



## NOTES ON THE FLORAL ANATOMY OF *DEERINGOTHAMNUS* SMALL (ANNONACEAE): CORTICAL VASCULAR SYSTEMS IN A CHAOTIC PATTERN

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**Abstract.** Floral anatomy of *Deeringothamnus* Small, an endemic and endangered annonaceous genus from Florida comprising 2 species, was studied. It is mainly distinguished by missing any sclerenchyma at anthesis – apart from anthers – and by a chaotic vasculature exhibiting a partial perianth-stamen cortical vascular system, appearing as an over-evolution of the pattern previously described in the neighbouring *Asimina* and other paracarpous and pseudosyncarpous genera. Such a pattern is amazingly similar to that previously recognized in the most basal annonaceous genera, as e.g. *Ambavia*, and might be considered as a reversion to an ancestral state. These alterations toward decrease are related to the receptacle flatness and lack of pollination chamber, and seem parallel with the dwarf habit of this genus.

**Key words:** *Asimina*, Magnoliales, cortical vascular systems (CVS), floral vasculature, Florida

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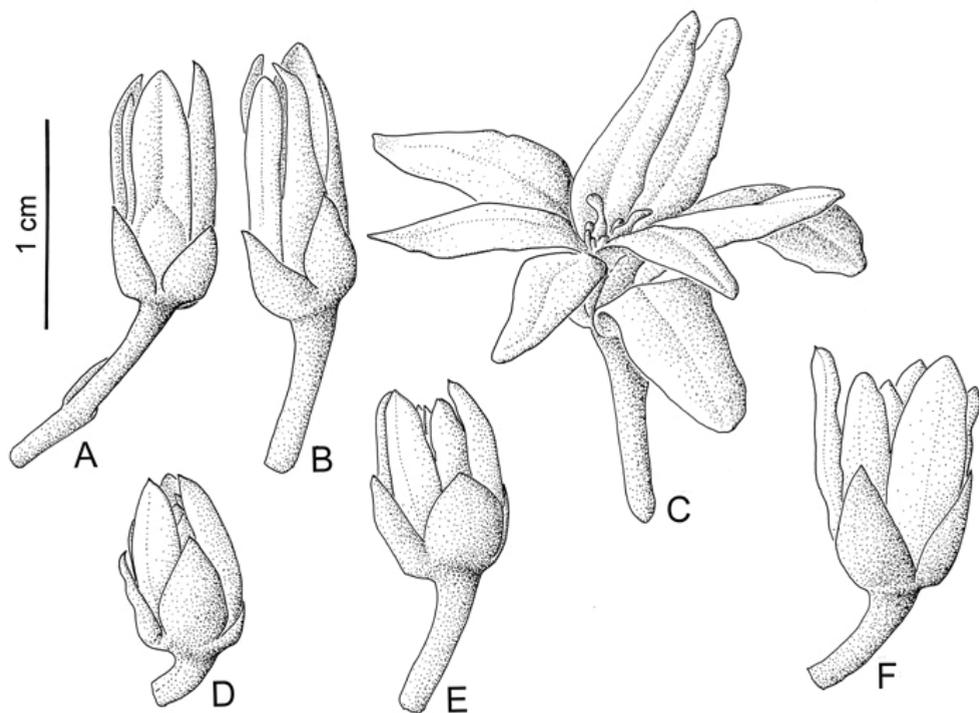
### Introduction

Among the Annonaceae, the genus *Deeringothamnus* Small is well characterized by its habit (dwarf shrub), diverging from its ally – Northern American too – *Asimina* Adanson by its diminutive stature, flat or depressed floral receptacle and narrow unsculptured petals (NORMAN 2003), as well as the petals number (6-12 vs. 6) and stamens number ( $\leq 25$  vs.  $\geq 30$ ), after KRAL (1960). It comprises two species, *D. pulchellus* Small and *D. rugelii* (B.L. Rob.) Small, both are more or less pyrophytic and endemic in open places of Florida, growing in poorly drained sandy soils, in flatwoods. About 27 populations were recognized for *D. pulchellus*, and 15 for *D. rugelii* (NORMAN 2007). A merging of the two species in one with two varieties was even proposed by WARD (2001), in fact based on the erroneous supposition intermediates occur on the field (NORMAN 2007). Two instances of natural intergeneric hybrids were recognized: *A. pygmaea* × *D. rugelii* and *A. reticulata* × *D. pulchellus* (NORMAN 2003), which appear however sterile (MERCER *et al.* 2016).

