Is a Ring-arranged Vascular Pattern Unique to Stems? A Three Dimensional Reconstruction of *Anaxagorea* (Annonaceae) Carpel Vasculature and its Evolutionary Implications

Running head: 3D Reconstruction of Carpel Vasculature

Ya Li,^{1,†} Wei Du,^{1,†} Ye Chen,² Shuai Wang,³ Xiao-Fan Wang^{1,*}

¹ College of Life Sciences, Wuhan University, Wuhan, Hubei 430072, China

² Department of Environmental Art Design, Tianjin Arts and Crafts Vocational
College, Tianjin, Tianjin 300250, China

³ College of Life Sciences and Environment, Hengyang Normal University,
Hengyang, Hunan 421001, China

*Author for correspondence: Xiao-Fan Wang, E-mail: wangxf@whu.edu.cn

† These authors have contributed equally to this work

16 **Abstract**

17 Elucidating the origin of carpel has been a challenge in botany for a long time. The
 18 Unifying Theory suggested that the carpel originate from a composite organ
 19 comprising an ovule-bearing shoot and a foliar part enclosing the shoot. A logical
 20 inference from this theory is that placenta in angiosperms should have
 21 radiosymmetrical vasculature, just like that in a young branch. *Anaxagorea* is the
 22 most basal genus of the primitive angiosperm family, Annonaceae. The conspicuous
 23 carpel stipe makes it an ideal material for exploring the carpel vasculature. In this
 24 study, serial sections of flower and carpel were delineated in *Anaxagorea luzonensis*
 25 and *A. javanica*, and a three-dimensional model of the carpel vasculature was
 26 reconstructed. The results show that (1) vascular bundles at both the carpel stipe and
 27 the ovule/placenta are in a radiosymmetrical pattern, (2) the amphicribal bundles
 28 would develop into ring-arranged bundle complex with the carpel maturation, (3) the
 29 ovule/placenta bundles were separated from the bundles of the carpel wall, and, (4) all
 30 the radiosymmetrical vasculature (including amphicribal bundles and ring-arranged
 31 bundle complexes) in the carpel were fed by a larger radiosymmetrical bundle system.
 32 These results suggest that the radiosymmetrical pattern of carpel vasculature are in
 33 line with the Unifying Theory.

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

36 INTRODUCTION

37 Based on common textbook knowledge (Esau, 1965; Rudall, 2007; Evert & Eichhorn,
38 2013), the arrangement of vascular bundles —in a radiosymmetrical pattern or lateral
39 pattern — is an important difference between the axial organ (such as stems), and the
40 lateral organ (such as leaves). Amphicribal bundles are frequently encountered in
41 stems of early land plants (Ogura, 1972; Fahn, 1990). An amphicribal bundle is
42 featured by xylem surrounded by phloem, while a collateral bundle has adaxial xylem,
43 and abaxial phloem. De Bary (1884) observed that amphicribal vascular bundles are
44 apparently rare in angiosperms. However, their occurrence has been reported in
45 flowers, especially in the placenta and the ovules of the carpel. They are fed
46 independently from lateral bundles which supply the carpel wall (e.g., *Actinidia* [Guo
47 et al., 2013], *Magnolia* [Liu et al., 2014]; *Michelia* [Zhang et al., 2017]; *Dianthus*
48 [Guo et al., 2017]; *Tapiscia* [Xin et al., 2019]). These studies, based on comparison of
49 vascular bundles, suggest that the widespread existence of amphicribal bundles in the
50 ovule/placenta is compatible with the Unifying Theory (Wang, 2010, 2018), which
51 states that the carpel in the classic sense is a composite organ comprising an
52 ovule-bearing shoot (ovule/placenta) and a foliar part (carpel wall) enclosing the
53 shoot.

54

55 Although vascular bundles are generally considered conservative, their anatomy
56 is influenced by the immediately adjacent histological environment, and its histology
57 is greatly influenced by the next older vascular bundle to which it connects (Endress,
58 2019). Could the carpel vasculature provide threads for speculate the evolutionary
59 origin of carpels? In the past, the material usually included limited developmental
60 stages, either flower or fruit. To account for the different development stages of

vascular bundles and the adjacent histological environment, two species of *Anaxagorea* were selected from both flowering and fruiting stages, and continuous serial sections were conducted of their receptacles and carpel, and a three dimensional (3D) -model of the carpel vasculature was reconstructed.

