionary origins, but

more solid evidence is still lacking. According to the above-mentioned hypotheses, it is critical to find clear evidences for the existence of axial homologs in the carpel for the origin of angiosperms. Following the logic that there is no doubt the carpel wall is derived from foliar homologs, as long as the axial homologs in the carpel are determined, the carpel should rather derived from an ovule-bearing axis fused with the leaf-like structure than derived from the megasporophyll.

To find a more dependable evidence for the existence of axial homologs, the carpel of *Anaxagorea* (Annonaceae) was selected for organogenesis and vascular anatomic examination. Annonaceae are one of the largest families of Magnoliales, which is one of the most important lineages in the early radiation of angiosperms (Sauquet et al., 2003). *Anaxagorea* is phylogenetically the most basal genus in Annonaceae (Doyle and le Thomas, 1996; Doyle et al., 2004), which live in dimly-lit riparian zones in forest slope understory habitats. In this genus, plants have simple leaves that are alternately arranged in two rows along the stems and the flowers usually have whorled phyllotaxis. Gynoecium are apocarpous (free carpels) throughout the life history (Deroin, 1988). Each carpel of *Anaxagorea* has a notable long stipe (Endress and Armstrong, 2011), which is one of the important characteristics of the genus. Our investigation is based on the considerations that morphological features shared by *Anaxagorea* are relatively primitive in 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

Study Species and Sites

Flower samples of *Anaxagorea luzonensis* and *Anaxagorea javanica* were collected in July 2017 at the Diaoluo Mountain (Hainan, China) and Xishuangbannan Tropical Botanical Garden (Yunnan, China), respectively. The former usually has two to four carpels, whereas the latter has a multicarpellate gynoecium.

SEM and Paraffin Slices

Flower samples were collected at different floral stages (from early bud to young fruit). Gynoeciums were isolated from the other parts of the flower and preserved in formalin–acetic acid–70% alcohol (5:5:90, v/v). The fixed material was dehydrated through a series of alcohol solutions ranging from 50% to 100%. To delineate the structure and development of carpel, some carpels were removed from the gynoeciums and passed through an iso-pentanol acetate series (SCR, Shanghai, China), and then critically point-dried, sputter-coated with gold, and were observed and photographed using a scanning electron microscope (Tescan VEGA-3-LMU, Brno, Czech). To illustrate the vasculature of carpel, some carpels were embedded in paraffin, sectioned serially into sections of 10- to 12-μm thickness, and then stained with Safranin O and Fast Green. Complete transverse and longitudinal series were examined and photographed under a light microscope (Olympus BX-43-U, Tokyo, Japan) and bright field optics.

RESULTS

Carpel Organogenesis

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

Carpel Vasculature

Main vascular bundles of *Anaxagorea* carpel are distinct as a dorsal bundle, a pair of ventral bundles, a pair of ovule bundles, and several lateral bundles (**Figures 2F, M, 3A, F**). Vascular system here are described from the base up to the carpel for easier comprehension of vascular transformation according to the schematic (**Figure 3**). At the base of each carpel stipe, discrete collateral (a collateral bundle has adaxial xylem and abaxial phloem) bundles form a ring (hereinafter abbreviated as ring-arranged collateral bundles, RACBs) around the pith-like cell groups (**Figures 2A, I, 3M**). At the slightly upper part of the carpel stipe, those discrete bundles tend to join adjacent bundles in phloem (**Figure 3L**), subsequently separate, and finally are gathered into three groups: a dorsal bundle and two sets of lateral bundle complexes (LBCs, **Figure 3K**). Each set of LBCs is assembled into an amphicribral (an amphicribral bundle has its xylem surrounded by the phloem) bundle in the young carpel (**Figure 2B**). While in the mature carpel, each set of LBCs is assembled as a set of RACBs, and thus there are two sets of RACBs in one carpel (**Figures 2E, K, 3J**). Below each placenta, each set of RACBs (or amphicribral bundle) is transformed to a set of “C”-shaped bundle complexes (CBCs), from which several collateral bundles separate from each set of CBCs, and merge with each other to form an amphicribral bundle, which is kept separate from other bundles (**Figures 2C, L, 3G–I**). Each set of CBCs allocates an ovule bundle into each ovule and other bundles into the carpel wall, where the ventral bundles are also separated (**Figures 2D, F, M, 3E–F**). On the cross section of the ovary locule, both ventral bundles and ovule bundles are amphicribral (**Figures 2G, H**). In the carpel wall, lateral bundles present as pinnate arranged collateral bundles, which are connected to form meshy complexes by poorly-differentiated strands (**Figures 2M, 3D, E**). The dorsal bundle is also connected with lateral bundles by poorly-differentiated strands (**Figures 2D, 3F**). Upon the ovule locule, bundles in the carpel wall join each other with xylem and phloem and finally diminish (**Figures 3B, C**).