As the reproductive biology of these two rare and endangered species (federally listed USFWS 1986) was analysed by the second of us, it was interesting to tackle their floral anatomy, so that comparisons could be made with the related genus *Asimina* (at present only *A. triloba* (L.) Dunal has been accurately studied, see SMITH 1928).

### Material and methods

Two advanced floral buds of each species (*D. pulchellus* and *D. rugelii*) were studied, from FAA gatherings (É. Norman *s.n.*, Florida: respectively Orange County, Christmas, IV.2005, and near Smyrna Beach, 15.XI.2004) kept at P (spirit collection) in a mixture of water, ethanol and glycerol (equal volumes). For each species, one bud (Fig. 1 B, E) was dehydrated by t-butanol, then embedded in paraffin (melting point = 60°C) upon the standard schedule (GERLACH 1984). Microtome cross sections were made at a thickness of 15 µm, stained by 0.5% Astrablue (5 mn) and 10% Ziehl's Fuchsin (5 mn), then dehydrated by acetone and mounted in Eukitt. Flower vasculature was



**Fig. 1.** Morphology of the studied *Deeringothamnus* flowers: **A-C** – *D. pulchellus*; **D-F** – *D. rugelii*.

reconstituted by drawings of the serial sections using a camera lucida. Mountings were deposited at the slide library (“histothèque”) of P, under the references: *Deroin 186* and *187*. Moreover, longitudinal hand sections of the other buds were made as a rough check of vascular diagram, and allow a better understanding of carpel morphology.

## Results

### Pedicle histology (Fig. 2 A, D)

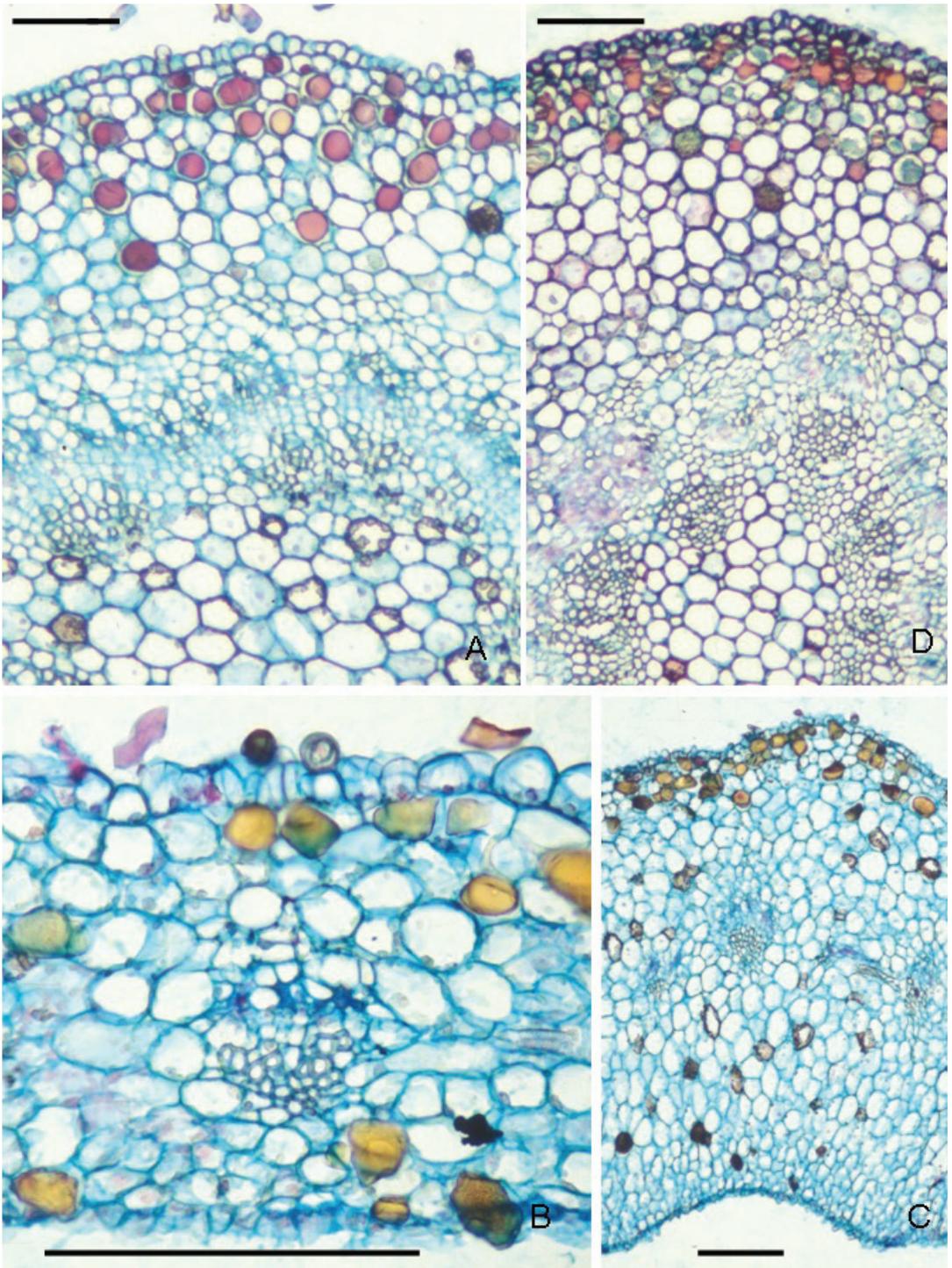
The epidermis is made up by small rounded and papillose cells, including some 2-celled simple hairs, and outlined by a hypodermis, slightly distinct from the cortex, which exhibits 8-12 layers of wider cells, with intercellular spaces and secretory cells, especially in the outer half. No sclereid is recognized at this stage, close to the anthesis. Phloem fibers show thin walls. There are *ca.* 20 bundles, elliptical in cross section and