Anaxagorea is the most basal genus of Annonaceae, one of the early radiations of angiosperms lineage (Doyle & Le Thomas, 1996; Doyle et al., 2004; Chatrou et al., 2012; Chatrou et al., 2018). *Anaxagorea* carpels are apocarpous throughout their life history (Derooin, 1988), and each has a notably long carpel stipe (Endress & Armstrong, 2011). If the Unifying Theory of carpel origin is practical, then it should also be applicable for *Anaxagorea*, i.e., (1) the ovule/placenta bundle of *Anaxagorea* should be amphi-cribral, (2) the ovule/placenta bundle should always be separated from the vascular bundle of the carpel wall, and hence, (3) the amphi-cribral bundle should come from a larger axial bundle system.

MATERIALS AND METHODS

Scanning electron microscopy and paraffin sectioning

Anaxagorea luzonensis flower samples at different floral stages (from early bud to young fruit) were collected from the Diaoluo Mountain, Hainan, China, in July 2017 and *Anaxagorea javanica* from the Xishuangbanna Tropical Botanical Garden, Yunnan, China in May 2017. The gynoecia were isolated and preserved in 70% formalin-acetic acid-alcohol (5:5:90, v/v), and the fixed specimens were dehydrated in

83 a 50% to 100% alcohol series. To delineate the structure and development of the
84 carpel, carpels were removed from the gynoecia, passed through an iso-pentanol
85 acetate series (SCR, Shanghai, China), critically point-dried, sputter-coated with gold,
86 observed, and photographed under a scanning electron microscope (Tescan
87 VEGA-3-LMU, Brno, Czech Republic). Flowers and carpels were embedded in
88 paraffin, serially sectioned into 10–12- μ m thick sections, and stained with Safranin O
89 and Fast Green to illustrate the vasculature. The transverse sections were examined
90 and photographed using a bright-field microscope (Olympus BX-43-U, Tokyo, Japan).
91 In addition, longitudinal hand-cut sections were made and observed for a rough check
92 and better understanding of the vasculature.

93

94 *Topological analysis of carpel vasculature*

95 Consecutive paraffin sections, 12- μ m each, of *A. javanica* were stained with aniline
96 blue, examined and photographed after excitation at 365 nm using an epifluorescence
97 microscope (Olympus BX-43-U, Tokyo, Japan) and a semiconductor refrigeration
98 charged coupled device (RisingCam, MTR3CMOS). Manual image registration of
99 each dataset was carried out using Photoshop CC 2017 (Adobe, San Jose, CA, US).
100 Forty-five images were selected equidistant from the 423 sections taken for the 3D
101 reconstruction. The figures were organized according to the vascular bundle outlines
102 of the sections by using Photoshop CC 2017 and Illustrator CC 2017 (Adobe). The
103 xylem and phloem contours were manually drawn, extracted as paths with the pen
104 tool, and exported in DWG format. The DWG files were imported into 3Ds Max 2016

105 (Autodesk, San Rafael, CA, US) and sorted according to the distance and order of the
106 sections. The paths were converted to Editable Spline curves to generate the basic
107 modeling contour. The Loft command of Compound Objects was used to get the
108 shape of the Editable Spline, and a complete 3D carpel vasculature model was
109 generated.

110

111 **RESULTS**

112 *Gynoecium structure and carpel organogenesis*

113 Flowers of two study species were trimerous with a whorl of sepals, two
114 morphologically distinct whorls of petals, and numerous stamens (and inner
115 staminodes of *A. Javanica*) (Figs. 1A–1D). *A. luzonensis* usually exhibits two to four
116 completely separate carpels (Figs. 1A, 1G). The carpel primordia are almost
117 hemispherically initiated and larger than the stamen primordia (Fig. 1F). Each carpel
118 consists of a plicate zone, a very short ascidiate zone (Figs. 3G, 5I, 5J), and a long,
119 conspicuous stipe (Fig. 2F). Carpel stipe ontogenesis occurs at the early stages of
120 carpel development (Fig. 2B). The continuous growth of the flanks on the ventral side
121 of the young carpel triggers its early closure; however, the closure does not extend to
122 the base of the carpel, where the carpel stipe was previously present (Fig. 2C).
123 Subsequently, the dorsal region of each carpel thickens markedly, and the stigma
124 forms (Figs. 2D, 2E). At anthesis, the carpels are widest at the basal region with an
125 arch on the abaxial side. The carpel stipe remains elongate, accounting for
126 approximately a quarter of the carpel length at anthesis, and continues to elongate

127 during the fruiting stage (Fig. 1F). Each carpel has two lateral ovules with the
128 placentae at the ovary base (Figs. 3H, 5L). *A. Javanica* exhibits a multicarpellate
129 gynoecium (Figs. 1B, 1J). The carpels are completely separate and appear whorled at
130 initiation (Fig. 1I); as the carpel volume increases, the whorled structure becomes less
131 obvious because the space in floral apex becomes limited. Each carpel consists of a
132 plicate zone and a conspicuous carpel stipe (Fig. 2J) but lacks the short ascidiate zone.
133 The carpel stipe ontogenesis occurs in the early stages of carpel development (Fig. 2H)
134 and remains elongate during the flowering and fruiting stages (Figs. 1D, 2I–2J). Each
135 carpel has two lateral ovules.