DISCUSSION

This study firstly investigated the organogenesis and the changes of vascular bundle in different development stages of the carpel stipe of *Anaxagorea*. The special arrangement of vascular bundles in the carpel stipe of *Anaxagorea* suggests that there are homologous structures of ovule axis exist in the carpels of modern angiosperms and constitutes an essential step to understand how the carpels evolved from female reproductive secondary shoot and the sterile bract.

It has been suggested that peltate carpels is plesiomorphic in Annonaceae (Deroin, 1988; Igersheim and Endress, 1997; Surveswaran et al., 2010; Couvreur et al., 2011). Carpels of *Anaxagorea* has been reported to have a short ascidiate zone in some studies (Deroin, 1988), while are completely plicate in others (Endress and Armstrong, 2011). In the present study, organogenesis shows that the carpel stipe of *A. luzonensis* and *A. javanica* occurs at the early stage of carpel development and continues to elongate with the development of the carpel. It is different from the mericarp stipes of other Annonaceae plants, the latter of which are not obvious until the fruit stage, and partly comes from the elongated receptacle (e.g., *Annona, Cananga, Uvari, Xylopia* [Deroin, 1999]). In flower stage, the ventral slit may continue for some distance along the ovule locule and terminated very close to the base of ovule locule. These variants of may suggest the transformation from peltate carpels to plicate carpels. The carpel stipe in *Anaxagorea* provides a buffer space for the drastic changes in structure of the vascular bundle at the base of 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 Annonaceae, it has been recognized that the distal parts of the cortical vascular system are used up in the formation of carpel traces, three to each carpel (Eames, 1961). However, in *A. luzonensis* and *A. javanica*, the number of vascular bundles entering the carpel is far more than three, and arranged in a radiosymmetric pattern. Radiosymmetric vasculature possessed universality in the stems of vascular plants (Metcalfe and Chalk, 1979; Evert, 2006; Beck, 2010; McKown and Dengler, 2010; Evert and Eichhorn, 2011). On the cross section of the stem in most angiosperms other than monocots, discrete collateral bundles form a single ring or a continuous hollow cylinder around the pith (e.g., Caprifoliaceae; Leguminosae; Tiliaceae; Ulmaceae). While the in the leaves, vascular distribution are lateral organized in most seed plants, a largest collateral bundle often extends along the long axis of the leaf as a midvein, from which smaller veins diverge laterally (e.g., Pinaceae; Gnetaceae; Magnoliaceae; Gramineae; Brassicaceae), even in the branching veins in *Ginkgo*, each of the veins is a single collateral bundle. In the cross sections of the carpel of *Anaxagorea*, the RACBs at the basal part acts in accordance with a typical form of the stem vascular anatomy of most angiosperms. In the distal region, a dorsal bundle separated from the basal RACBs, and the remainder gathered into two sets of

amphicribral bundles below each placenta, which are developed into two sets of RACBs in the more mature carpel. This vasculature transition may be an important indication that (1) the basal part of the *Anaxagorea* carpel stipe is an axial homolog in nature, and (2) the carpel is integrated axial-foliar originated from the ovular axis with its bract. The branching of RACBs in each carpel seems to be an evidence for the existence of axial homologs in the carpel because the foliar homologs (lateral organs) 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 branching RACBs (one set of RACBs branch into two sets of RACBs) in the carpel of *Anaxagorea* are equivalent to a three-way tunnel.