2-3 cambial layers are seen in them. Pith cells appear similar to cortical ones. Main differences between the two species occur in the parenchymas (lignified in *D. rugelii*), and in the arrangement of phloem fibers (rather continuous ring in *D. pulchellus*; discrete crescent massifs in *D. rugelii*).

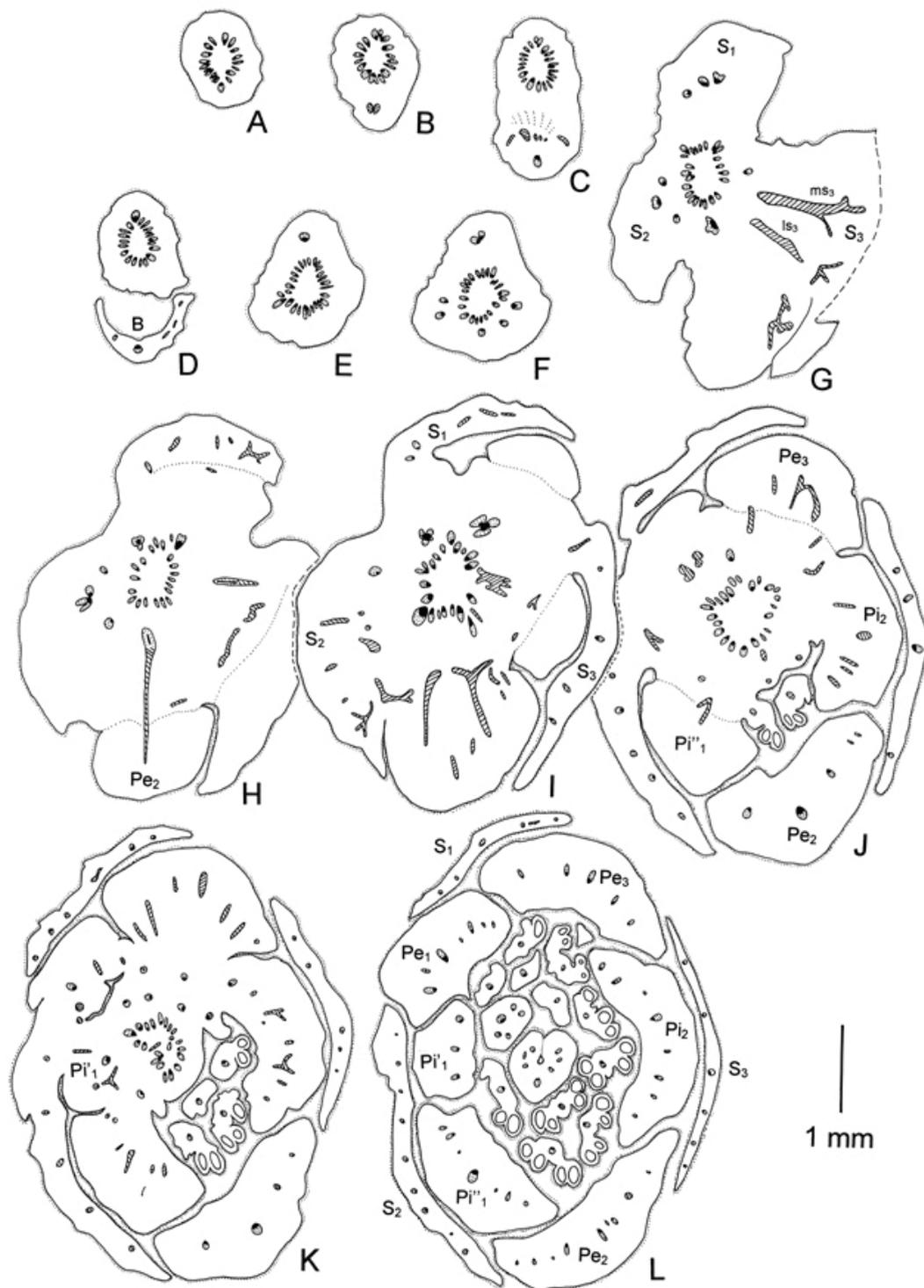
With a diameter of 1000-1250  $\mu\text{m}$  and *ca.* 20 xylem poles, *Deeringothamnus* pedicels may be compared with those previously described in *Cananga*, *Isolona*, *Rollinia* or *Uvariopsis* (DEROIN 1997), i.e. in fairly derived genera.