136

137 ***Vasculature from receptacle to carpel***

138 In the *A. luzonensis* cross-sections, the receptacle base presented a hexagon of 18
139 bundles from the pedicel stele (Fig. 3A). The hexagon had six breaks, which built up a
140 crown of the cortical vascular system to supply the sepals and the two whorls of petals
141 and the stamens (Figs. 3B). The central stele, composed of 18 bundles, finally broke
142 into two nine-bundle groups at the floral apex and ran into the two-carpel gynoecium
143 (Figs. 3C, 3D). Each group of nine bundles assembled as a basal ring around the
144 parenchyma at each carpel base (Figs. 3E). At the slightly upper part of each carpel,
145 several bundles emerged on the lateral side, and the basal ring broke, from which the
146 dorsal bundle separated and the lateral bundles reorganized into two groups of lateral
147 bundle complexes (Figs. 3F). In each of the lateral bundle complexes, the adjacent
148 bundles tended to join, assembling into an amphiobribral pattern (Fig. 3G). Below each

149 placenta, each of the amphi-cribral lateral bundle complexes transformed into a set of
150 “C”-shaped lateral bundle complexes, from which the ovule bundles separated, while
151 the other bundles ran into the ovary wall. There were no horizontal connections
152 between the dorsal and other bundles (Fig. 3H).

153 The pseudosteles at the base of the *A. Javanica* receptacle were triangular, with ~
154 45 bundles. The outer six cortical traces were cylindrical and served the sepals and
155 petals (Figs. 4A, 4B). At a slightly higher level, the androecial bundles emerged and
156 served the stamens by repeated branching, and the staminode bundles emerged as a
157 crown around the central stele (Fig. 4C). Before entering the gynoecium, the central
158 stele enlarged and broke up into ~ 70 bundles to supply the nine carpels, and each
159 carpel was served by 7–10 bundles (Figs. 4D–4E). The vascular bundle arrangement
160 was similar to ascending sections in *A. luzonensis*, with the basal ring and
161 amphi-cribral lateral bundle complexes presented in each carpel (Figs. 4F–4H).

162

163 ***Three dimensional (3D)-reconstruction of carpel vascular topology***

164 At the base of a mature *A. luzonensis* carpel, 15 discrete bundles were arranged in a
165 radiosymmetrical pattern, forming a basal ring around the central parenchyma (Fig.
166 5A). At the slightly upper part, the basal ring curved inward on the ventral side and
167 broke away from the invagination (Figs. 5B, 5C). The bundles (except the dorsal)
168 divided into two groups on each side of the carpel, each forming a lateral bundle
169 complex, which was also ring-arranged. At the flowering stage, the lateral bundle
170 complexes corresponded to the above-mentioned sections of the amphi-cribral

complexes (Figs. 5D–5F). Below each placenta, bundles of each lateral bundle complex broke up on the dorsal side and transformed into a “C”-shaped lateral bundle complex (Figs. 5G, 5H). The bundles on the ventral side of each lateral bundle complex gathered together (excluding the ventral bundle) and entered each ovule, while other bundles entered into the ovary wall. The ovule bundles were amphicribal. (Figs. 5I–5L).

177

Consecutive cross-sections of *A. Javanica* were similar in vasculature to those of *A. luzonensis* (Figs. 6A–6D). The base of the mature *A. Javanica* carpel exhibited 16 distinct bundles forming the basal ring (Fig. 6A, 6F). The 3D model showed that (1) the basal ring and lateral bundle complex were cylindrical (Figs. 6F, 6H). (2) The ovules were fed directly by bundles from the base of the carpel through the lateral bundle complex. (3) Each ovule bundle was formed from several non-adjacent lateral bundles, and two bundles of them that fed each ovule joined on the ventral side (Figs. 6G, 6I). (4) The dorsal bundle remained independent throughout ontogenesis, without any link to other bundles (Appendix S1, S2; see Supplemental Data with this article).