Development of amphicribral bundles into RACBs was observed in the carpel of *A. luzonensis* at different stages. The ovule bundles are also appeared amphicribral, which is separated from the CBCs directly. Amphicribral bundles are frequently seen 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 the carpels of *Magnolia* (Liu et al., 2014) and *Michelia* (Zhang et al., 2017), amphicribral bundles were observed supplying the ovules and derived from cortical amphicribral bundles. Whereas in pistils of *Actinidia* (Guo et al., 2013) and *Dianthus* (Guo et al., 2017), amphicribral bundles were reported in the placenta, distinct from the collateral bundles in the carpel wall. Beyond that, amphicribral bundles seems widespread in the 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]). Combined with our above-mentioned findings, this would be a reasonable result since the ovule and placenta in the carpel is originated from the ovular axis.

Considering the relationship between angiosperms and other seed plants lineages has been a lasting challenge. Taylor (1991) once set a goal for research on the origin of angiosperms, that is, correlation between carpel in angiosperms and reproductive structure in other seed plants. The branching RACBs of *Anaxagorea* carpels implies that the carpel might comparable to a compound shoot and provide an understanding of how the bract-bracteole-terminal ovule system in gymnosperms evolved to an angiosperm carpel (**Figure 4**). The Unifying Theory (Wang, 2010, 2018) suggests that angiosperm carpels are derived from the sterile bracts (formed carpel wall) attached to the ovule-bearing secondary shoot (formed ovule/placenta) of Cordaitales, a common ancestor of conifers and gnetophytes (Eames, 1952; Bold, 1973; Hickey and Taylor, 1996; Doyle, 2008). Taking Cordaitales as the common ancestor of angiosperms and other gymnosperms may resolve the contradiction between the similar morphological characters and the distant phylogenetic relationship between angiosperms and gnetophytes (Hajibabaei et al., 2006; Wickett et al., 2014; Wan et al., 2018). However, in Doyle's (2008) strict consensus of 18 most parsimonious trees of 364 steps obtained from the analysis with the arrangement of living taxa fixed to a molecular

backbone constraint tree, when Cordaitales was putted in the ancestral position of conifers, gnetophytes, and angiosperms, the most parsimonious trees with *Amborella* basal in angiosperms (as in molecular analyses) would be of the anthophyte type, which is conflicts with molecular data. Since those potential ancestors include Cordaitales are fossils which still have many unclear or misinterpreted characters, more evidences were needed from different approaches.

In conclusion, our results provide a more reliable evidence that the placenta with ovule and the carpel wall has separated evolutionary origin, and thus support the idea that carpels originated from sterile bracts attached to the ovule-bearing shoot. However, this study is limited to only a single genus. We hypothesize that the axial homologs might be a regular presence in angiosperms carpel. However, these homologs may be diverse organized because the vascular bundle in carpel is usually simplified. We hope that more extensive studies, especially that on early diverging angiosperm groups with carpel stipe, such as Cabomboideae, Illicieae, and Schisandreae, in the future will test the validity of this hypothesis. Nature follows the principle that selecting a simple mechanism or module as building blocks for a complex system and then using that module repeatedly in other systems. The presentation of axial homologs in carpel seems the embodiment of this rule. Interestingly, the word “carpel” was defined in the Annonaceae family firstly by Dunal (1817) two hundred years ago, and now new enlightenment was brought to us in this big family once more. Attempting to explain the origin of angiosperms requires integrating morphological and molecular evidence from a wide range of fossil and extant seed plant data. A full theory of flower origins must account for all the major morphological innovations of the flower other than a focus on only one organ innovation (Frohlich, 2006). More robust answers will come from more integrative models with consideration of multiple traits at once from both extant plants and fossil records, high-quality taxonomic data, molecular mechanism of character evolution, and an abundance of databases cataloguing georeferenced occurrences.

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LITERATURE CITED

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.

Bold, H. C. (1973). *Morphology of plants*, 3rd edn. New York: Harper & Row.

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.

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

Deroin, T. (1999). Functional Impact of the Vascular Architecture of Flowers in Annonaceae and Magnoliaceae, and Its Bearing on the Interpretation of the Magnoliaceous Gynoecium. *Systematics and Geography of Plants* 68, 213–224. doi: 10.2307/3668602

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.

Eames, A. J. (1952). Relationships of the Ephedrales. *Phytomorphology* 2, 79–100.

Eames, A. J. (1961). *Morphology of the angiosperms*. New York: McGraw-Hill.