### Perianth histology (Fig. 2 B, C)

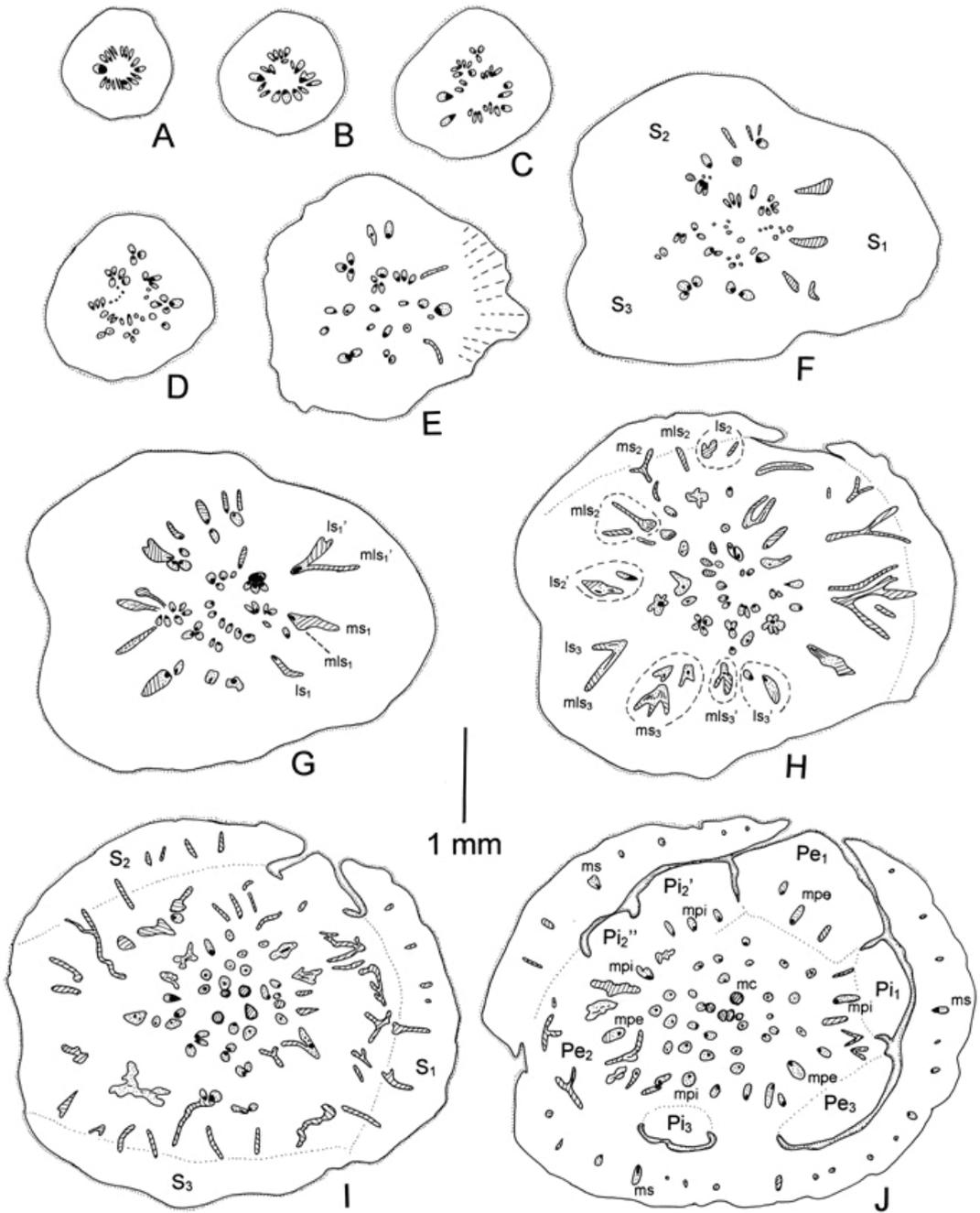
Sepals are third (*D. pulchellus*) to half (*D. rugelii*) the thickness of petals, exhibit a homogeneous parenchyma with scattered secretory cells, and bear simple hairs outside like the pedicel. In petals parenchyma cells decrease in size towards the inner side, which is covered by a papillose epidermis, devoided