187

188 **DISCUSSION**

Elucidating the origin of carpel has been a challenge in botany for a long time (Takhtajan, 1969; Doyle, 1978; Cronquist, 1979; Thorne, 1996; Hickey & Taylor, 1996; Doyle, 2008). The carpel has been assumed as a conduplicate leaf-like structure bearing marginal ovules in the traditional interpretation. However, research was carried out to investigate whether the reproductive module (ovule/placenta) in the

194 carpel and the carpel wall had evolved from two independent organs (Satina &
195 Blakeslee 1941, 1943; Satina 1944, 1945; Angenent et al., 1995; Roe et al., 1997;
196 Wynn et al., 2014; Zhang et al., 2019). Many hypotheses exist where the
197 ovule/placenta of the carpel originated from the female reproductive branches of
198 gymnosperms or seed ferns (e.g., the Gonophyll Theory [Melville, 1960]; the
199 Neo-pseudanthial Theory [Doyle, 1994; Nixon et al., 1994]; the
200 *Caytonia*-Glossopterid Model [Doyle, 2006]; the Unifying Theory [Wang 2010,
201 2018]). A logical inference from these hypotheses is that the placenta in angiosperms
202 should have amphicribal vascular bundle, just like that in a young branch (Liu et al.,
203 2014). Favoring this inference, amphicribal bundles have been documented in the
204 placenta of many angiosperms (e.g., *Papaver* [Kapoor, 1973], *Psoraleae* [Lersten &
205 Don, 1966], *Drimys* [Tucker, 1975], *Nicotiana* [Dave et al., 1981], *Whytockia* [Wang
206 & Pan, 1998], *Pachysandra* [Von Balthazar & Endress, 2002], *Actinidia* [Guo et al.,
207 2013], *Magnolia* [Liu et al., 2014]; *Michelia* [Zhang et al., 2017]; *Dianthus* [Guo et
208 al., 2017]; *Tapiscia* [Xin et al., 2019]). However, regarding the amphicribal bundle as
209 the evidence to support the axial nature of the carpel is considered to be flawed. As
210 Endress (2019) summarized: (1) a vascular bundle often changes its anatomy along its
211 length; the anatomy is influenced by its immediately adjacent histological
212 environment; (2) the structure of a vascular bundle is not organ-specific; thus, it
213 cannot be used to determine the kind of organ in which it was formed; and, (3) a
214 vascular bundle is connected to other vascular bundles in the plant body, and its
215 histology is greatly influenced by the next older vascular bundle to which it connects.

216

217 Considering the variability of the vascular bundles mentioned above, it is

218 necessary to take the histological environment around the amphicribal ovule/placenta
219 bundle into account. On the basis of confirming the existence of amphicribal
220 ovule/placenta bundles in the carpel of *A. luzonensis* and *A. javanica*, this study also
221 found that, vascular bundles are arranged in rings in different parts of the *Anaxagorea*
222 carpel: (1) the basal ring maintains spatiotemporal continuity throughout the carpel
223 stipe, and (2) the ring-arranged lateral bundle complexes at each placenta; these
224 ring-arranged lateral bundle complexes developed from the amphicribal bundle
225 complexes in the placenta with carpel maturation.

226

227 In most groups of Annonaceae, gynoecium is fed by an enlarged central stele, and
228 each carpel is usually fed by three bundles, one median and two lateral (Derooin, 1989;
229 Ronse De Craene, 1993; Derooin & Norman, 2016; Derooin & Bidault, 2017). This
230 feature allows the carpel to be interpreted as foliar organ. Because in eudicots, usually
231 one to three bundles connect the leaf with the vascular system of the stem (Beck,
232 2010). However, in *Anaxagorea*, the basal ring seems inconsistent with the traditional
233 interpretation. In other sister branches, *Cananga* represents the relatively basal
234 position. In the carpel stipe of *Cananga*, although three xylem bundles can be
235 identified, the phloem of the vascular bundle is connected together (Appendix S3; see
236 Supplemental Data with this article). This seems to represent a transitional pattern
237 between *Anaxagorea* and other latter divided clades.

238

239 It has been reported that in *Anaxagorea*, the ovules are served by the lateral
240 bundle complex from the base of the carpel [e.g., *A. luzonensis* (Derooin, 1997); *A.*
241 *crassipetala* (Endress & Armstrong, 2011)]. In our study, the 3D model showed that

the topological structure of the ring-arranged lateral bundle complexes plays a key role in forming the ovule bundles and that it facilitates the non-adjacent bundles to approach each other and merge. The dorsal bundle remained independent throughout, and there were no horizontal connections between dorsal bundle and the lateral bundle complexes. The ventral bundle participated in the formation of the spatial ring- arrangement of the lateral bundle complexes; however, it was not involved in forming of ovule bundles. In the basal ring, there are two lateral bundles which are fed to both ovules, which makes the topological structure of the basal ring unable to be flattened into a leaf-like structure bearing marginal ovules.