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

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

Hajibabaei, M., Xia, J., and Drouin, G. (2006). Seed plant phylogeny: Gnetophytes are derived conifers and a sister group to Pinaceae. *Molecular Phylogenetics and Evolution* 40, 208–217. doi: 10.1016/j.ympev.2006.03.006

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.

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. (1981). "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.

Taylor, D. W. (1991). Angiosperm ovules and carpels: their characters and polarities, distribution in basal clades and structural evolution. *Postilla* 208, 1–40.

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

Wan, T., Liu, Z. M., Li, L. F., Leitch, A. R., Leitch, I. J., Lohaus, R., et al. (2018). A genome for gnetophytes and early evolution of seed plants. *Nature Plants* 4, 82–89. doi: 10.1038/s41477-017-0097-2

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.

Wettstein, R. (1907). Uber des vorkommen zweigeschlectiger Infloreszenzen bei Ephedra. *Festschrifter Naturwissenschaften Vereins Universitiit Wien* 25, 21–28.

Wettstein, R. (1935). *Handbuch der systematischen Botanik*, 4th edn. Leipzig: Franz Deuticke.

Wickett, N. J., Mirarab, S., Nguyen, N., Warnow, T., Carpenter, E., Matasci, N., et al. (2014). Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences of the United States of America* 111, E4859–E4868. doi:10.1073/pnas.1323926111

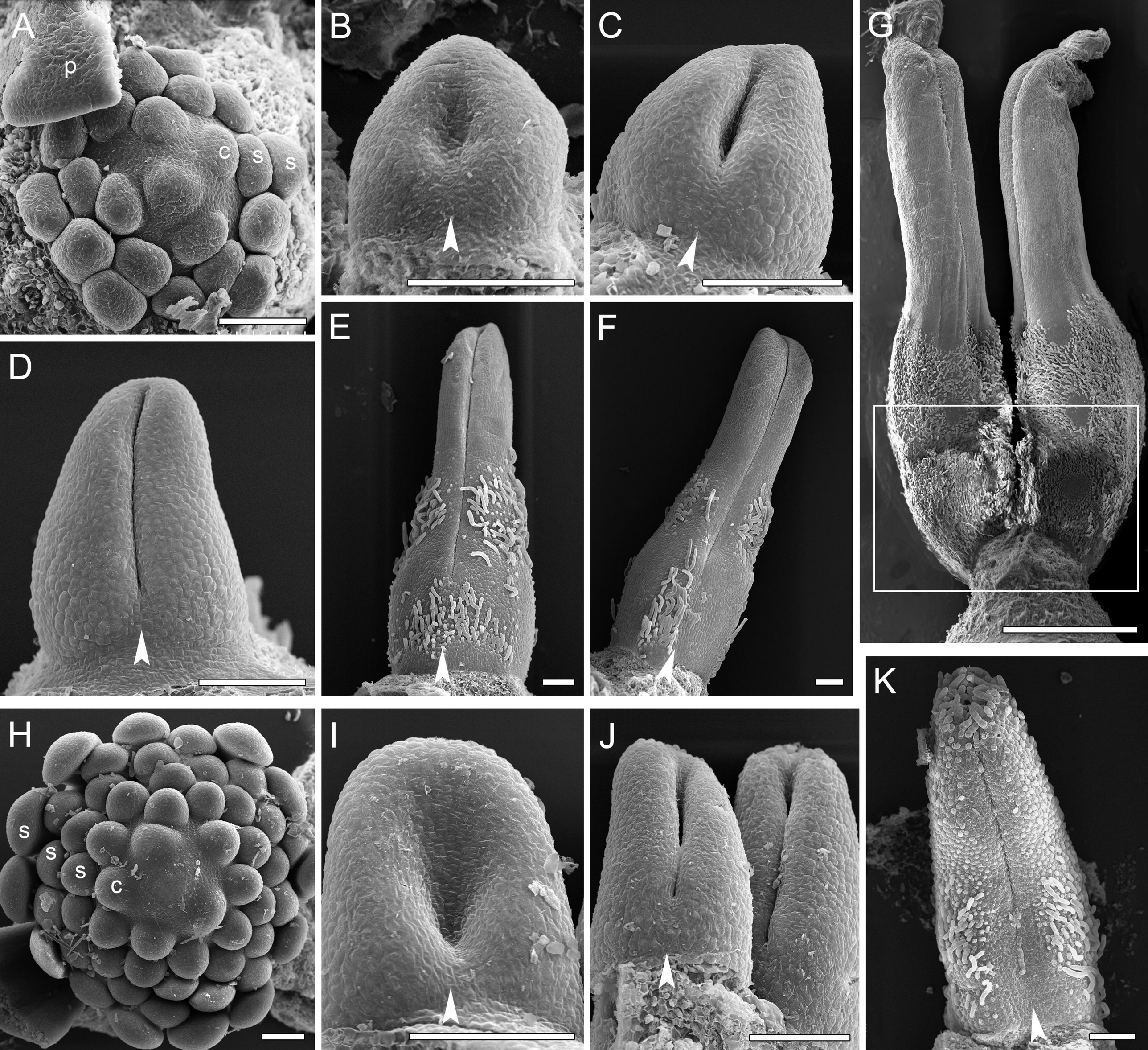
Zhang, X., Liu, W., and Wang, X. (2017). How the ovules get enclosed in magnoliaceous carpels. *PLoS One* 12, e0174955. doi:10.1371/journal.pone.0174955