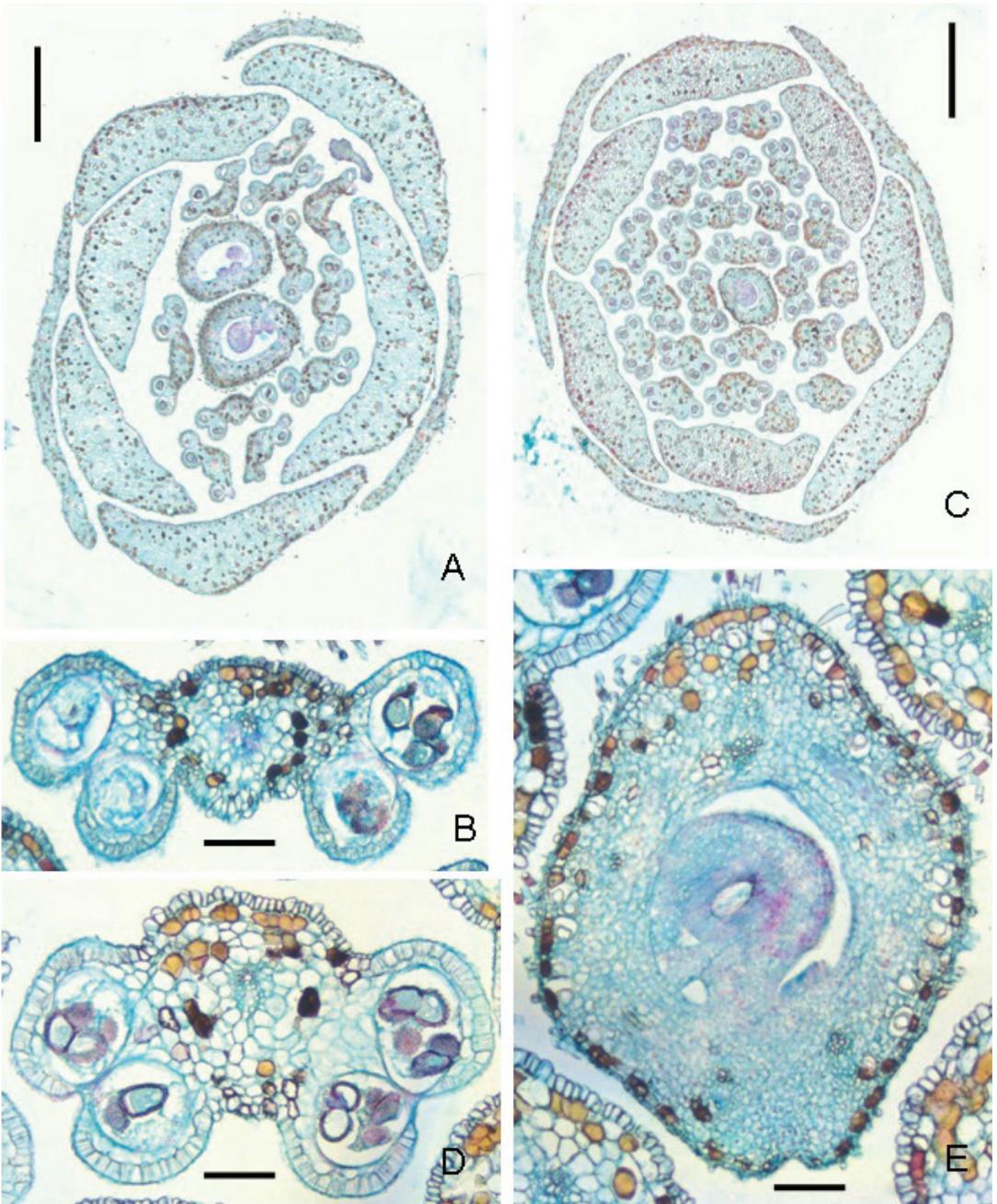
**Fig. 2.** Flower histological features in *Deeringothamnus pulchellus* (A – cross section of pedicel (detail); B – sepal; C – outer petal) and in *D. rugelii* (D – pedicel). Stained by the combination of aqueous Astrablue 0.5% and Ziehl's Fuchsin 10%. All scale bars = 100  $\mu$ m.