251

In conclusion, (1) the ovule/placenta bundles of *Anaxagorea* are amphicribal, (2) the ovule/placenta bundles are separated from the bundles of the carpel wall, and, (3) radiosymmetrical vasculature (including amphicribal bundles and ring-arranged bundle complexes) in the carpel of *Anaxagorea* are fed by a larger radiosymmetrical bundle system. Radiosymmetrical vasculature is a universal feature in vascular plant stems (Metcalf & Chalk, 1979; Evert, 2006; Beck, 2010; McKown & Dengler, 2010; Evert & Eichhorn, 2013). Recent results show that a similar pattern of ovule/placenta bundles might be common in angiosperms. It is plausible that the carpel is the product of the fusion between an ovule-bearing axis and the phyllome that subtends it, and the placenta bundles originate from a former branch. However, comparable information on the ring-arranged bundles of carpels is still lacking. It is necessary for further investigation to understand whether the radiosymmetrical vasculature are universal in angiosperm carpel.

265 **ACKNOWLEDGMENTS**

266 The authors thanks Profs. Lars Chatrou, Xin Wang, and Xin Zhang for their helpful
 267 suggestions to the manuscript. We also thank Chun-Hui Wang, Shi-Rui Gan, and
 268 Yan-Lian Qiu for their help in searching for the target species in the field, Qing-Long
 269 Wang for his assistance in caring for the transplant materials in Hainan province,
 270 Qiang Liu for his support during sampling, and Lan-Jie Huang and Ke Li for their
 271 advice on manuscript writing. We would also like to thank Editage for English
 272 language editing.

273

274 **FUNDING**

275 This study was supported by the National Natural Science Foundation of China (grant
 276 number 31270282, 31970250).

277

278 **AUTHOR CONTRIBUTIONS**

279 YL planned and designed the research, performed the experiments, collected the
 280 images, drew the illustrations, and wrote the article; WD performed the experiments
 281 and complemented the writing; YC developed the 3D model; SW complemented the
 282 writing; X-FW supervised the experiments and complemented the writing.

283

284 **SUPPORTING INFORMATION**

285 Additional supporting information may be found online in the Supporting Information
 286 section at the end of the article.

287

288 S1 Serial Section-Based Carpel Vasculature 3D Reconstruction of *A. javanica* (3ds

289 file)

290

291 S2 Serial Section-Based Carpel Vasculature 3D Reconstruction of *A. javanica* (movie)

292

293 S3 Ascending paraffin transections of *Cananga odorata* flower.

294

295 REFERENCES

- 296 Angenent, G. C., Franken, J., Busscher, M., van Dijken, A., van Went, J. L., Dons, H. J.
297 & van Tunen, A. J. (1995). A novel class of MADS box genes is involved in ovule
298 development in *Petunia*. *Plant Cell* **7**, 1569–1582.
- 299 Beck, C. B. (2010). *An introduction to plant structure and development: plant anatomy*
300 *for the twenty-first century*, 2nd edn. Cambridge, New York: Cambridge University
301 Press.
- 302 Chatrou, L. W., Pirie, M. D., Erkens, R. H. J., Couvreur, T. L. P., Neubig, K.M., Abbott,
303 J. R., Mols, J. B., Maas, J. W., Saunders, R. M. K. & Chase M.W. (2012). A new
304 subfamilial and tribal classification of the pantropical flowering plant family
305 Annonaceae informed by molecular phylogenetics. *Botanical Journal of the*
306 *Linnean Society* **169**, 5–40.
- 307 Chatrou, L. W., Turner, I. M., Klitgaard, B. B., Maas, P. J. M. & Utteridge, T. M. A.
308 (2018). A linear sequence to facilitate curation of herbarium specimens of
309 Annonaceae. *Kew Bulletin* **73**, 39.
- 310 Cronquist, A. (1979). The evolution and classification of flowering plants.
311 *Brittonia* **31**(2), 293–293.
- 312 Dave, Y. S., Patel, N. D. & Rao, K. S. (1981). Structural design of the developing fruit
313 of *Nicotiana tabacum*. *Phyton* **21**, 63–71.
- 314 De Bary, A. & Scott, D. H. (1884). *Comparative anatomy of the vegetative Organs of*
315 *the phanerogams and ferns*. Oxford, UK: Clarendon.
- 316 Ronse De Craene, L. P. & Smets, E. F. (1993). The distribution and systematic
317 relevance of the androecial character polymery. *Botanical journal of the Linnean*
318 *Society* **113**, 285–350.
- 319 Derooin, T & Norman, É. M. (2016). Notes on the floral anatomy of *Deeringothamnus*