FIGURE 1. SEM of *Anaxagorea* carpel. **(A–G)** *A. luzonensis.* **(A)** Carpel primordia. **(B–C)** Appearance of the basal stipe. **(D)** Incomplete stipe closure. **(E–F)** Carpel thickening and stigma formation. **(G)** Carpel stipes at anthesis (box). **(H–K)** *A. javanica* shows similar carpel developmental features correlating with changes depicted in **(A, C, D, F)**. Carpel stipes are marked by arrows. Abbreviations: c, carpel; s, stamen; p, perianth. Scale bars: **(A–F, H–K)** = 100 μm; **(G)** = 1 mm.

FIGURE 2. Paraffin sections of carpels stained with Safranin O and Fast Green. **(A–D)** Anthetic carpels of *A. luzonensis*, from base up. **(A)** The ring-arranged collateral bundles (RACBs) at the base of carpel, ventral sides are marked by arrows. **(B)** Bundle complexes with xylem surrounded by phloem in each carpel (ring marked). **(C)** A short ascidiate zone. **(D)** Ovule bundles separate from the CBCs. **(E–H)** Mature carpel of *A. luzonensis*. **(E)** Two sets of RACBs at the level of **(B)**. **(F)** Ventral bundles (vb) and ovule bundles (ob). **(G)** Detailed view of a vb from **(F)**. **(H)** Detailed view of an ob from **(F)**. **(I–M)** Mature carpel of *A. javanica*, from base up. **(I)** RACBs at the base of carpel, a distinct collateral bundle marked by ring. **(J–K)** Bundles rearranged into two sets of RACBs. **(L)** The CBCs with phloem joint each other. **(M)** The base of ovule locule. Abbreviations: db, dorsal bundle; vb, ventral bundle; ob, ovule bundle. Scale bars = 200 μm.

FIGURE 3. Drawings of sequential sections of a mature *Anaxagorea* carpel. Vasculature colored with phloem dark green and xylem red; distinct vascular bundles within the vascular complex are outlined. **(A)** Schematic longitudinal section with ventral side at the left. Vertical zones labeled with letters correspond to the transverse sections of panels to the right. **(B–M)** Transverse sections (ventral side up) here are described from the base up to the carpel in the following. **(M)** The ring-arranged collateral bundles (RACBs) at the base of carpel. **(J–L)** Vasculature changes from a set of RACBs at the base of the carpel to two sets of RACBs. **(H–I)** Vasculature changes from each set of RACBs to the "C"-shaped bundle complexes (CBCs). **(E–G)** Ovule bundles are separated from the CBCs, while others into the carpel wall. **(B–D)** Meshy bundles in the carpel wall. Abbreviations: db, dorsal bundle; vb, ventral bundle; ob, ovule bundle. Scale bars = 500 μm.