**Fig. 3.** Ascending transverse sections of the flower of *Deeringothamnus pulchellus*: **A-D** – insertion of the bract; **E-L** – from the pedicel to the gynoecium base level. Abbreviations: **B** – bract; **ls** – sepal lateral bundle; **ms** – sepal median bundle; **Pe** – outer petal; **Pi** – inner petal; **S** – sepal.



**Fig. 4.** Ascending transverse sections of the flower of *Deeringothamnus rugelii*: A-J – from the pedicel to the receptacle top level. Abbreviations: same as in Fig. 3, with **mc** – carpel median bundle; **mls** – sepal mediolateral bundle.



**Fig. 5.** Histological features in *Deeringothamnus pulchellus* (A – cross section of flower; B – anther base) and in *D. rugelii* (C – cross section of flower; D – anther base; E – carpel (second ovule level in ascending order)). Stained by the combination of aqueous Astrablue 0.5% and Ziehl's Fuch sine 10%. Scale bars: A, C = 1 mm; B, D, E = 100  $\mu$ m.

of any corrugation unlike *Asimina* (NORMAN *et al.* 1992). Secretory cells are located as in the pedicel. In *D. rugelii*, petals bundles show some phloem fibers.

#### Anther histology (Fig. 5 C, D)

Epidermis is somewhat thin and discontinuous on the pollen sacs, much thicker

and cutinized around the connective. Fibrous layer appears weakly lignified and very distinct from the hypodermis, parenchymatous on the adaxial side, sclerenchymatous on the abaxial one. At the anthesis tapetum seems 1-layered with flattened cells. Pollen grains are more or less in tetrads, exine is brightly stained by Fuchsin.

Connective contains an abundant parenchyma, including a crown of secretory cells. The collateral bundle exhibits protoxylem and metaxylem vessels and a rather wide phloem. Differences between the both species are very slight: in *D. rugelii*, hypodermis is much more lignified on the abaxial side, and connective contains larger cells.

### Receptacle vasculature (Figs 3 & 4)

While subtending bract is 1-trace (Fig. 3 A-D), sepals have basically 3 traces with all stages of fusion between them (Fig. 3 E-G,  $S_1$ : one strand; Fig. 4 F-G,  $S_1$ : 3 free strands). All petals are basically 1-trace in *D. pulchellus* (Fig. 3 H-I,  $Pe_2$ ,  $Pe_3$ ), 3-trace in *D. rugelii* (Fig. 4 H-J). Outer corolla is always regularly trimerous, inner one being altered both by loss of petal (*D. pulchellus*: Fig. 3 J-L, no  $Pi_3$ ) and duplication of inner petal (Fig. 3 J-L,  $Pi'_1$ ,  $Pi''_1$ ; Fig. 4 J,  $Pi'_2$ ,  $Pi''_2$ ). Near the top of the receptacle, androecial and gynoecial traces are inserted on a chaotic vascular plexus. Some fusions occur between bundles from different whorls, and so an irregular cortical vascular system (CVS) might be recognized, androecial strands being not arranged in a distinct outer crown in cross section, as usual in Annonaceae.

By comparison with *Asimina triloba* (SMITH 1928), the vascular pattern in *Deeringothamnus* is somewhat irregular (Fig. 7), and bundle fusions are scarcer, at once inside each whorl (e.g. between lateral bundles of sepals or petals) and between neighbouring whorls. Thus only a partial CVS is built, but it belongs to the same perianth-stamen pattern fully expressed in *Asimina*. Androecial vasculature is double in origin (from CVS and from stele), as in e.g. the paracarpous genus *Monodora* (DEROIN 1988). In fact, *Deeringothamnus* shows a reduction of CVS extent, linked to a disorganization of

the inner corolla (leading to the lack of any pollination chamber, see NORMAN 2003), and a drastic decrease in stamen and carpel numbers. In *D. pulchellus* floral vasculature appears most condensed, each petal fed by a unique trace. Moreover it should be quoted that receptacle is flat in *Deeringothamnus*, but rather bulging in *Asimina* (VAN HEUSDEN 1992).