- 320 Small (Annonaceae): cortical vascular systems in a chaotic pattern. *Modern*
321 *Phytomorphology* **9**, 3–12.
- 322 Derooin, T. (1988). Aspects anatomiques et biologiques de la fleur des Annonacées.
323 Ph.D. dissertation, Centre d’Orsay, France: Université de Paris-Sud.
- 324 Derooin, T. (1989). Définition et signification phylogénique des systèmes corticaux
325 floraux: l’exemple des Annonacées. In *Comptes Rendus de l’Académie des*
326 *Sciences Série III, Sciences de la vie*, pp. 71–75. Paris, France: Elsevier.
- 327 Derooin, T. (1997). Confirmation and origin of the paracarp in Annonaceae, with
328 comments on some methodological aspects. *Candollea* **52**, 45–58.
- 329 Derooin, T. & Bidault, E. (2017). Floral anatomy of *Pseudartabotrys* Pellegrin
330 (Annonaceae), a monospecific genus endemic to Gabon. *Adansonia* **39**, 111–123.
- 331 Doyle J. A. Origin of the angiosperm flower—A phylogenetic perspective. In Early
332 evolution of flowers, Endress, P. K. & Friis, E. M. (eds.), Plant Systematics and
333 Evolution Supplement 8, vol 8. Austria, Vienna: Springer.
- 334 Doyle J. A. (2006). Seed ferns and the origin of angiosperms. *Journal of the Torrey*
335 *Botanical Society* **133**, 169–209.
- 336 Doyle, J. A. (1978). Origin of angiosperms. *Annual Review of Ecology and Systematics*
337 **9**, 365–392.
- 338 Doyle, J. A. (2008). Integrating molecular phylogenetic and paleobotanical evidence on
339 origin of the flower. *International Journal of Plant Sciences* **169**, 816–843.
- 340 Doyle, J. A. & Le Thomas, A. (1996). Phylogenetic analysis and character evolution in
341 Annonaceae. *Adansonia* **18**, 279–334.
- 342 Doyle, J. A., Sauquet, H., Scharaschkin, T. & Le Thomas, A. (2004). Phylogeny,
343 molecular and fossil dating, and biogeographic history of Annonaceae and
344 Myristicaceae (Magnoliales). *International Journal of Plant Sciences* **165**,

345 S55–S67.

346 Endress, P. K. (2019). The morphological relationship between carpels and ovules in
 347 angiosperms: pitfalls of morphological interpretation. *Botanical Journal of the*
 348 *Linnean Society* **189**, 201–227.

349 Endress, P. K. & Armstrong, J. E. (2011). Floral development and floral phyllotaxis in
 350 *Anaxagorea* (Annonaceae). *Annals of Botany* **108**, 835–845.

351 Esau, K. (1965). *Plant anatomy*, 2nd edn. New York, USA: Wiley.

352 Evert, R. F. (2006). *Esau's Plant Anatomy. Meristems, Cells, and Tissues of the Plant*
 353 *Body: Their Structure, Function, and Development*, 3rd edition. New Jersey, USA:
 354 John Wiley & Sons, Inc, Hoboken.

355 Evert, R. F. & Eichhorn, S. E. (2013). *Raven Biology of Plants*, 8th edition. New York,
 356 USA: Palgrave Macmillan: W.H. Freeman Press.

357 Fahn, A. (1990). *Plant Anatomy*, 4th edition. Oxford, UK: Pergamon Press.

358 Guo, X. M., Xiao, X., Wang, G. X. & Gao R. F. (2013). Vascular anatomy of kiwi fruit
 359 and its implications for the origin of carpels. *Frontiers in Plant Science* **4**, 391.

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

363 Hickey, L. J. & Taylor, D. W. (1996). Origin of the angiosperm flower. In Flowering
 364 Plant Origin, Evolution & Phylogeny, D. W. Taylor & L. J. Hickey (eds.), pp.
 365 176–231. New York, USA: International Thomson publishing.

366 Kapoor, L. D. (1973). Constitution of amphicribal vascular bundles in capsule of
 367 *Papaver somniferum*. Linn. *Botanical Gazette* **134**, 161–165.

368 Lersten, N. R. & Don, K. W. (1966). The discontinuity plate, a definitive floral
 369 characteristic of the *Psoraleae* (Leguminosae). *American Journal of Botany* **53**,

370 548–555.