FIGURE 4. Homology comparison between *Anaxagorea* carpel vasculature and female reproductive shoot of seed plants. The color implies homologies. Cordaitales is a Carboniferous to Permian gymnosperm group. On the female reproductive shoot of Cordaitales, the ovules (yellow) are interspersed with sterile scales (light green), and ovular axis bearing bracteoles (yellow). The bracteoles on the ovular axis may aggregate to the ovule and the ovular axis become shorter. Arrangement of the ovules on the shoot apex (dark green) and the of lengths ovular axis are relatively flexible. *Ephedra* as a genus represents gnetophytes, which shows its female reproductive shoot with subtending bracts (light green), have a pair of ovules (yellow) at their apical end with the abortive apex of the shoot axis (dark green) lying in between. The axis bearing a pair of ovules and their envelope is called gynocladal axis.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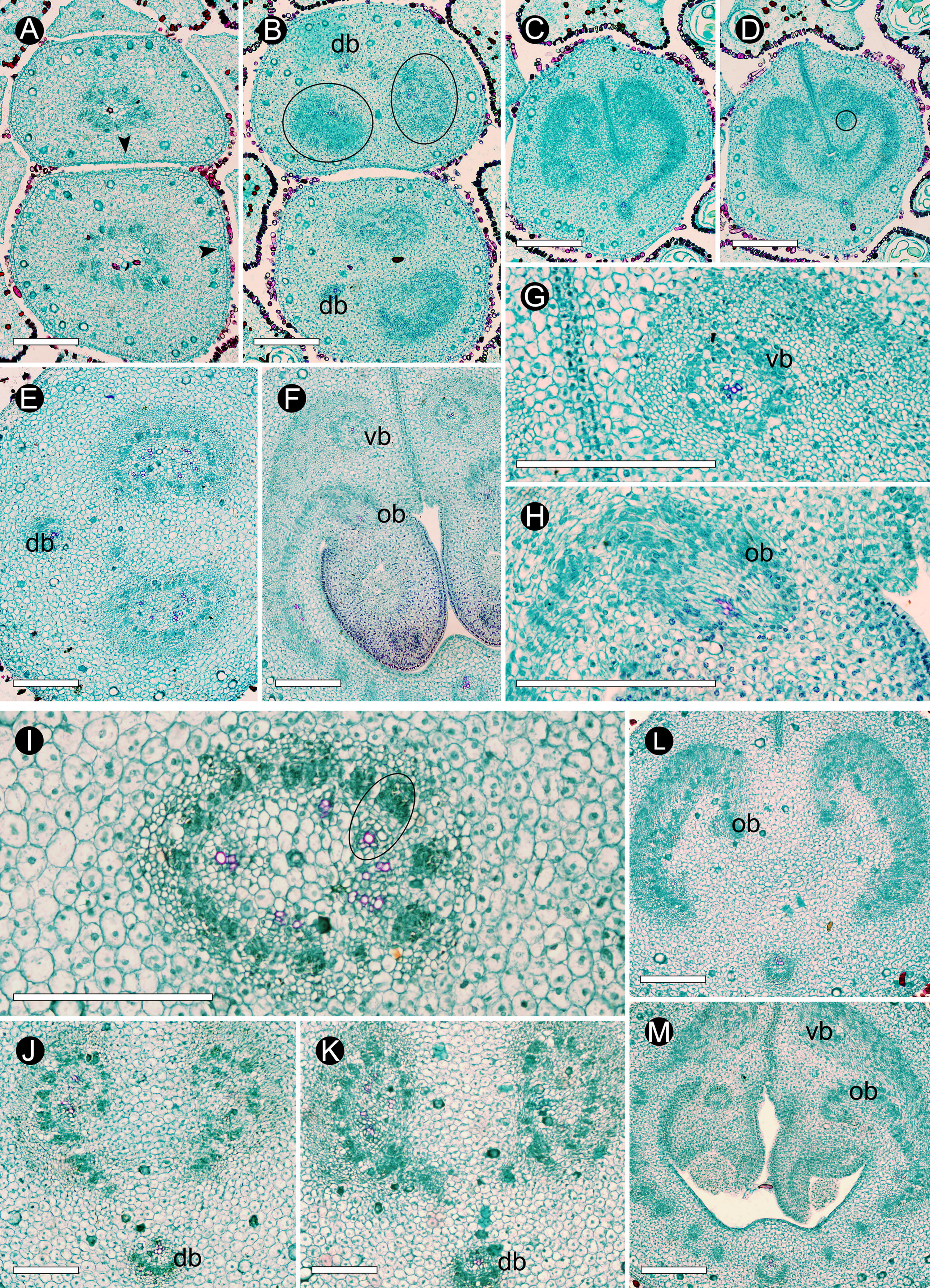