### Carpel vasculature (Fig. 6)

In the two species, carpels are very few (1-3), not peltate and supplied by 3 bundles. The 5-6 ovules are 1-seriate and fed during anthesis – as the rule in Annonaceae, and even Magnoliales – by median bundle, through a vascular network in the ovary wall. Lateral bundles fade at the stylar level. As usual, median bundle only enters the stigma, splitting at the top for providing the two lobes. Carpel histology is very like that of sepal or petal, but epidermis bears longer simple 2-celled hairs.

### Discussion and conclusions

Between the two *Deeringothamnus* species, histological differences appear very slight. The most striking features vs. *Asimina* are probably the lack of any sclereid at anthesis (outside anther), whereas the secretory cells are mainly crowded at the abaxial side of floral phyllomes. Interestingly, anther histology is homogeneous, although connective heads are obviously different (tongue-like in *D. pulchellus*, bulbous in *D. rugelii*). Inflorescence architecture and petal thickness revealed also to be reliable diagnostic characters (NORMAN 2003). Thus a disjunction occurs between histology (at the genus level) and external morphology, the latter much more plastic.

On the other hand, vascular diagrams are consistent with a tight relationship with *Asimina*, wholly strengthened by a recent study (MERCER *et al.* 2016): same CVS pattern involving 4 whorls, double supply of androecium, 3-trace carpel (without mediolateral bundles – a derived condition for the family – see DEROIN 1997). This advanced syndrome is wholly comparable to that in other paracarpous (*Isolona*,

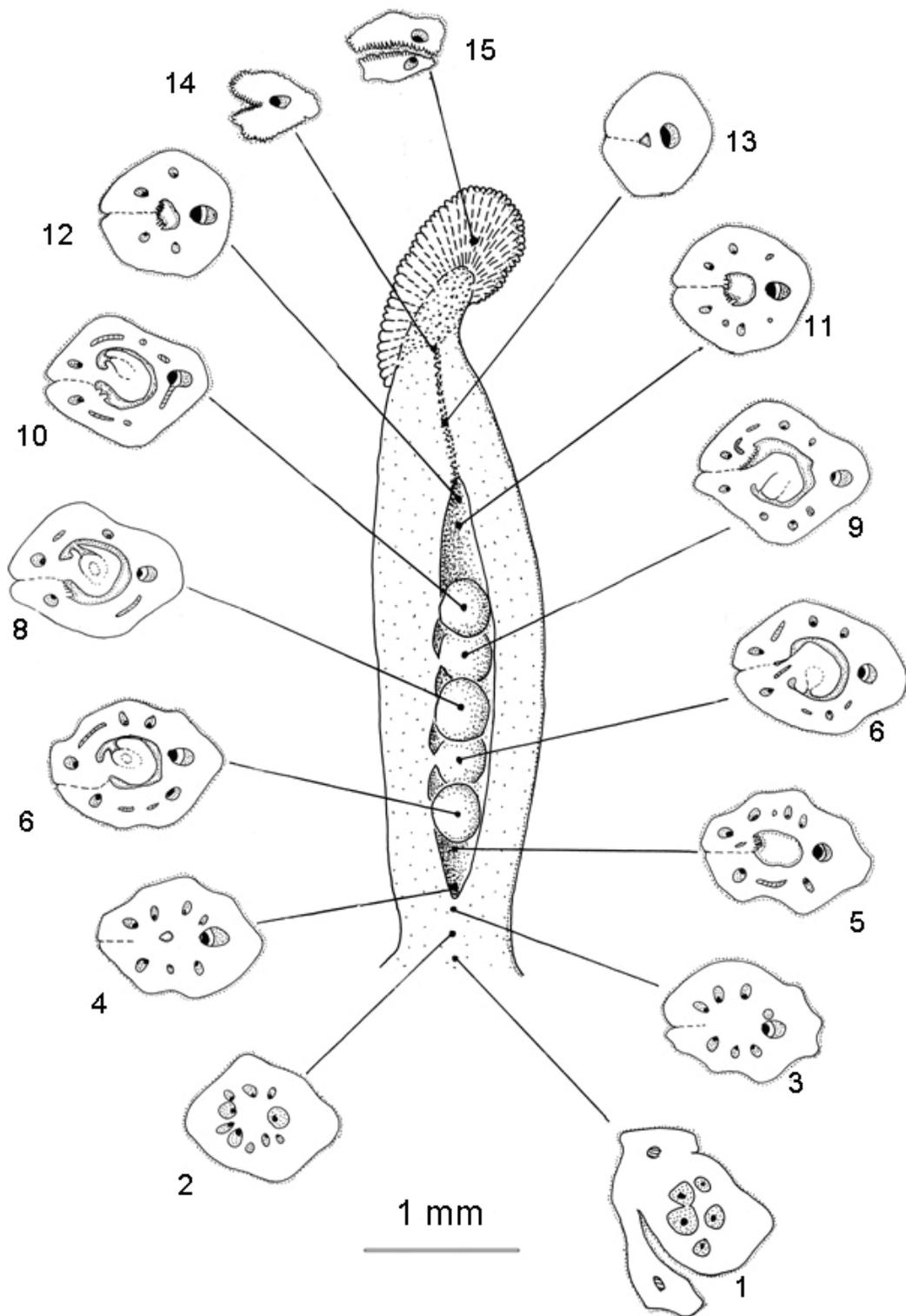
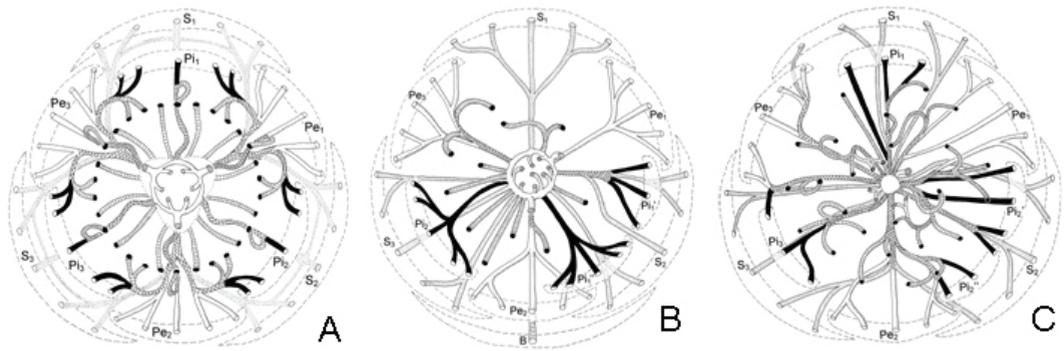