371 Liu, W. Z., Hilu, K. & Wang, Y. L. (2014). From leaf and branch into a flower:
372 *Magnolia* tells the story. *Botanical Studies* **55**, 28.

373 McKown, A. D. & Dengler, N. G. (2010). Vein patterning and evolution in C4 plants.
374 *Botany* **88**, 775–786.

375 Melville, R. (1960). A new theory of the angiosperm flower. *Nature* **188**, 14–18.

376 Metcalfe, C. R. & Chalk, L. (1979). *Anatomy of the dicotyledons, vol. 1, systematic*
377 *anatomy of leaf and stem, with a brief history of the subject*, 2nd edition. Oxford,
378 UK: Clarendon Press.

379 Nixon, K. C., Crepet, W. L., Stevenson, D. & Friis, E. M. (1994). A reevaluation of
380 seed plant phylogeny. *Annals of the Missouri Botanical Garden* **81**, 484–533.

381 Ogura, Y. (1972). *Comparative anatomy of vegetative organs of the pteridophytes*.
382 Berlin. Germany: Gebrueder Borntraeger.

383 Roe, J. L., Nemhauser, J. L. & Zambryski, P. C. (1997). TOUSLED participates in
384 apical tissue formation during gynoecium development in *Arabidopsis*. *Plant Cell* **9**,
385 335–353.

386 Rudall, P. J. (2007). *Anatomy of flowering plants. An introduction to structure and*
387 *development*. Cambridge, UK: Cambridge University Press.

388 Satina, S. (1944). Periclinal chimeras in *Datura* in relation to development and structure
389 (A) of the style and stigma (B) of calyx and corolla. *American Journal of Botany* **31**,
390 493–502.

391 Satina, S. & Blakeslee, A. F. (1943). Periclinal chimeras in *Datura* in relation to the
392 development of the carpel. *American Journal of Botany* **30**, 395–450.

393 Satina, S. & Blakeslee, A. F. (1941). Periclinal chimeras in *Datura stramonium* in
394 relation to development of leaf and flower. *American Journal of Botany* **28**,

395 862–871.

396 Satina, S. (1945). Periclinal chimeras in *Datura* in relation to the development and

397 structure of the ovule. *American Journal of Botany* **32**, 72–81.

398 Takhtajan, A. (1969). *Flowering plants, origin and dispersal*. Edinburgh, UK: Oliver &

399 Boyd Ltd.

400 Thorne, R. F. (1996). The Least Specialized Angiosperms. In *Flowering Plant Origin,*

401 Evolution & Phylogeny, Taylor, D.W. & Hickey, L. J. (eds.), pp.286-313. New

402 York, USA: Chapman & Hall.

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

404 *American Journal of Botany* **62**, 191–197.

405 Von Balthazar, M.& Endress, P. K. (2002). Reproductive structures and systematics of

406 Buxaceae. *Botanical Journal of the Linnean Society* **140**, 193–228.

407 Wang, X. (2010). *The Dawn Angiosperms*. Heidelberg, Germany: Springer.

408 Wang, X. (2018). *The Dawn Angiosperms*, 2nd edition. Heidelberg, Germany,

409 Springer.

410 Wang, Y. Z. & Pan, K. Y. (1998). Comparative floral anatomy of *Whytockia*

411 (Gesneriaceae) endemic to China. In *Floristic Characteristics and Diversity of East*

412 Asian Plants, Zhang, A. L. & Wu, S. G. (eds.), pp. 352–366. Beijing, China: China

413 Higher Education Press.

414 Wynn, A. N., Seaman, A. A., Jones, A. L. & Franks, R. G. (2014). Novel functional

415 roles for PERIANTHIA and SEUSS during floral organ identity specification,

416 floral meristem termination, and gynoecial development. *Frontiers in Plant Science*

417 **5**, 130.

418 Xin, G., Ni, X. & Liu, W. (2019). Anatomy and Development of Gynoecium in

419 *Tapiscia sinensis* Oliv. and its Implications for the Origin of Carpels. *Bangladesh*

- 420 *Journal of Botany* **48(4)**, 933-941.
- 421 Zhang, X., Liu, W. & Wang, X. (2017). How the ovules get enclosed in magnoliaceous
- 422 carpels. *PLoS One*, 12, e0174955.
- 423 Zhang, X., zhang, Z. X. and Zhao, Z. (2019). Floral ontogeny of *Illicium Lanceolatum*
- 424 (Schisandraceae) and its implications on carpel homology. *phytotaxa* 416, 200–210.
- 425 doi: 10.11646/phytotaxa.416.3.1
- 426
- 427

Fig. 1. Floral morphology and gynoecium development in two *Anaxagorea* species.