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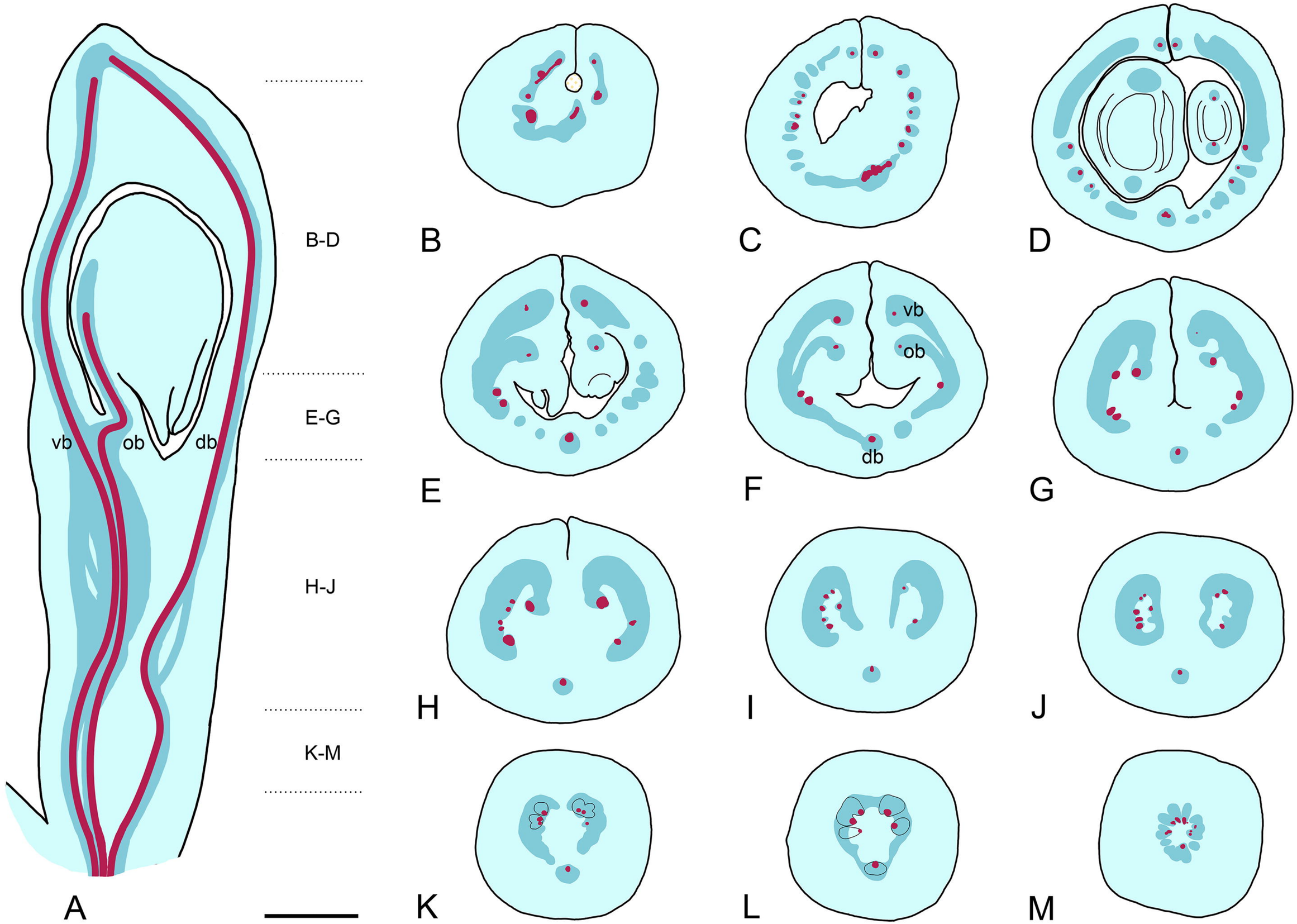
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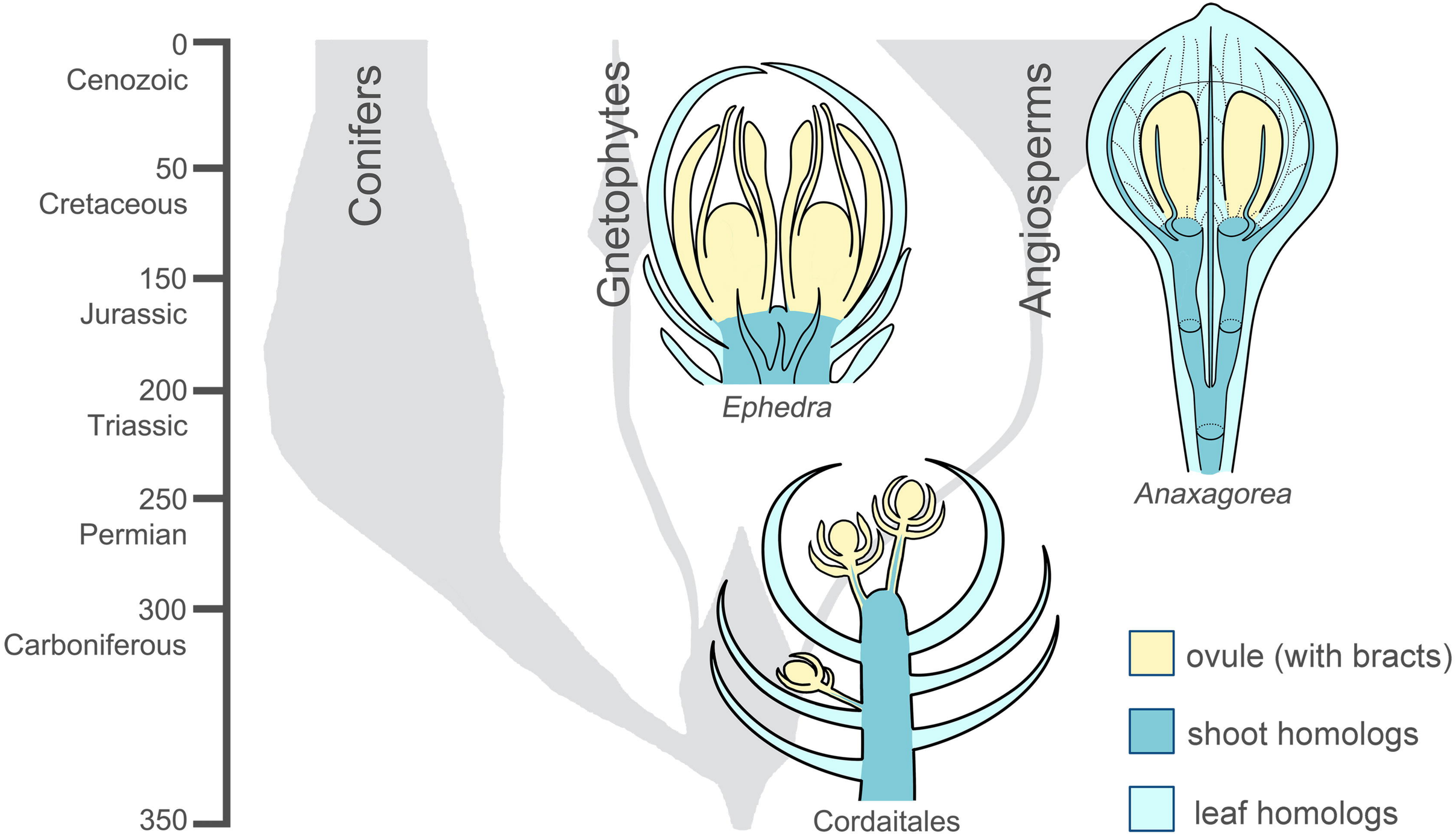
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Cenozoic
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Triassic
250
Permian
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Carboniferous
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Conifers
Gnetophytes
Angiosperms
Ephedra
Anaxagorea
Cordaitales
ovule (with bracts)
shoot homologs
leaf homologs