Fig. 6. Carpel architecture of *Deeringothamnus pulchellus* – longitudinal section with related ascending transverse ones.



**Fig. 7.** Vascular diagrams of flowers: **A** – *Asimina triloba*, after SMITH (1928); **B** – *Deeringothamnus pulchellus*; **C** – *D. rugelii*. Abbreviations: same as in Fig. 3. Sepal bundles **stippled**, outer petal bundles **white**, inner petal bundles **black**, stamen bundles **hatched with black tips**, carpel bundles **stippled with hatched tips**, fused bundles (cortical vascular system) **crossed**.

*Monodora*, see DEROIN & COUVREUR 2008) and pseudosyncarpous (*Annona*, *Rollinia*) genera, and close phylogenetical relationships are now well supported by molecular analysis (COUVREUR *et al.* 2015: 5). However *D. pulchellus* differs significantly from *D. rugelii* by lacking lignified parenchymas, its highly reduced CVS, and the lack of vascular fusion between members of a same whorl. Combining these features with differences in petal thickness and connective head, it seems more appropriate not to merge the two species, as proposed by WARD (2001), although they are undoubtedly very near.

Conversely the vascular architecture of *Deeringothamnus* flower appears as an over-evolution of the *Asimina* pattern, its irregular character is reminiscent of that previously described in the East-African genus *Sanrafaelia* Verdc., in which the flat receptacle exhibits even a trend to inferovary (DEROIN 2000), fully expressed in most *Xylopia* species (VAN HEUSDEN 1992). Such a chaotic vascular pattern was yet described in the Malagasy endemic *Ambavia gerrardii* (Baill.) Le Thomas, combined with a wide intra-individual variation in perianth arrangement and lack of any CVS, a relevant feature of the other basal genera in the family (DEROIN & LE THOMAS 1989). So floral vasculature of the most derived annonaceous genera exemplifies a reversion to an ancestral condition with a recovery of evolutive potentialities.

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