(A) *Anaxagorea luzonensis* flower. (B) *Anaxagorea javanica* flower. (C) Young *A. luzonensis* fruit. (D) Mature *A. javanica* fruit. (E–G) *A. luzonensis* floral development. (H–J) *A. javanica* gynoecium development. s, sepal; pe, outer petal; pi, inner petal; st, stamen; si, staminode; c, carpel. Scale bars =200 μ m.

Fig. 2. Carpel organogenesis in two *Anaxagorea* species.

(A–F) *Anaxagorea luzonensis*. (A) Carpel primordia. (B–C) Carpel stipe emergence.

(D–E) Carpel thickening and stigma formation, showing carpel stipe elongation. (F)

Mature carpels. (G–J) *A. javanica* shows similar carpel developmental features to

changes depicted in (A–E), and (F). Ventral slit end indicated by arrows. Scale bar =

200 μ m.

Fig. 3. Ascending paraffin transections of the *Anaxagorea luzonensis* flower.

(A) Base of receptacle. (B) Mid-section of androecia, showing stamen bundles and central stele. (C) Top of receptacle, showing central stele divided into two groups (* mark the breaks). (D) Bundles from the central stele enter carpels. (E) Base of carpels, showing basal ring. (F) Upper part of carpel stipes, showing the basal ring breaks (marked as *). (G) Bottom of ovary locule, showing amphicribal lateral bundle complexes (left), and “C”-shaped lateral bundle complexes (right). (H) Base of ovary locule. st, stamen; db, dorsal bundle; lbc, lateral bundle complex; vb, ventral bundle; ob, ovule bundle. Scale bar = 500 μm.

Fig. 4. Ascending paraffin transections of the *Anaxagorea javanica* flower.

(A) Base of receptacle, showing six groups of vascular bundles and sepal connections. (B) Points of petal connection to receptacle, showing perianth bundles. (C) Androecial bundles serving stamens by repeated branching. (D–E) Base of gynoecium, showing enlarged central stele breaks and bundles distributed into carpels. (F–G) Carpel vasculature at different positions. (F1) Detailed view of (F), showing basal ring of carpel. (H) Amphicribal lateral bundle complexes in carpel. st, stamen; si, staminode; c, carpel; db, dorsal bundle; lbc, lateral bundle complex. Scale bar = 500 μ m.

463

464 **Fig. 5.** Ascending paraffin transections of the mature *Anaxagorea luzonensis* carpel.

465 (A) Carpel base, showing basal ring. (B–C) Basal ring breaks on ventral side. (D–F)

466 Ascending carpel stipe sections, showing lateral bundles reconstituted to two sets of

467 ring-arranged lateral bundle complexes. (G–H) Top of carpel stipe, showing

468 “C”-shaped lateral bundle complex. (I–K) Below ovary locule, showing formation of

469 ovule bundles. (L) Base of ovary locule. db, dorsal bundle; lb, lateral bundle; vb,

470 ventral bundle; ob, ovule bundle. Scale bar = 500 μm.

Fig. 6. 3D construction of *Anaxagorea javanica* vasculature.

Bundle outlines colored green, xylem red, and purple, among which bundles associated with ovule bundles are colored purple. (A–D) Aniline blue-stained *A. javanica* sections for modeling. (E) Longitudinal section of mature *A. javanica* carpel (left), and 3D vasculature model, dotted lines on longitudinal section indicate vasculature position in carpel. (F) Perspective from base of carpel vasculature. (G) Perspective from base of carpel (xylem only). The arrow indicates the intersection of two lateral bundles which feed two ovules respectively. (H) Cross-section of 3D model corresponding to (C), showing ring-arranged lateral bundle complexes. (I) 3D

481 model section showing distribution of vascular bundles at base of ovary. db, dorsal

482 bundle; vb, ventral bundle; ob, ovule bundle, lb, lateral bundle. Scale bar = 500 μ m.

483

484

485 **Appendix S3.** Ascending paraffin transections of the *Cananga odorata* flower. (A)

486 Bundles of the receptacle. (B) The base of gynoecium. (C) The base of carpel,

487 showing the connected phloem. **(D)** The sunken carpel stipes. **(E–F)** The ovary locule,
 488 showing ovules are fed by the dorsal bundle. db, dorsal bundle; lb, lateral bundle; mlc,
 489 carpel mediolateral bundle. Scale bars (**A–E**) = 1000 μm ; **F, G** = 500 μm .

490